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Abstract  Cortical spreading depression (CSD), an underlying mechanism of migraine aura, propagates to the hippocampus, and might explain hippocampus-associated symptoms during migraine attack. We hypothesised that this process is, some parts, mediated by NMDA receptors. By using a rat model, CSD was elicited by solid KCl for 45 minutes prior to electrophysiological and quantitative analyses. The result from electrophysiological study was the ratio of glutamate NMDA receptor 2A and 2B subunits (GluN2A/B). Total NMDA receptor response was isolated using an AMPA antagonist, prior to a GluN2B receptor antagonist. The GluN2A/B ratio was calculated by dividing the remaining NMDA-mediated field-excitatory synaptic potentials (fEPSP) with the subtracted difference of NMDA-mediated fEPSP. Western blot analysis of the hippocampus was performed to confirm the quantitative change of GluN2A/B ratio. In hippocampal slice study (n = 12), the GluN2A/B ratio of hippocampal fEPSP was significantly increased in CSD group. Western blot analysis (n = 30) revealed an increase in GluN2A subunits and a decrease in GluN2B subunits in the hippocampus ipsilateral to the CSD induction. Our current study revealed that GluN2A/B ratio was shown to be elevated following CSD stimulation by increasing the total number of GluN2A while reducing the total number of GluN2B subunits. This ratio was demonstrated to be associated with synaptic plasticity of the hippocampus in numerous studies. In conclusion, we showed that CSD increased GluN2A/B ratio, in turn, would result in altered synaptic plasticity. Our findings provide a probable implication on the correlation of migraine aura and hippocampus-associated symptoms.

Keywords  NMDA receptors • migraine aura • transient global amnesia (TGA) • hippocampal spreading depression • long-term potentiation (LTP) • AMPA receptors

Introduction  Various cerebral insults (e.g. epileptic crises, trauma, ischemia, haemorrhage, and migraine) were shown to produce a transient disturbance in cortical activity, so-called cortical spreading depression (CSD). Cortical spreading depression is caused by massive redistribution of ions, particularly potassium and hydrogen ions between intracellular and extracellular compartments [1] resulting in cortical depolarisation that can spread to the adjacent areas. By adopting a model of migraine with aura, spreading depression (SD) in each cortical area is accountable for different clinical manifestations seen in the patients. For instance, CSD in the occipital cortex can cause visual metamorphopsia [2, 3], whereas those occur in the somatosensory cortex result in paraesthesia or hemi-amaesthesia [4].

Spreading depression propagated to the hippocampus is believed to cause amnesia, emotional and behavioural changes (e.g. hyperactivity, yawning) during migraine attack [5]. In case of acute amnesia, it seems to be transient (4-8 hours in duration) and may impair both anterograde and retrograde memory. Long-term association of migraine and amnesia was also demonstrated in a retrospective cohort study suggesting that migraine is a risk factor of developing transient amnesia.
global amnesia (TGA) [6]. For decades, Olesen and Jorgensen proposed that the association between migraine and TGA may be explained by presence of spreading depression in the hippocampus [7]. The hypothesis was later proven by groups of ex vivo and in vitro experiments showing that CSD propagated to the hippocampus. Limited studies were published regarding the presence of SD in the in vivo hippocampus following CSD except in familial hemiplegic migraine type 1 (FHM1) mutant mice [8]. Although the molecular mechanisms underlying the correlation of migraine and TGA are still unclear, existing evidence suggested that the process may involve actions of glutamatergic receptors [9].

Glutamatergic transmission is known to play an important role in inducing plastic change in the hippocampal synapse. Repetitive activation of the hippocampal synapse can result in a long-lasting change in synaptic activity known as long-term potentiation (LTP). This process is an important step in the registration and consolidation of new memories. Various classes of glutamatergic receptors are involved in LTP development, specifically α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartic acid (NMDA) receptors. Previously, our group showed that CSD significantly reduced LTP magnitude by decreasing post-synaptic AMPA receptor response [9]. However, the effect of CSD on hippocampal NMDA receptor activity were not demonstrated. Differential subunits of NMDA receptors mainly detected in the hippocampus are GluN2A and GluN2B subunits [10]. Thus, these receptors are of our particular interest. Additional studies have demonstrated that GluN2A/B response ratio is strongly associated with synaptic plasticity by modifying LTP induction threshold [11-13].

In this study, we aimed to demonstrate the changes in synaptic transmission of NMDA receptors. We identified existence of SD observed in the rat hippocampus and compared their differences in electrical properties with the original CSD. Sequential changes in NMDA receptor activity were reported in terms of GluN2A/B response ratio. Quantitative assays of GluN2A and GluN2B subunits were also performed using Western blot analysis. The findings of this study may imply a clinical correlation between migraine and hippocampus-associated symptoms.

Methods

Animals

Adult male Wistar rats (National Laboratory Animal Centre, Mahidol University, Nakorn-Pathom, Thailand) weighing 200-350 g were recruited in this study (n = 50). The animals were acclimatised to the housing facility for at least seven days prior to the experiments. The study was conducted according to the guideline for experimental animals suggested by the National Research Council of Thailand. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University (No.012/2553).

Animal preparations

Each rat was anaesthetised with 60 mg/kg of sodium pentobarbital (Ceva Sante Animale, Libourne, France) intraperitoneally. We avoided using inhaled isoflurane or IV dexmedetomidine as surgical anaesthetics due to their effects on suppressing CSD frequency [14]. Physiological parameters were monitored and only animals that were in stable condition throughout the preparation were included in the experiment. All anatomical landmarks guided by Paxinos & Watson’s brain atlas [15].

CSD induction

The rat’s head was fitted to a stereotaxic apparatus (Narishige, Tokyo, Japan). After the right parietal bone had been exposed, a 2-mm craniotomy was performed at 6 mm posterior to the bregma and 2 mm lateral to the sagittal suture using an electric dental driller (NSK, Tokyo, Japan). Since propagation of CSD into the hippocampus usually occurred under hyper-excitible conditions, induced either pharmacologically or genetically, increased dose of KCl was employed in our study. For CSD induction, 3 mg of solid KCl (Sigma-Aldrich, St. Louis, MO, USA) was topically applied onto the dura mater for 45 minutes. In control rats, 3 mg of solid NaCl (Merck, Darmstadt, Germany) was used instead of solid KCl.

In vivo cortical DC recording

For the DC recording (n = 4), a 2-mm diameter craniotomy was performed in the right frontal bone (from bregma: anterior-posterior, +3 mm; lateral, 2 mm; and dorsal-ventral, 0.5 mm). A recording glass microelectrode for detecting the DC potential was inserted into the frontal neocortex to a depth of 500 μm. Analogue data were converted into digital format using. The data were then analysed using MP100 (Biopac Systems Inc., Goleta, CA, USA) and AcqKnowledge acquisition software (Biopac Systems Inc., Goleta, CA, USA). The measured variables included the area under the curve (AUC) and the amplitude of each CSD wave as well as the number of CSD waves that occurred within a 45-minute period.
In vivo hippocampal DC recording

In a separated set of experiments (n = 4), the DC potential was recorded in the CA1 region of the hippocampus instead of the neocortex (from bregma: anterior-posterior, -4 mm; lateral, 2 mm). A recording glass microelectrode was inserted with a depth of 2.2 mm with the aid of rat brain atlas [15] and previous electrophysiological study [16]. The histological position of the electrode was confirmed microscopically.

Hippocampal slice preparation

After 45 minutes of CSD stimulation using solid KCl, the animals (n = 6, each group) were decapitated and their ipsilateral hippocampal tissues were entirely dissected. These tissues were then quickly loaded and sectioned using a Vibratome tissue slicer (Vibratome, Richmond, IL, USA). The tissues were processed in cooled artificial CSF solution (119 mM NaCl, 26.2 mM NaHCO3, 11 mM glucose, 2.5 mM KCl, 2.5 mM CaCl2, 1.3 mM MgSO4, 1.0 mM NaH2PO4, 0.1 mM picrotoxin; a GABA_A receptor antagonist), bubbled with carbogen (95 % O2, 5 % CO2). Fresh slices were moved to a humidified interface-type holding chamber and recovered for at least 1.5 hours prior to the performance of the electrophysiological study.

Electrophysiological recording

A continuation between the CA1 and CA3 region was terminated in order to prevent epileptiform activity originating from the CA3 region. A bipolar tungsten-stimulating electrode was placed in the Schaffer collaterals to evoke a postsynaptic response by delivering a square-pulse stimulus at 0.1 Hz for 0.2 msec. Discharges from presynaptic fibres followed by fEPSPs were recorded using a glass microelectrode. Only slices that produced fEPSP amplitudes of more than 1 mV and were stable for at least 15 minutes were included in this study.

Response ratio of GluN2A/B

After stable baseline fEPSPs were recorded for at least 15 minutes, NMDA receptor-mediated fEPSPs were isolated by bath application of a potent AMPA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione; CNQX (10 µM in 0.1 % DMSO; Tocris Bioscience, Bristol, UK) to exclude signals from AMPA components. Ten minutes after application of the drug, input stimulation was delivered at 0.033 Hz and its intensity was adjusted to evoke stable NMDA receptor-mediated fEPSPs. Ten minutes later, the GluN2A component of NMDA receptor-mediated fEPSPs was isolated by bath application of CNQX (10 µM) and GluN2B subunit-selective NMDA receptor antagonist, ifenprodil (3 µM in 0.1 % DMSO; Tocris Bioscience, Bristol, UK) for 1 hour.

Total component of NMDA receptor responses was the averaged AUC of the NMDA receptor-mediated fEPSPs during 10 minutes prior to ifenprodil application. The amplitude of NMDA receptor-mediated fEPSPs was normalised in the range of 0.5-1.5 mV. GluN2A component of NMDA receptor responses was the averaged AUC of the NMDA receptor-mediated fEPSPs during 50-60 minutes after ifenprodil application (i.e., ifenprodil-insensitive component). GluN2B component (i.e., ifenprodil-sensitive component) was the total component of NMDA receptor responses subtracted with GluN2A component. GluN2A/B ratio was then calculated by dividing GluN2A component with GluN2B component.

Western blot analysis

Another set of adult male Wistar rats was divided into control and CSD group (n = 15, each group). Protocols for CSD stimulation using 3 mg of solid KCl for 45 minutes, combined with solid NaCl in control groups, were repeated.

After 45 minutes of SD stimulation by solid KCl, the isolated hippocampi were then homogenised in a solution containing RIPA buffer (lysis buffer; 150 mM NaCl, 20 mM Tris-HCl, 2 mM EDTA, 1 % Triton X-100, 0.05 % SDS, 1 mM PMSF, pH 8; Cell Signaling Technology, USA) for 1 hour.

Table 1 Comparison between CSD and Hippocampal SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CSD (n = 4)</th>
<th>Hippocampal SD (n = 4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of SD (waves/45 min)</td>
<td>9.23 ± 1.74</td>
<td>3.67 ± 0.58</td>
<td>0.004*</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>34.62 ± 6.78</td>
<td>24.79 ± 3.51</td>
<td>0.04*</td>
</tr>
<tr>
<td>Wave interval (min)</td>
<td>5.10 ± 1.49</td>
<td>10.53 ± 2.27</td>
<td>0.03*</td>
</tr>
<tr>
<td>Duration (s)</td>
<td>69.67 ± 19.60</td>
<td>74.14 ± 28.93</td>
<td>0.83</td>
</tr>
<tr>
<td>AUC (mV•s)</td>
<td>712.35 ± 187.77</td>
<td>810.46 ± 217.11</td>
<td>0.56</td>
</tr>
</tbody>
</table>

AUC, area under the curve; CSD, cortical spreading depression; SD, spreading depression; *P < 0.05 (independent sample two-tailed t-test).
Beverly, MA, USA) and Protease Inhibitor Cocktail (Cell Signaling Technology, Beverly, MA, USA). Tissue homogenates were centrifuged (Sigma-Aldrich, St. Louis, MO, USA) at 12,000 rpm; 4 ºC for 15 minutes.

Fifteen micrograms of protein were loaded onto a 7.5 % SDS-PAGE and electroblotted onto a polyvinylidene difluoride (PVDF) membrane (GE Healthcare Life Sciences, Little Chalfont, UK). Membranes that were intended to determine the quantity of GluN2A were blocked with 5 % bovine serum albumin (BSA) prior to incubation with the primary antibody in 5 % BSA at 4ºC overnight and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000 dilution; anti-rabbit IgG antibody; Sigma-Aldrich, St. Louis, MO, USA) for 1 hour. For GluN2B detection, the membranes were blocked with 5 % TBST-MLK at room temperature for 1 hour and incubated with a primary antibody in 5 % TBST-MLK at 4 ºC overnight, and incubated with a HRP-conjugated secondary antibody (1:10,000 dilution; anti-mouse IgG antibody; Sigma-Aldrich, St. Louis, MO, USA) for 1 hour. The dilutions of the primary antibodies were 1:500 for both GluN2A (rabbit monoclonal antibody; Millipore, Billerica, MA, USA) and GluN2B (mouse monoclonal antibody; Millipore, Billerica, MA, USA) and 1:2,000 for β-actin (mouse monoclonal antibody; Sigma-Aldrich, St. Louis, MO, USA). Protein bands were sequentially detected using enhanced chemiluminescent (ECL) reagents (GE Healthcare Life Science, Little Chalfont, UK) exposed onto a hyperfilm (GE Healthcare Life Science, Little Chalfont, UK). The quantity of GluN2A and GluN2B were eventually measured using Image J software (NIH, Bethesda, MD, USA). These signals were normalised against β-actin bands.

Statistical analysis

All data were reported in the format of mean ± standard error of the mean (SEM). Statistical analysis was performed using IBM SPSS software version 20. Independent sample t-test was adopted in the analyses to establish a statistical correlation. Only probability values less than 0.05 (P < 0.05) were considered to be statistically significant.

Results

In vivo cortical and hippocampal DC recordings

Our data indicated that CSD propagated and reached the CA1 area of the hippocampus in an in vivo model with alteration of electrical properties. Several parameters characterising electrical properties of CSD and SD measured at the hippocampus (e.g. total number of SD waves, amplitude, duration, AUC, and wave interval) are displayed in Table 1. In the cortical DC recording, the results showed that multiple shifts of negative DC characterised as SD were detected in the frontal neocortex in all rats (n = 4) which indicated that solid KCl application consistently induced multiple waves of CSD (Fig. 1A). In hippocampal DC recording, we illustrated that a series of negative DC potentials characterised as SD was detected in the hippocampal CA1 in all rats (n = 4; Fig. 1B).

Ratio of GluN2A/B response

Analysis of our current study revealed that GluN2A/B ratio of the CSD group significantly increased in comparison with the control group. After application of ifenprodil, NMDA receptor-mediated fEPSPs were reduced in both control and CSD slices (Fig. 2A). The reduced magnitudes of NMDA receptor-mediated fEPSPs in CSD and control slices were 23.5 ± 2.1 and 34.4 ± 5.5 %, respectively (P = 0.092; independent samples two-tailed t-test). However, the extent of this reduction was not significantly different between the CSD and control groups. In control slices, ifenprodil decreased NMDA receptor-mediated fEPSPs on the hippocampal CA1 by a degree comparable to other studies [17]. The GluN2A/B ratios in CSD and control groups were 3.376 ± 0.361 and 1.968 ± 0.346, respectively (P = 0.018; n = 6 each group; independent

![Cortical and hippocampal DC recordings. (A) A representative tracing showing the DC shift in frontal cortex surface induced by KCl application (Scale bar: 5 min; 10 mV). (B) A representative tracing showing the DC shift in hippocampus induced by KCl application (Scale bar: 5 min; 10 mV). These tracings illustrated that SDs originated from the neocortex were shown to be able to spread to the hippocampus. The waves also appeared to be morphologically different between CSD and hippocampal SD. Recorded parameters were previously described in Table 1.](image-url)
samples two-tailed t-test; Fig. 2B). This result showed that CSD altered the responses of NMDA receptors in the hippocampal CA1 towards greater GluN2A/B response ratio. After treatment with ifenprodil, we also demonstrated that isolated fEPSPs were mediated by NMDA receptors, because the remaining fEPSPs were abolished by the NMDA receptor antagonist, APV (25 µM). Importantly, this GluN2A/B ratio represents the ‘response’ of GluN2A over GluN2B subunits on the neuronal plasma membrane.

Quantitative assay of GluN2A and GluN2B receptors

Our results obtained from Western blotting analysis showed that the total number of both ipsilateral GluN2A and GluN2B subunits of the NMDA receptor were significantly altered in CSD groups (n = 15; right KCl-placed hippocampus) compared to control groups (n = 15). We observed a significant increase in total number of GluN2A subunits and a reduction of those GluN2B subunits. The averaged intensity of GluN2A protein band relative to β-actin in ipsilateral CSD and control groups was 0.589 ± 0.027 and 0.713 ± 0.024, respectively (P = 0.002; independent samples two-tailed t-test). We also compared the total number of GluN2A and GluN2B subunits of the NMDA receptor between ipsilateral and contralateral sides of the hippocampus in CSD group. Total number of GluN2A subunits was significantly increased in the ipsilateral side of the hippocampus (P = 0.042; independent samples two-tailed t-test). The averaged values of protein intensity in both ipsilateral and contralateral sides of the hippocampus were 0.777 ± 0.040 and 0.650 ± 0.047, respectively. In contrast, a significant reduction in the total number of GluN2B subunits was demonstrated with an averaged band intensity in both ipsilateral and contralateral side of 0.589 ± 0.027 and 0.699 ± 0.031, respectively (P = 0.012; independent samples two-tailed t-test). In addition, we compared a number of GluN2A and GluN2B subunits in contralateral CSD-induced hippocampi with those control hippocampi. These results were undoubtedly insignificant for both GluN2A and GluN2B subunits (P = 0.826 for GluN2A component; P = 0.805 for GluN2B component; independent samples two-tailed t-test).
Discussion

Our study demonstrated that induction of CSD resulted in trains of DC shifting, compatible with hippocampal spreading depression. The results are consistent with previous ex vivo studies that CSD was induced by 2 M KCl microinjection [18, 19], or 3 mg solid KCl [9]. However, microinjection of 0.5 M KCl was shown not to produce DC shifting in the hippocampus [20]. Another study revealed that single CSD induced by 300 mM KCl topical application resulted in waves of SD in in vivo hippocampus only in FHM1 mutant mice, but not the wild-type mice [8]. These evidence support that propagation of SD from the neocortex into the hippocampus is increased in dose-dependent fashion.

According to the DC recordings, wave frequency and amplitude of hippocampal DC waves were diminished, while there were no significant changes in both duration and AUC. The duration of SD refers to how long ion channels remain open to enable prolongation of depolarisation. The AUC is the sum of the amplitude and duration. Wave frequency, which refers to the induction threshold, and amplitude were significantly diminished in hippocampal SD. Possible explanations may lie in anatomical difference [18, 21] and conduction sensitivity of the two structures.

Based on our electrophysiological studies, we observed an enhanced GluN2A/B ratio secondarily to CSD stimulation. Combining this information with our previous research [9], we pointed that LTP magnitude was significantly reduced in CSD group compared to the control group. These findings suggest that CSD may be able to alter hippocampal synaptic transmission by interfering GluN2A/B response ratio. Some evidence strongly suggest that ratio of GluN2A/B response governs bidirectional modification of LTP induction threshold in the CA1 of hippocampus [23, 24]. An increase in GluN2A/B ratio was shown to impair LTP, in which an increase of GluN2A/B ratio by one unit is associated with approximately 9 % reduction of LTP. [12, 25]

Findings from Western blotting analysis support our electrophysiological result of enhanced GluN2A/B ratio. We demonstrated that the total number of GluN2A subunits of the NMDA receptor was elevated whilst those of GluN2B were significantly diminished in the CSD group. Because CSD was elicited for only 45 minutes, we hypothesised that CSD causes post-translational modifications to GluN2A and GluN2B proteins rather than interfering with transcriptional processes. Although little is known regarding the precise mechanism by which GluN2A/B ratio alters the plasticity threshold, we propose that it involves the individual properties of both GluN2A and GluN2B subunits. Furthermore, we also showed that CSD may not travel to the contralateral hippocampus, because we observed no

![Fig. 3 Total number of GluN2A and GluN2B subunits of the NMDA receptor measured by Western blot analysis](image-url)
significant changes of ipsilateral (right) and contralateral (left) in either the total number of individual GluN2A or GluN2B subunits.

Evidence from molecular experiments suggests that GluN2B receptors have longer activation duration than GluN2A, which results in a greater Ca$^{2+}$ influx [26]; thus, overexpression of GluN2B led to the enhanced LTP in the hippocampus [27]. In addition, activation of GluN2B subunits of the NMDA receptor could generate LTP in GluN2A-knockout mice [23, 28] and hippocampal LTP was not observed in GluN2B-knockout mice [29].

Some limitations should be considered. First, since we used CSD as a model, the interpretation of our study may not only be constrained to migraine with aura, since various cerebral insults has been shown to produce CSD. Second, although the knowledge obtained from this study may explain hippocampus-associated symptoms during migraine aura, the behaviour or memory in animals were not evaluated. This, however, are being studied in our further research.

Taken together, our study revealed that CSD increased GluN2A/B ratio by modifying the numbers of GluN2A and GluN2B subtypes. Our previous studies [9] showed that repetitive CSD resulted in a reduction of LTP which, in turn, is correlated to impaired memory processes. Thus, it is suggested that increased GluN2A/B ratio is associated with reduced LTP. This physiological finding may be used to imply a temporal correlation between migraine with aura and hippocampus-associated symptoms. However, our study was conducted in animals, whether or not the possibility of our findings hold true in human remains unanswered.

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Conflict of interest The authors declare that they have no conflict of interest.

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Abstract

Functional role of the striatum in motor control has been widely studied. In addition, its involvement in reward function as a brain area in the dopamine system has also been mentioned. However, neural signaling in the striatum in response to consumption of emotional enhancing substances remained to be explored. This study aimed to investigate local field potential (LFP) of the striatum following morphine administration. Male Swiss albino mice implanted with electrode into the striatum were given an intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). LFP and locomotor activity of individual animals were simultaneously recorded in the recording chamber following the administration. The inspection of LFP tracings revealed the increase in fast wave induced by morphine particularly at a high dose. Statistical analyses were performed using a one way ANOVA followed by Tukey post hoc test. Frequency analysis using Fast Fourier transform also confirmed a significant elevation of low gamma (30-44.9 Hz) activity. When analyzed in time domain, significant increase in low gamma power was observed from the 15th to 65th min following 15 mg/kg morphine treatment. Moreover, morphine treatment also exhibited a stimulating effect on locomotor speed. However, regression analyses revealed no significant correlation between low gamma power and locomotor speed. In summary, this study demonstrated the increase in low gamma oscillation in the striatum and this effect was not associated with locomotor activity of animals. Thus, it is possible that low gamma oscillation induced by morphine treatment is related with the reward function.

Keywords

Striatum • Local field potential • Low gamma wave • Morphine

Introduction

Drugs abuse are naturally rewarding which is the reason why they are self-administered by laboratory animals or consumed by humans [1]. In 1954, the brain was demonstrated to have specialized centers for reward functions [2]. The confirmation was made when these brain regions were electrically stimulated and highly rewarding response was obviously produced. In term of mechanism, the midbrain dopaminergic system was particularly sensitive to electrical brain self-stimulation, the operant conditioning method used to determine rewarding effects [for review see 3].

Dopamine is a neurotransmitter important for the rewarding effects of drugs abuse. The dopamine circuits have been extensively studied for neuronal networks of reward and addiction. Most of them focused on the role of the mesolimbic and mesocortical dopamine pathways. These pathways consist of dopamine cells in the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc) and the frontal cortex respectively. Moreover, the other dopamine system has been studied in Parkinson’s disease topics that investigate the nigrostriatal dopamine system with dopamine cells in substantia nigra (SN) projecting into the dorsal striatum. These are two separate dopamine systems with different brain areas.

Currently, a new challenge has been focused on the hypothesis whether both systems participate in reward hypothesis and addiction [4]. Anatomically, there is no clear boundaries that completely separate these two midbrain dopamine systems [5, 4]. In addition, tracing study demonstrated that the SN and VTA dopamine cells have overlapping, not distinct, projection fields [6]. Ultimately, brain stimulation has been applied to study the mapping of reward-related circuitry in the brain by using direct electrical stimulation to certain brain regions. The population of midbrain dopamine neurons was seen as a final common pathway for the rewarding effect of the medial forebrain bundle (MFB) stimulation [4].
However reward sites are found both in the SN and the VTA [7]. Movable electrode mapping studies also indicated reward related brain sites within the areas of the dopaminergic cell body regions of the SN and the VTA [8]. Previously, the substantia nigra pars compacta (SNC) was demonstrated to produce dopamine to innervate the dorsal striatum, the brain area involved in motor and reward processes [9]. Taken together, the nigrostriatal dopamine system has been found to possess similar properties to that of the mesolimbic dopamine system in participation of reward function and addiction [4]. Until recently, no direct pattern of electrical brain wave in the striatum has been explored in rewarding events.

This study aimed to investigate local field potentials (LFPs) of the dorsal striatum, the brain area that receives dopaminergic inputs from the SN during morphine administration. Male mice were used for electrode implantation into the striatum. Following morphine treatment, LFPs were recorded. Fast Fourier transform is used for the analysis of frequency spectrum. Changes in some frequency ranges would reflect the activity of the nigrostriatal pathway during morphine administration.

**Materials and Methods**

Experiments were performed using 3 groups (n = 6-9) of adult male Swiss albino mice (approximately 35 g at the start of the experiment) from Southern Laboratory Animal Facility of Prince of Songkla University (PSU), (Songkhla, Thailand). Animals were housed in standard environmental conditions (24 ± 1 °C and 12 hr light/dark cycle). They had freely access to standard commercial food pellets and filtered tap water. The experimental protocols for care and use of the experimental animals in the present study were approved and guided by the Animals Ethical Committee of the PSU.

For surgical procedure animals underwent stereotaxic implantation of electrode for local field potential recording. Surgery was performed under ketamine/xylazine (150/15 mg/kg) by intramuscular (i.m.) injection. Therefore, animal’s head was fixed with stereotaxic frame through ear pieces as described previously (Fig. 1 a-e) [10]. Briefly, the scalp was shaved and swabbed with betadine. After lidocaine (20 mg/ml) was injected subcutaneously, a midline incision was made at on the scalp. The electrodes were stereotaxically implanted overlying the left striatum area (AP: +1.1 mm, ML: 1.5 mm, DV: 3.5 mm) using bregma as the landmark and the cerebellum (AP: -6.5 mm, DV: 2 mm) as a reference and ground electrode. Additional holes were drilled for stainless steel anchor screws. All the electrodes were linked to a female connector fixed to the skull by dental cement. After surgery, animals were placed in a clean cage with a heating pad and monitored until ambulatory behavior was observed. Antibiotic (100 mg/kg ampicillin) was applied intramuscularly for 3 days to prevent infection. They were allowed to fully recover for at least 7-10 days before the start of the experiment.

Experimental procedure and local field potential (LFP) recording before LFP recording in response to acute morphine administration, the animals were habituated with the recording condition in a chamber for 4 hrs per day for 3 consecutive days. Then, baseline recording for one hour was required before intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). Post-drug recording was performed for 3 hrs following the
injection. LFP signals were amplified with low-pass 200 Hz, high-pass 1 Hz and digitized at 2 kHz by a PowerLab 16/35 system (AD Instruments, Castle Hill, NSW, Australia) with 16-bit A/D. Data were stored in a PC through the LabChart 7 program software. 50 Hz notch filtering was applied to remove the noise from power line artifacts. All LFP signals were processed through 1–200 Hz band-pass digital filter (raw filtered signal). Locomotor activity of animals was recorded by using a video camera mounted on the top of the recording chamber. The recording method and analysis of locomotor speed were done as previously described [10].

For spectral power analysis, power spectral density (PSD) was generated by LabChart 7 software using Hanning window cosine with 50% window overlapping and 0.976 Hz frequency resolution. Then, the PSD in each frequency bin was expressed as the percentage of total power (1-100 Hz). The average spectral power were constructed in discrete frequency bands of each group and expressed in frequency domain. In this study, power spectrum in the striatum LFP was divided into slow wave (1-4 Hz), theta (4-8 Hz), alpha (9.7-12 Hz), beta1 (13.6-18 Hz), beta2 (19.5-29.3 Hz), low gamma (30-44.9 Hz) and high gamma (60.5–100 Hz).

All data were averaged and expressed as mean ± Standard Error of Mean (S.E.M.). Differences between the saline and morphine (5 mg/kg or 15 mg/kg) were analyzed by using one-way analysis of variance (ANOVA) followed by multiple comparisons using Tukey’s post hoc test to indicate specific points of significance. In addition, linear regression analyses between striatum LFP power and locomotor speed were also analyzed. Levels of significance were set at $P < 0.05$.

**Results**

Following the administration of saline or morphine (5 and 15 mg/kg), LFP signals from individual mice were continuously recorded for 3 hrs (Fig. 2a). Representative raw LFP tracings of saline, 5 mg/kg morphine and 15 mg/kg morphine were displayed in time-domain. (c) Power spectrums of striatum LFP are expressed in frequency domain. (d) Averaged percent total power of low gamma range are expressed as mean ± S.E.M. * $P < 0.05$ compared with the saline control group (one-way ANOVA followed by Tukey’s post hoc test).
analysis of raw LFPs during a period of 25-35 min was conducted for percent total power in a broad frequency range from 1 to 100 Hz (Fig. 2c). Obviously, morphine treatment (15 mg/kg) appeared to specifically increase power in a range of low gamma oscillation. Statistical analysis also confirmed that significant increase in low gamma (30-44.9 Hz) power was seen in the group of high dose of morphine (Fig. 2d). No significant difference was produced by 5 mg/kg morphine.

Therefore, effects of morphine treatment on low gamma oscillation in the striatum were particularly analyzed in time domain (Fig. 3). Data were converted to percent total power and analyzed every 5 mins. Differences in percent total power of low gamma frequency compared to control levels were determined by using one-way ANOVA followed by Tukey’s post hoc test. It was found that 15 mg/kg morphine began to produce significant increases from the 15th until 65th min. Peak effect was observed during the 30th min. No significant change in percent total power of low gamma was induced by 5 mg/kg morphine.

For the effects of morphine administration on locomotor activity, the results showed that morphine dose dependently increased averaged speed and travelled distance in comparison to saline control group (Fig. 4a and b). One-way ANOVA revealed that locomotor speed \( F(2, 24) = 11.522; P < 0.001 \) and travelled distance \( F(2, 24) = 6.868; P < 0.001 \) were significantly increased in 15 mg/kg morphine group. No significant change was observed in 5 mg/kg morphine group for both locomotor parameters.

In addition, regression analyses were performed to evaluate the correlation between striatum low gamma power and locomotor speed (Fig. 4c) or travelled distance (Fig. 4d) following morphine administration. The results showed no significant correlation between low gamma power and these two parameters. Locomotor speed and travelled distance of animals did not predict low gamma power for both doses of morphine.

Discussion

The present study demonstrated low gamma oscillation in the striatum induced by morphine administration in mice.

It has been well established that most addictive drugs produce their effects through activity of the dopamine neurotransmitter system as a common mechanism [11]. Their effects on the dopamine system were dominant as the administration of these drugs was found to increase midbrain dopamine neuron firing [12] and dopamine release preferentially in the NAc [13]. In contrast, drugs with aversive properties were demonstrated to reduce dopamine release in the NAc [13]. In terms of mechanism, the opiates have been proposed to activate dopamine cells via non-dopamine cells, through \( \mu \)-opiate receptors located on GABAergic midbrain interneurons that have inhibitory tone on dopamine cell firing [14]. Activation of these inhibitory \( G_{\alpha i} \)-coupled \( \mu \)-opiate control group using one-way ANOVA followed by Tukey’s post hoc test. *, **: \( P < 0.05 \) and \( P < 0.01 \), respectively.

![Graph showing the average percent total power of low gamma range were analyzed every 5 min period after injection of morphine (5 and 15 mg/kg) or saline. Data were compared with that of saline control group using one-way ANOVA followed by Tukey’s post hoc test. *, **: \( P < 0.05 \) and \( P < 0.01 \), respectively.](image-url)
receptors was found to withdraw the GABAergic tone from midbrain dopamine neurons which, in turn, resulted in increasing firing rate and the amount of dopamine released in the NAc [11]. Moreover, additional research findings also demonstrated that morphine increased cell firing levels in both 2 origin dopaminergic areas, the VTA and the SNc [12] and extracellular dopamine concentrations in 2 terminal dopaminergic areas, the NAc and the striatum [13].

The striatum is among main components of the basal ganglia complex. Its principal functions are primarily related to motor control. The nigrostriatal dopamine pathway (with dopamine cells locating in the SN projecting their axons to the striatum) is one of neural circuits that also has important roles in movement [15]. Dopamine is produced by cells in the pars compacta of SN. Nigrostriatal axon terminals release dopamine into the striatum to produce an excitatory effect upon cells in the striatum [15]. The deficits of dopamine pathway are associated with movement disorders such as Parkinson’s disease [16]. Basically, Parkinson patients have considerable difficulties in initiation and termination of movement. Later, the involvement of this pathway in reward processes has been studied [17]. Therefore, it has been discussed that the nigrostriatal dopamine pathway also plays a significant role in reward in addition to that of the mesolimbic and mesocortical dopamine pathways [for review see 4].

The present study clearly demonstrated that a significant increase in low gamma power was observed from the 15th to 65th min following 15 mg/kg morphine treatment. It has been well established for the rewarding properties of morphine [11, 18]. Most of classical studies of reward function have focused on the activity of the ventral striatum, also known as the NAc [for review see 3]. Previously, the study of local field potentials in the ventral striatum demonstrated reward-associated gamma oscillations [19]. On the other hand, gamma oscillations in the dorsal striatum were partially correlated with movement initiation [20]. However, the stimulation of the SN, the brain areas that projects neural pathway mainly to the dorsal striatum, also produced rewarding effect [8]. Previously, lesions of the dorsal striatum were found to reduce reward response to either cocaine or morphine [21]. In particular, brain imaging study using positron emission tomography (PET) in human cocaine addicts demonstrated an increase in dopamine release within the dorsal striatum in response to cocaine associated cues [22]. Taken together, these findings suggest some degree of involvement of the dorsal striatum in drug reward and addiction. Therefore, it is likely that the enhanced gamma oscillation seen in the present study might be associated with reward induced by morphine treatment. Until recently, no direct link

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**Fig. 4 Morphine induced averaged speed and travelled distance.** (a) Averaged speed and (b) travelled distance during morphine administration expressed as mean ± S.E.M. (c) Regression analyses between striatum low gamma oscillation and averaged speed and (d) travelled distance during morphine administration. Data were analyzed using one-way ANOVA followed by Tukey’s *post hoc* test. ***: *P < 0.001* compared with the saline control group.
between gamma oscillation and reward has been established. In general, the increase in gamma activity is involved in information processing [for review see 23]. For example, it is dominant in learning related brain areas during cognitive performance [24]. It means that information signaling is processed to mediate functional roles of the brain areas.

Following the administration of morphine, either reward or motor functions could be affected. Previously, reduced locomotor activity was seen as a result of either dopamine D1 [25] or D2 [26] receptor knockout (D1R-KO or D2R-KO respectively) in the nigrostriatal system. The present data also exhibited a stimulating effect of morphine on locomotor speed that would confirm its psychomotor properties. However, regression analyses confirmed that the induction of low gamma oscillation was not correlated with locomotor activity. The increase in locomotor speed or travelled distance did not predict low gamma power induced by morphine. Thus, it is possible that low gamma activity induced by morphine is associated with reward function.

In conclusion, this study showed the effects of morphine administration on LFP oscillation in the striatum and locomotor activity in mice. The increase in low gamma activity was not correlated with motor function. Therefore, it was proposed to reflect rewarding process of morphine. Altogether, these findings emphasized the involvement of the striatum in reward function and demonstrated a highlight of low gamma oscillation in response to morphine treatment.

Ethical approval All procedures performed in this study involving animals were in accordance with the ethical standards of the Animals Ethical Committee of the PSU.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Gamma wave oscillation and synchronized neural signaling between the lateral hypothalamus and the hippocampus in response to hunger

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Abstract The lateral hypothalamus plays an important role in homeostasis. It is sensitive to negative energy balance and believed to interact with other brain regions to mediate food seeking behavior. However, no neural signaling of hunger in the lateral hypothalamus has been studied. Male Swiss albino mice implanted with intracranial electrodes into the lateral hypothalamus and the hippocampus were randomly treated with drinking water for control condition, 18-20 h deprivation of food for hunger condition, and fluid food for satiety condition. Therefore, local field potential (LFP) and locomotor activity of animals were simultaneously recorded. One way ANOVA with Tukey’s post hoc test was used for statistical analysis. Frequency analysis of LFP revealed that food deprivation significantly increased the power of gamma oscillation (65-95 Hz) in the lateral hypothalamus and the hippocampus. However, satiety did not change the oscillation in these regions. Moreover, no significant difference among groups was observed for locomotor count and speed. The analysis of coherence values between neural signaling of these two brain areas also confirmed significant increase within a frequency range of 61-92 Hz for hunger. No change in coherence value was induced by satiety. In summary, this study demonstrated neural signaling of the lateral hypothalamus in response to hunger with differential power spectrum of LFP and the interplay with the hippocampus. The data may suggest critical roles of the lateral hypothalamus in detection of negative energy balance and coordination of other higher functions for food related learning or behaviors through the connectivity with the hippocampus.

Keywords Lateral hypothalamus · Hippocampus · Local field potential · Hunger · Energy balance

Introduction

A drive of eating behavior is one of crucial survival strategies. Physiologically, it is critical to maintain the energy balance through homeostatic processes. The detection of energy levels in the body by specific brain areas is highly sensitive to ensure the equivalence between energy intake and expenditure. For example, whenever negative energy balance is likely to progress, specific neuronal circuits are excited in a process to increase motivation that would trigger feeding behavior.

The lateral hypothalamus located on either side of the third ventricle has been considered as a key player in the regulation of food intake in mammals [1]. Bilateral destructions of this area in rats or cats led to complete inhibition of spontaneous eating [2]. This area is called the ‘feeding center.’ On the other hand, an increase in food intake has been induced by electrical stimulation of the lateral hypothalamus [3]. Similar responses were also induced by anticipation of food [4] or treatment with opioids agonist [5] or some glutamate agonist [6]. Moreover, blockade of excitatory amino acid receptors in the nucleus accumbens shell also exhibited feeding [7]. Therefore, this connection was hypothesized as the functional pathway between accumbens shell and the lateral hypothalamus. The study of neural bases of eating behavior has also been focused on the interactions between the lateral hypothalamus and other brain areas such as the nucleus accumbens and ventral pallidum in controlling feeding motivation [8]. This includes the connection with the hippocampus through the
hippocampal fibers reported to project to the lateral hypothalamus [9].

In reticulo-hypothalamic systems, a stimulation of the lateral hypothalamus was reported to affect the hippocampal theta oscillation [10]. Electrolytic lesion of the lateral hypothalamus activated aphagic mechanisms including the absence of normal atropine-resistant EEG activity in the hippocampus [11]. Changes in the hippocampal brain oscillation were also observed in response to stimulation or blockade of the lateral hypothalamus [12]. These findings appeared to confirm the connections between brain areas that some of them might function in association with feeding behaviors. However, the relationship between the lateral hypothalamus and the hippocampus in food intake mechanism remains unclear. In particular, the neural signaling and the interplay between these brain areas in association with feeding have not been studied.

This study aimed to investigate LFP oscillations in the lateral hypothalamus and the hippocampus of mice. LFP power spectrum in each brain areas and coherence between these two areas were evaluated during negative and positive energy balances in comparison to control energy levels.

**Materials and Methods**

**Animals and electrode implantation**

Fifteen male Swiss albino mice weighing 40-45 g were supplied by the Southern Animal Laboratory Facility of Prince of Songkla University, Songkhla, Thailand. Electrodes were implanted according to the procedures described by Cheha et al. [13]. Briefly, animals were anesthetized with a cocktail of ketamine and xylazine via intramuscular injection and positioned in a stereotaxic apparatus. Lidocaine was applied under the dorsal scalp as a local anesthesia to make midline incision. Therefore, intracranial electrodes were implanted on the left side of the brain to the lateral hypothalamus (anteroposterior (AP) to bregma -1.5 mm, mediolateral (ML) to bregma -1 mm, dorsoventral (DV) to bregma 5.2 mm), dorsal hippocampus CA1 (AP -2.5 mm, ML -1.5 mm, DV 1.5 mm) and cerebellum (AP -6.5 mm, Midline, DV 2 mm as a reference) according to mouse brain atlas [14]. Thereafter, electrodes were connected with the skull by using dental cement. The antibiotic ampicillin was given intramuscularly once a day for 3 days to prevent infection. Animals were allowed to recover for at least 2 weeks.

**LFP data acquisition and data analysis**

LFP signals and locomotor activity were recorded in a black cylinder chamber (33 cm diameter and 40 cm height). LFP signals were recorded by a PowerLab 16/35 system (AD Instruments, Australia) with 16-bit A/D, and stored in a PC computer through Lab Chart 7.3.7 Pro software. All LFP signals were processed through 1 - 200 Hz band-pass digital filter with a sampling frequency of 1 kHz. Recorded files were overviewed by using visual inspection and only noise-free signals were used for the analysis. 50 Hz notch filtering was applied to remove the power line noise. For data analysis, power spectral density (PSD) was generated by Lab Chart 7.3.7 Pro

![Fig. 1](image-url) Representative spectrograms of LFP signals from the lateral hypothalamus (a-c) and the hippocampus (d-f). Signals were recorded from animals in 3 conditions which included control (a and d), hunger (b and e) and satiety (c and f).
software using a Hanning window cosine (window size = 0.976 s, overlaps = 0.488 s). The PSD was calculated as the percent total power. The average spectral power was expressed in frequency domain (1 – 100 Hz) indicated for each condition. LFP coherence was analyzed using MATLAB R2012b (Version 8.0) expressed as the average of coherence value. Additionally, the software was used to analyze animal images detected via a video camera. The translocation of animal was caught with sensitivity at 2-mm threshold as previously described [13].

Experimental procedure

Baseline oscillation of the lateral hypothalamus and the hippocampus was obtained before the testing day. All mice were individually habituated in the test box for 30 min per day for 3 consecutive days. Prior to placing mice in the box, they were treated with an oral gavage (blank). On the testing day, control mice were put into the test box after feeding through gavage with 0.1 ml/10 g body weight of drinking water. For a group with hunger condition, animals with 18-20 h food deprivation were treated with blank gavage. For satiety condition, animals received an oral gavage with 0.1 ml/10 g body weight of

Fig. 2  Percent total power in 1-100 Hz range are expressed as mean ± S.E.M. One-way ANOVA followed by Tukey’s post hoc test were used to indicate significant effect for hunger at 65-95 Hz relative to control both in the lateral hypothalamus (a) and the hippocampus (b).

Fig. 3  Locomotor activity of animals in 3 different conditions. Data are analyzed and expressed in terms of the number of locomotion (a) and velocity (b) using One-way ANOVA statistical analysis.
liquid food. Therefore, animals were individually placed in the box for EEG and locomotor activity recordings. Individual mice were assigned randomly for these 3 conditions in 3 consecutive days for repeated measures.

Statistical analysis

All data were averaged and expressed as mean ± Standard Error of Mean (S.E.M.). Changes in spectral power, locomotor activity and coherence values among 3 conditions were analyzed by using one-way ANOVA followed by Tukey’s post hoc method. P value < 0.05 was accepted to be statistically significant.

Results

Prior to frequency analysis of LPFs, signals were visually inspected for their general appearances. Power spectrograms of the signals from the lateral hypothalamus and the hippocampus during hunger and satiety period were shown with respect to that of control periods (Fig. 1). Obviously, different patterns between LFP spectrograms of the lateral hypothalamus and the hippocampus were seen. By using a gray scale code reference of power, the lateral hypothalamus appeared to have relatively lower power than that of the hippocampus. It could be seen by visual inspection that the hippocampus had a dominant activity of theta oscillation. However, it seemed unable to distinguish the difference among signals of different energy states within each brain area by using the spectrograms. Therefore, frequency analysis was performed to quantitate percent total power of the LPFs during a 20-25 min period in frequency domain. The results showed that lateral hypothalamic LPFs were characterized by the prominent peak within delta frequency band (2-5 Hz) (Fig. 1a-c) whereas hippocampal LPFs were characterized by the prominent peak within theta band (8-12 Hz) (Fig. 1d-f). One-way ANOVA revealed significant differences between lateral hypothalamic LFP powers during hunger condition and control condition in a range of 65-95 Hz \[ F(2, 44) = 3.300, P < 0.05 \] also known as gamma band (Fig. 2a). No change in lateral hypothalamic LFP was induced by satiety condition. Moreover, power spectrums of hippocampal LPFs were analyzed. Significant increase was induced by hunger in the same frequency range to that found in the lateral hypothalamus (Fig. 2b). By the way, satiety condition had no effect on hippocampal LFP oscillation.

Locomotor activity of animals during a 20-25 min period was analyzed in terms of number of locomotion and velocity. Values of hunger and satiety conditions were compared to control levels. Neither hunger nor satiety was found to have significant effect on these 2 parameters (Fig. 3).

The interplay between the lateral hypothalamus and the hippocampus was examined. Coherence values of LFP from these brain areas were analyzed for 1-100 Hz during a 20-25 min period. In comparison to control
values, significant increase was induced by hunger in a 61-92 Hz [F (2, 44) = 5.747, P < 0.05] (Fig. 4). No significant change in coherence value was induced by satiety.

Discussion

Altogether, this study highlighted the application of signal analysis to understand neural processes of feeding. The confirmation of brain detection of negative energy balance in the lateral hypothalamus by LFP power spectrum was consistent with previous studies [3, 15]. The response found in the lateral hypothalamus was specific to hunger but not satiety. In general, the brain has been found to be involved in physiological mechanisms of energy homeostasis. Specific neural circuits in the lateral hypothalamus detect the alteration of energy deposits and expenditures to provide the optimized energy balance [4, 16].

However, an additional highlight proposed by the present study was the coherence between the lateral hypothalamus and the hippocampus arisen during a period of hunger. The coherence values hinted the active neuronal circuits for some homeostatic processes. In this study, the coherence analysis revealed the interplay between the lateral hypothalamus and the hippocampus following food deprivation for 18-20 h. The communication between these two brain areas might be hypothesized as a neural circuit for motivation of feeding behavior. The specificity of this coherence to hunger condition was confirmed when the coherence between these two areas was not influenced by satiety.

The present study identified the high spectral power density at delta band (2-5 Hz) in the lateral hypothalamus and theta band (8-12 Hz) in the hippocampus. These patterns are proposed to be area specific. Therefore, in hunger condition, the increases in gamma synchronization during a period of negative energy balance were seen. In general, gamma oscillation has been observed during cognitive performance tests [17, 18]. The enhancement of gamma activity was not due to hyperactive locomotor activity. In terms of mechanism, the activation of specialized GABAergic interneuron networks has been shown to produce gamma oscillations [19]. Moreover, gamma rhythm is also maintained by fast excitations through activation of glutamate receptors [20]. It was found that gamma oscillations are necessary for functional connectivity among various brain areas [21]. During hungry period, gamma oscillation was proposed to support the essential communication between the lateral hypothalamus and the hippocampus in response to hunger but not satiety. Based on various anatomical and physiological findings, pacemaker neurons that produce gamma wave oscillation in the hippocampal and neocortical networks might enhance synchronized network oscillations within the gamma band by entraining other cells with their firing patterns.

In conclusion, the data confirmed a sensitive role of the lateral hypothalamus to hunger with LFP power spectrum. The interaction of the lateral hypothalamus with the hippocampus also suggests neural mechanism that would trigger feeding related learning or behavior.

Ethical approval All procedures performed in this study involving animals were in accordance with the ethical standards of the Animals Ethical Committee of the PSU.

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Conflict of interest All authors declare that they have no conflict of interest.

References


Antinociceptive effects of \textit{Rhus coriaria} L. extract in male rats

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Abstract It is well known that the tendency toward the medicinal plants is increasing in recent years. They have low side-effects and high varieties of efficient components. This study was designed to investigate the analgesic effect of hydro alcoholic leaf extract of \textit{Rhus coriaria} (HRCLE) in a rat model. For this purpose, 42 adult male rats were divided into 7 groups: control, HRCLE (80, 100 and 300 mg/kg, i.p.), morphine (1 mg/kg, i.p.), aspirin (1 mg/kg, i.p.), and HRCLE 300 mg/kg plus naloxone (1 mg/kg, i.p.). The analgesic effects of HRCLE were assessed with writhing, tail flick and formalin tests. The data were compared with control by one-way ANOVA and Tukey post hoc test. All dose levels of HRCLE inhibited the number of contractions induced by acetic acid in the writhing test significantly. None of the dose levels of HRCLE have been showed antinociceptive activity in the formalin test except the dose of 100 mg/kg (at chronic phase) and the dose of 300 mg/kg (at chronic– acute phase). In the tail flick model, the highest effect was at the dose of 300 mg/kg of HRCLE ($P < 0.01$). Utilization of naloxone plus extract inhibited the antinociceptive effect of HRCLE. In this study, our findings suggest that analgesic effect for the HRCLE may be mediated via both peripheral and central mechanisms. The presence of flavonoids might be responsible for the antinociceptive activity of this plant.

Keywords Pain · Formaldehyde · \textit{Rhus coriaria} · Medicinal plants · Rat

Introduction

Pain is a somatic sensation such as touch, pressure and proprioception. Always pain has been a serious challenge in medicine as which has an important protective role in avoiding or treatment of actual or potential tissue damages. Although, nonsteroidal anti-inflammatory or opioid drugs are mostly used to control pain, but these drugs have a many adverse effects and cause gastrointestinal and renal disorders sometimes. Therefore, most people looks for new drugs that have fewer side effects and are cheaper and easily available [1].

There is also increasing evidence that in traditional medicine prescribing medicinal plants to treat a pain and inflammation is prevalently but the origin and structure of such plants have often remained unknown. Therefore, information about the pharmaceutical effects of these plants can be applied as a logical research approach in order to discover new drugs [2, 3].

It believes that plants belongs to Anacardiaceous family have a variety pharmaceutical effects as an analgesic, anti-inflammatory and antipyretic [4-6]. Sumac with scientific name \textit{Rhus coriaria}, is a perennial plant that contain over 250 individual species of flowering plants belong to Anacardiaceous family [7], its name is derived from the word of sumaga that means red [8]. This plant have a latex stems, simple or compound leaves, small flowers, fruits and dense cluster [9] which grows over the wide in Mediterranean, Iraq and Iran.

Sumac is used in traditional medicine as an antibacterial, anti-spasmodic, anti-virus and anti-inflammatory agent and a drug for treatment fever, diarrhea, gastrointestinal diseases and dermatitis [10, 11]. It also has anti-microbial and anti-oxidant effects and these properties have been proven in modern medicine [12-14]. The old claim of traditional medicine for anti-inflammatory properties of sumac due to the strong relationship between inflammatory and pain processes. Previous studies have clearly not showed the analgesic effects of this plant. On this foundation, this study was
designed to investigate antinociceptive effect of hydro alcholic leaf extract of *Rhus coriaria* using formalin, writhing and tail flick tests in rat model.

**Methods**

**Plant material collection**

Some of fresh *Rhus coriaria* leaves were prepared and authenticated by a botanist and then a voucher specimen number of the plant was deposited in the herbarium of the department of biology, faculty of basic sciences, Abu Ali Sina University of Hamadan, Iran. For preparation of hydro alcholic extract, *Rhus coriaria* leaves were shed dried at room temperature in the shade and were pulverized mechanically using a grinder. One hundred grams of powdered *Rhus coriaria* leaf was placed in one liter of 80 % methanol for 72 hours to extract the required active ingredients. The obtained mixture was placed in a rotary device to remove the solvent and then to dehydrate the substance, it was put in a dish and under a hood for one week. After that, the residual material at the bottom of the container, extract, was dissolved in the appropriate amount of saline (0.9 % saline) to treat rats with different doses.

**Animal experiments and drug administration**

Forty-two adult male Wistar rats (200 –250 g) were purchased from Pasteur’s institute of Iran. The animals were housed three to four per cage and kept at a controlled temperature of 23 ± 1 °C under a light/dark cycle of 12:12 h with food and tap water available ad libitum. All experiments were conducted between 10:00 and 16:00. All rats were treated humanely and were conducted in concordance with the IASP guidelines on the use of laboratory animals [15]. The animals were randomly divided into seven equal groups (*n* = 6 rats per group): control, HRCLE (80, 100, and 300 mg/kg, i.p.), morphine (1 mg/kg, i.p.), aspirin (1 mg/kg, i.p.) and 300 mg/kg of HRCLE plus naloxone (1 mg/kg i.p.). Sulfate morphine, naloxone and aspirin were purchased from Darou Pakhsh (Iran), and acetic acid and formalin from Merck Inc (Germany).

**Tests of pain**

**Writhing test:** On the experiment day, 30 minutes before running the experiments, the animals were sent into a standard experiment glass box to get used to the conditions. The HRCLE was solved in sterile physiologic serum and injected intraperitoneally in doses of 80, 100, and 300 mg /kg. After 15 minutes, acetic acid on the scale of 1 mg/kg of the body weight with the density of 6 % was injected and immediately after the intraperitoneal injection of the acetic acid, the number of abdominal contractions was counted for 30 minutes (both legs stretched). It is also necessary to mention that each animal was used only once [16]. In the control group, after intraperitoneal injection of saline, the writhing test was run. It is worth mentioning, based on CPCSEA report in 2004 which was stated that laboratory animals used for the experimentation should be properly used and pain and sufferers inflicted in animals should be avoided or minimized if avoidance is not possible so, we proceeded on the basis that experimental procedures that cause pain or sufferers in human beings will also cause similar pain or sufferings in animals be minimize [17].

**Tail Flick Test:** The tail flick test is a test of the pain response in animals, similar to the hot plate test. It is used in basic pain research and to measure the effectiveness of analgesics, by observing the reaction to heat. It was first described by D’Amour and Smith in 1941 [18]. Most commonly, a light beam is focused on the animal’s tail and a timer starts. When the animal flicks its tail, the timer stops and the recorded time (latency) is a measure of the pain threshold. For the tail flick test, we used a tail flick analgesimeter apparatus made in Borj Sanat Iran Company. Animals were separately put in a restrainer and 30 minutes after ingestion, the baseline reaction time was measured by focusing a light beam on the distal one-third portion of the animal’s tail. Each 15 minutes interval, the reaction time was recorded until 2 hours. However a 15 seconds cut off time was used for preventing tissue damage. Percent of maximum possible antinociceptive effect was calculated for each time.

**Formalin test:** In this experiment, suggested model of Dubuisson and Dennis, was used in order to evaluate the chronic pain. One hour before the test, the animals were sent into the special box of formalin test in order to get used to the experiment condition. The box was made of Plexiglas in the dimensions of 30 × 30 × 30 cm. Positioned in 45°, a mirror was inserted below the box and in front of the observer to observe the animal’s behaviors more clearly. Thirty minutes after the intraperitoneal injection of the drugs, there was a subcutaneous injection of 50 μl of 2.5 % formalin solution into the sub plantar surface of the left hind paw; then the animal was sent to the test special box again. The animal’s behavior was observed and labled for 60 minutes as following: once every 15 seconds, the motor response to pain was rated and recorded on a scale of 0, 1, 2, and 3. The numbers indicate the following reactions: number 0, the animal moves with complete balance and its weight distributed equally on both feet; number 1, the animal could not tolerate its body weight on the being-injected foot or take care of that foot; number 2, the animal raised the painful claw and has no contact with...
the box floor; and number 3, the animal licks the painful claw, chewed or moved severely. The average of first 5-minute grades was considered as phase 1 (acute phase) and the average of minutes 15 to 60 was considered as the phase 2 (chronic phase) [19].

Lethal dose (LD50)

The acute toxicity was determined by the previous laboratory model [20]. Various doses of the extract were injected separately and intraperitoneally to the male rats. The number of deaths of the animals was counted within the next 72 hours and the LD50 of the plant extract was determined.

Data analysis

All data were expressed as mean ± S.E.M. Foe analysis of data, one-way ANOVA followed by Tukey’s post hoc test was used and a calculated $P < 0.05$ was considered statistically significant.

Results

Writhing test: Statistical analysis of this study revealed that injection doses of 80, 100 and 300 mg/kg of the beneficial effect of HRCLE caused a significant reduction in comparison with the control group ($P < 0.05$ and $P < 0.01$, respectively). As it was shown in Fig. 1, there was a very significant reduction in morphine and aspirin groups in comparison with control group ($P < 0.001$ and $P < 0.01$, respectively).

Tail flick test: According to Fig. 2, group of the HRCLE 300 mg/kg showed a very significant increase of tail flick latency when compared to the control group ($P < 0.01$). Injection of morphine and aspirin were increased tail flick latency as well as ($P < 0.001$ and $P < 0.01$, respectively).

Regarding formalin test, injection of 300 mg/kg of HRCLE was strongly decreased pain score in both acute and chronic phase in comparison with control group ($P < 0.01$), while injection dose of 100 mg/kg of HRCLE led to the significant decrease of the pain score in chronic phase ($P < 0.05$). Although, injection dose of 80 mg/kg of HRCLE was shown no significant change in pain score. Groups of aspirin and morphine were also significantly decreased pain score when compared to the control.
control group ($P < 0.001$; Fig. 3). At the all of tests of pain, the treatment of naloxane plus HRCLE (300 mg/kg) inhibited the antinociceptive effect of HRCLE. There was also no significant change in weight of animals in each group. Lethal dose of the plant intraperitoneally was 5100 mg/kg.

Discussion

The proper management of pain has been identified as a primary indicator of quality assurance. Pain and substance abuse co-occur frequently, and each can make the other more difficult to treat [21]. Non-steroidal anti-inflammatory drugs (NSAID) such as aspirin is widely used in the treatment of pain, but often of these cause gastrointestinal injury [22]. The present research indicates antinociceptive effect of hydro alcoholic extract of Rhus coriaria leaf (HRCLE) on an animal model. Standard tests of writhing, tail flick and formalin were used in order to investigate the antinociceptive effects of HRCLE.

One of the most important tests is writhing test which usually used to screen possible antinociceptive mixtures. In this test acetic acid is a chemical stimulation which is extensively used to evaluate peripheral antinociceptive activity [23]. The HRCLE prevented abdominal constriction caused by acetic acid therefore, it is imagined that its alleviative effects are supported by the environmental mechanisms. Intraperitoneal injection of acetic acid can cause the acute inflammation of the peritoneum [24]. In this model, it seems that peripheral antinociceptive effects of HRCLE are indirectly due to internal mediators such as bradykinin, serotonin, histamine, substance P, and prostaglandin. It is justified that all of these mediators are associated with the stimulation of peripheral nociceptive neurons [23].

The tail flick assay or tail flick test uses a high-intensity beam of light aimed at a rodent’s tail to detect nociception [18]. In this study, injection of the extract high dose decreased pain. Experimental tests of the tail flick testing method showed that the temperature of the skin of the tail plays a major role in the critical temperature, i.e., the temperature at which the tail flicks in response to pain. Since tail flick test performed to evaluate the spinal reflexes and the central analgesic pathways [25] therefore, it seems that the antinociceptive effect of the extract involves a central nervous component which may be elicited from several defined areas in the CNS.

Among the several models of persistent nociception, formalin test has well been established as a valid model for screening of anti-inflammatory and antinociceptive agents that act through central pain route from peripheral pain [26]. Intraplantar injection of formalin evokes signs of nociception such as flinching and licking of the injected paw early (phase 1), and subsequently a quiescent period was characterized by fewer pain behaviors and late-hyperalgesic components (phase 2) that last for approximately 1 hour. The early phase or neurogenic nociception results direct activation of peripheral nociceptors whereas the late phase due to inflammatory nociception that reflect induction of a spinal state of facilitation, central sensitization, development of inflammation and enlargement of receptive fields and also the concurrent presence of low level input from both large and small afferents [27]. The results showed that HRCLE have an inhibitory effect over the pain. The HRCLE under investigation in this study showed antinociceptive activity in both phases of formaldehde-induced pain in rat. It was found that its decreasing effect is more potent in the chronic phase than the acute phase. Inhibition of the chronic phase of the formalin test by HRCLE can be a result of inflammation, so that part of the antinociceptive effect seems to be mediated by releasing compounds like prostaglandins F2α and E2 in some amounts sensitized by central nociceptive neurons [28].
To evaluate opioid system interference with antinociceptive effect of this extract, we were used one of the antagonist drugs in opioid system i.e., naloxane, which prevents the activation of opioid receptors [29]. The results indicate that naloxone attenuates the antinociceptive effect of HRCLE. Therefore, it seems that the effect of HRCLE in pain relief is due to the opioid receptors.

However, the analgesic activity of HRCLE is mediated by opioid receptors and may produce the dependence effect similar to opioids therefore, the further studies and suitable methods need to be established for the identification the dependence effects.

Biologic or therapeutic activity of herbs has a close relationship with their chemical combinations [30]. It has been known that Rhus coriaria contains several compounds such as organic acids, phenolic acids and derivatives, hydrolysable derivatives, anthocyanins and derivatives, flavonoids and isoflavonoid derivatives, terpenoid derivatives and other compounds [10, 31, 32] which possess antioxidant, anti-inflammation and antinociception properties [33]. The active ingredients or major bioactive components of Rhus coriaria which produce morphine-like antinociceptive effect are the flavonoids and isoflavonoid derivatives. A total of 61 flavonoid derivatives were detected and characterised in sumac. Apigenin-7-O-(6′-O-galloyl)-β-D-glucopyranoside which corresponds to apigenin in structure. This compound was reported as an active compound in Euphoria. In the same manner, dihydroramarixetin galloyl-hexoside was tentatively proposed as this effect [31, 32]. As far as we know, there is no alkaloids compound with structures similar to morphine in this plant [10, 30, 34].

Previous studies have been shown that inhibition of N-methyl-D-aspartate receptor cut intracellular calcium down. Consequently, the synthesizer enzyme of calcium-related nitric oxide and phospholipase A 2 decreases too and with the reduction of nitric oxide and prostaglandins, especially the prostaglandin E2 and F2α reveals its antinociceptive effects [35]. Many flavonoids and tannins are able to chelate free radicals such as hydroxyl radicals and subsequently dismutate ROS. In this regard, there are reports that tannins have roles in creating antinociceptive and anti-inflammatory effects [36]. Therefore, another part of antinociceptive effect of this extract is due to tannins inside the plant.

Conclusions

The results of the present study suggest that the antinociceptive effect of this extract may be due to their content of flavonoids. In this study reduction in writhing, increase in tail flick and inhibition of both phases of the formalin test approved antinociceptive effect of Rhus coriaria.

Finally, we conclude that extract possess analgesic effect that is probably due to inhibition of prostaglandin synthesis and inhibition of the central and peripheral nervous system. So this extract could potentially be used to control the painful disease.

Acknowledgements We thank Masoud Mohammadi for his assistant in this work and all who helped us in conducting this research are highly appreciated. This paper was extracted from master thesis of Saeed Mohammadi that submitted at Islamic Azad University of Hamadan in Iran and financially supported by grant no. 91223 from this university.

Conflict of interest The authors declare that they have no conflict of interest.

References

8. Wetherilt H, M Pala (1994) Herbs and spices indigenous to Turkey, Amsterdam: Elsevier Science BV.
Abstract  Migraines typically occur more frequently in women than men because of the effects of estrogen on both the frequency and severity of migraine attacks. Many women suffer from migraine attacks during menstruation, which are known as menstrual migraines. The pathophysiology of menstrual migraines can be explored by using the rat estrous cycle, which shows a cyclical fluctuation of estrogen level that resembles the menstrual cycle. The aim of this study was to investigate whether different stages of the estrous cycle are involved in migraine development by comparing the excitability of trigeminal ganglion (TG) neurons in four different stages of the estrous cycle by using action potential (AP) parameter assessments. The stages of the estrous cycle were identified by a vaginal smear and measuring the estrogen levels in collected blood. The proestrus and estrus stages had higher estrogen levels compared with the diestrus and metestrus stages. Whole-cell patch clamp recordings demonstrated that TG neurons in the proestrus and estrus stage had lower AP threshold, lower rheobase, higher AP height, shorter AP falling time and deeper after-hyperpolarization (AHP) depth. Hence, our results revealed that the high level of estrogen in the proestrus and estrus stage alters the AP properties of TG neurons. Estrogen may increase membrane excitability and the summation of cellular responses, which alters the AP properties. The alterations of the AP properties in the proestrus and estrus stage may relate to a modification of voltage-gated ion channels in TG neurons, which is a pathogenesis for menstrual migraine. No COI.

Keywords  Menstrual migraine • Estrous cycle • Trigeminal ganglion (TG) neurons • Whole-cell patch clamp recording • Voltage-sensitive ion channels

Introduction  Migraine occurs more often in women than in men. More than 50% of women experience menstrual cycle-related migraine [1, 2]. The menstrual cycle enhances various parameters of a migraine, such as the severity, duration and frequency of painful migraine attacks. Previous research suggests that fluctuation of estrogen level during the menstrual cycle can affect menstrual migraine [3]. The incidence of migraine attacks peaks on the days before and after the onset of menstruation [4]. Estrogen levels fluctuate during each stage of the menstrual cycle, which is divided into follicular and luteal phases. In female rats, the menstrual cycle is called the estrous cycle and occurs in four stages, diestrus, proestrus, estrus and metestrus. Estrogen levels peak during proestrus and estrus. The duration of estrous cycle is very short compared with human menstrual cycle (4-5 days and 28 days, respectively). Hormonal fluctuation in estrous cycle of rat is quite similar to human menstrual cycle [5]. In both cycles, there were marked increases of estrogen level at the latter half of preovulatory phase followed by rapid decreases before the initiation of ovulation. Estrogen level gradually fluctuates again during postovulatory phase, and then returns to initial level at the end of ovarian cycle. Thus, the rat estrous cycle is used to model the effects of estrogen during the menstrual cycle in women. Cyclical fluctuation of estrogen level occurs during estrous cycle progression. Estrogen level steadily rises during the diestrus stage and peak during the proestrus stage. Subsequently, estrogen level rapidly drops and then slowly rises to reach a plateau at the estrus stage. From the plateau during the estrus stage, the estrogen level steeply decreases during the metestrus stage and...
then increases during the progression towards the diestrus stage. Estrous cycle activity has been implicated as a cause of migraine development because high levels of estrogen during the proestrus and estrus stage can enhance the excitability of neurons in the trigeminal nucleus caudalis (TNC), which results in migraine attacks [6]. Furthermore, estrogen exposure also increases the sensitization of the temporomandibular branch, which is innervated by TG neurons [7]. These results demonstrated that estrogen can affect the trigeminal system.

In the trigeminal nociceptive system, estrogen activates the estrogen receptor (ER) on TG neurons via either genomic or non-genomic pathways. Estrogen modulates neuronal activity through the expression of ion channels or by increasing intracellular cascades, such as extracellular-signal-regulated kinase (ERK) signaling, which phosphorylates various types of ion channels [8, 9]. In addition, previous research has demonstrated that voltage-gated Na channels [10-12] and voltage-gated K channels [13] in dorsal root ganglion (DRG) cells are activated by exogenous estrogen. In other words, activation of voltage-gated ion channels affects AP development in TG neurons, which is a major nociceptive signal from the periphery to higher cortical neurons. Thus, fluctuation of estrogen level during the estrous cycle may alter AP development in TG neurons which correlate to the change of nociceptive inputs from trigeminal system.

Our study aimed to investigate whether the estrous cycle is involved in neuronal excitability of trigeminal system by comparing AP properties of TG neurons in four different stages of estrous cycle. The AP properties reflect the activation of voltage-gated ion channels that affect the excitability of TG neurons. Our results suggested that modulation of the trigeminal system underlies the pathophysiology of menstrual migraine.

Methods

Animals

Female Sprague–Dawley rats, 6-8 weeks old, had a sufficient estrogen level for observing the estrous cycle [14, 15]. Animals used in all experiments were purchased from the National Laboratory Animal Center, Mahidol University, Nakorn-Pathom, Thailand. Rats were housed in stainless cages in a ventilated room under a 12-hour dark-light cycle and were fed ad libitum. All of the protocols were approved by the Animal Care and Use Committee of the Faculty of Medicine, Chulalongkorn University, Thailand (No. 4/58).

Immunooassay of Estrogen levels

Immediately after decapitation, the arterial blood was collected from the left cardiac ventricle for storage in a 1.5-ml microcentrifuge tube. The collection tubes were centrifuged at 3,200 rpm for 10 min. Then, serum was collected and stored at -20 °C. The serum concentration of estradiol (E2) was measured using the Chemiluminescent Microparticle Immunoassay (CMIA) method.

Estimation of estrous cycle stages

Using a dropper, the vagina was flushed by normal saline. Subsequently, one drop of vaginal fluid was placed on a slide and stained with 1% methylene blue. In this manner, vaginal smears were observed under a light microscope (with a 40X objective lens). Four different stages of the estrous cycle were determined by observing

<table>
<thead>
<tr>
<th>Variables</th>
<th>Diestrus (n = 52)</th>
<th>Proestrus (n = 24)</th>
<th>Estrus (n = 35)</th>
<th>Metestrus (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>-46.57 ± 1.13</td>
<td>-47.88 ± 2.38</td>
<td>-49.39 ± 2.46</td>
<td>-41.98 ± 1.86</td>
</tr>
<tr>
<td>Threshold (mV)</td>
<td>-19.14 ± 1.76</td>
<td>-27.46 ± 0.52*</td>
<td>-27.00 ± 2.20*</td>
<td>-29.95 ± 3.14</td>
</tr>
<tr>
<td>Rheobase (pA)</td>
<td>73.33 ± 3.54</td>
<td>55.63 ± 0.27*</td>
<td>49.58 ± 2.70*</td>
<td>66.36 ± 8.00</td>
</tr>
<tr>
<td>AP height (mV)</td>
<td>109.21 ± 3.35</td>
<td>116.66 ± 1.16*</td>
<td>98.24 ± 4.97*</td>
<td>92.53 ± 6.40</td>
</tr>
<tr>
<td>AV overshoot (mV)</td>
<td>52.41 ± 2.76</td>
<td>67.41 ± 1.02*</td>
<td>51.63 ± 3.84</td>
<td>39.10 ± 6.56</td>
</tr>
<tr>
<td>AP rising time (ms)</td>
<td>1.52 ± 0.63</td>
<td>1.26 ± 0.39</td>
<td>1.24 ± 0.62</td>
<td>1.95 ± 0.93</td>
</tr>
<tr>
<td>AP falling time (ms)</td>
<td>5.21 ± 0.40</td>
<td>3.22 ± 0.56*</td>
<td>2.43 ± 0.31*</td>
<td>4.41 ± 0.66</td>
</tr>
<tr>
<td>AP duration (ms)</td>
<td>6.46 ± 0.46</td>
<td>4.48 ± 0.20*</td>
<td>3.67 ± 0.31*</td>
<td>5.99 ± 0.82</td>
</tr>
<tr>
<td>AHP depth (mV)</td>
<td>-5.10 ±0.49</td>
<td>-12.15 ± 2.36*</td>
<td>-13.56 ± 1.00*</td>
<td>-6.85 ± 1.78</td>
</tr>
<tr>
<td>AHP duration (ms)</td>
<td>7.46 ± 1.10</td>
<td>7.92 ± 1.12</td>
<td>5.20 ± 0.82</td>
<td>7.62 ± 2.23</td>
</tr>
</tbody>
</table>
the population of three types of cells in the vaginal smear (i.e., nucleated epithelial cells, cornified epithelial cell and leukocyte, Fig. 2B, 2C and 2D, respectively) [16]. Total 138 female rats were used in the experiment. There were 52 rats in diestrus stage, 24 rats in proestrus stage, 35 rats in estrus stage and 27 rats in metestrus stage (Table 1).

Primary culture of TG neurons

Primary dissociated TG neurons were cultured as described previously [17-19]. Briefly, rats were euthanized with an overdose intra-peritoneal injection of sodium pentobarbital (1 ml/1 rat) before decapitation. Both trigeminal ganglia were removed and placed in a 35-mm culture dish of ice-cold Hank’s Balance Salt Solution (HBSS) with penicillin/streptomycin (10-20 µl; 10,000 U penicillin and 10 mg streptomycin/mL), washed twice in HBSS, and sectioned into small pieces with a sterile razor blade in 1 ml of HBSS. Collagenase (100 µl; 2 mg/mL) and dispase (200 µl; 50 U/mL) were filtrated using a 0.22-µm filter and then added to the sample. Immediately following filtration, the sample was incubated at 37 °C for 20 min. Papain was filtered and added, and then the sample was incubated at 37° C for 20 min again. Afterwards, the sample was centrifuged at 1,500 rpm for 2 min, and the supernatant was removed. The precipitate was triturated 3 times in L-15 complete medium using a glass pipette. Next, the sample was centrifuged at 1,500 rpm for 8 min. The precipitate was collected and washed twice with F-12 complete medium. Finally, 400 µl of F-12 complete medium was added to the sample and placed into a 35-mm Laminin/PDL dish for incubation in an incubator (37 °C, 5% CO2) for 3 hours. The cell diameter was evaluated as the average of the longest and shortest axis in a BX51WI upright microscope (Olympus, Tokyo, Japan). Only cells with diameter < 38 µm were analyzed.

Electrophysiological recording

Whole-cell patch clamp recording was used to record the electrophysiological and AP properties of dissociated TG neurons that were maintained in primary culture for 3 hours. Plastic chambers containing trigeminal neurons were placed on the microscope sample stand (Olympus BX51WI microscope, Olympus, USA), after which the cells were superfused with extracellular solution flowing into the plastic chamber at a flow rate of 1 ml/min at room temperature. The extracellular solution was composed of 145 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 10 mM D-Glucose and 10 mM HEPES; the pH value was adjusted to 7.40 with 1 M NaOH, and the osmolality was adjusted to 320 ± 5 with glucose. Glass microelectrodes with an outer diameter of 1.5 mm and an inner diameter of 0.86 mm (Sutter Instruments, Navato, CA, USA) were pulled on a microelectrode puller (Sutter Instrument) and heat polished using a microforge (Narashige, Tokyo, Japan) to a diameter of 1-2 µm.

Next, microelectrodes were filled by an intracellular solution (composed of 140 mM K-gluconate, 1 mM CaCl2, 2 mM MgCl2, 10 mM EGTA, 10 mM HEPES and 10 mM ATP; osmolality adjusted to 280 ± 5 with glucose). Then, microelectrodes were inserted to the headstage of an Axopatch amplifier (Axon, Sunnyvale, CA, USA). In current-clamp recording, to evaluate AP properties in response to the different stages of estrous cycle, the membrane potential was manually held at -60 mV and injected with a current of 10 pA/step with 100 ms duration. The criteria for successful recording were a minimum 10 min recording time, with a stable RMP of more negative than -40 mV; an amplitude of the action potential that was greater than 70 mV; and an input resistance that was higher than 100 mega-ohm. The protocol was adapted from previous reports [21, 22]. The cell diameter was evaluated as the average of the longest and shortest axis in a BX51WI upright microscope (Olympus, Tokyo, Japan). Only cells with diameter < 38 µm were analyzed.

Assessment of the AP properties

The current clamp injected with brief (100 ms) current pulses from a holding potential at -60 mV is shown in Fig. 1A. The threshold (mV) was the lowest membrane potential that yielded the first depolarization phase of an AP. Rheobase (pA) was the minimal current injection that was able to cause the depolarization phase of an AP. The AP height (mV) was measured as the elevation of an AP measured from holding potential to peak amplitude of the AP. The AP overshoot (mV) was measured as the elevation of an AP measured from 0 mV to peak amplitude of the AP. The rising time (ms) was measured as the elevation of an AP measured from the holding potential to peak amplitude of the AP. The AP overshoot (mV) was measured as the elevation of an AP measured from 0 mV to peak amplitude of the AP. The rising time (ms) was

Fig. 1 Assessment of the AP properties. (A) Currents in each step are incremented by 10 pA (total 7 currents and 2 spikes). (B) 1 = threshold, 2 = AP rising time, 3 = AP falling time, 4 = AP duration, 5 = AP overshoot, 6 = AP height and 7 = AHP depth. (C) 7 = AHP depth and 8 = AHP duration
measured as the duration of the rapid depolarization phase, which was measured from the threshold to peak amplitude of an AP. The AP falling time (ms) was measured as the duration of the less positive phase, which was measured from a peak amplitude of an AP to the holding potential (Fig. 1B). The after-hyperpolarization depth (AHP depth mV) is the de-escalation of an AP measured from the holding potential to the negative peak of an AHP. The AHP duration (ms) is the duration time from the negative peak of an AHP to 50% of the recovery of the holding potential (Fig. 1C).

Data analysis

All data are presented as mean ± standard errors of the mean (SEM). Statistical analysis was performed using IBM SPSS Statistics data editor. A one-way ANOVA was used to detect the change among groups that are varied due to the stages of estrous cycle. An independent Bonferroni post hoc test was used to determine intergroup differences. A $P < 0.05$ was accepted as statistically significant.

Results

After decapitation, blood serum was immediately collected to analyze the estrogen level. A comparison of the estrogen levels at each stage of the estrous cycle (Fig. 2A) demonstrated that the estrogen level at the proestrus stage (61.00 ± 1.41 pg/ml, n = 5) was the highest among the estrous cycle stages (comparing with diestrus, 31.00 ± 2.18 pg/ml, n = 5; post hoc test, $P < 0.05$). Furthermore, the estrogen level at the estrus stage was significantly higher than the level at the diestrus stage (46.33 ± 2.89 pg/ml, n = 5; post hoc test, $P < 0.05$), whereas the estrogen level at the metestrus stage was significantly lower than the level at the diestrus stage (21.00 ± 2.65 pg/ml, n = 5; post hoc test, $P < 0.05$). The results showed that the overall estrogen level among four different stages of estrous cycle was significantly changed (one-way ANOVA, $P < 0.05$).

The vaginal smear demonstrated that the cytological properties of the vaginal cells changed according to the estrogen level at different stages of the estrous cycle. At the diestrus stage, there were more leukocytes than nucleated epithelial cells (Fig. 2E). In the proestrus stage, there were only clusters of round nucleated epithelial cells, which included a granular cytoplasm and a nucleus (Fig. 2F). In the estrus stage, there were only clusters of cornified epithelial cells (Fig. 2G). In the metestrus stage, there were more leukocytes than cornified epithelial cells (Fig. 2H). However, there were no differences in the morphology of TG neurons at four different stages of
estrous cycle stage (Figs. 2I, 2J, 2K and 2L).

At each stage of the estrous cycle, the dissociated TG neurons in the primary culture were recorded using a whole-cell patch clamp configuration, of which depolarizing current steps were used to stimulate TG neurons to analyze the AP characteristics (Fig. 3A, 3B, 3C and 3D). The TG neurons at each stage of the estrous cycle had similar RMP values (Table 1, RMP, one-way ANOVA, \( p = 0.207 \)). The threshold at the proestrus and estrus stages was significantly lower than thresholds at the diestrus stage (Table 1, Threshold, post hoc test, \( P < 0.05 \), \( P < 0.05 \), respectively). However, threshold in metestrus stage was invariant to diestrus stage (Table 1, Threshold, \( p = 0.422 \)). The rheobase at the proestrus and estrus stages was also lower than the rheobase at the diestrus stage (Table 1, Rheobase, post hoc test, \( p < 0.05 \), \( p < 0.05 \), respectively). The AP height and overshoot at the proestrus stage were significantly higher compared to the diestrus stage (Table 1, AP height and AP overshoot, post hoc test, \( P < 0.05 \), \( P < 0.05 \), respectively). The rising time of the AP was not significant at any stage (Table 1, AP rising time, one-way ANOVA, \( P = 0.124 \)), and the falling time of the AP at the proestrus and estrus stages were significantly shorter compared to the diestrus stage (Table 1, AP falling time, post hoc test, \( P < 0.05 \), \( P < 0.05 \), respectively). Moreover, the duration of the AP at the proestrus and estrus stage were also significantly shorter compared to the diestrus stage (Table 1, AP duration, post hoc test, \( P < 0.05 \), \( P < 0.05 \), respectively). The depth of the AHP at the proestrus and estrus stages was significantly deeper compared to the diestrus stage (Table 1, AHP depth, post hoc test, \( P < 0.05 \), \( P < 0.05 \), respectively), whereas the duration of the AHP was not changed at any stage (Table 1, AHP duration, one-way ANOVA, \( P = 0.131 \)).

**Discussion**

The present study investigated the effects of four different stages of estrous cycle on the alteration of the AP properties linked to the trigeminal nociceptive system. Each stage of the estrous cycle was associated with differences in the morphology of the vaginal epithelium, which was influenced by the level of estrogen. Our findings are consistent with Goldman, et al. [17]; however, the morphology of the TG neurons did not change, while the properties of the AP were changed.

Estrogen, which binds to ER-alpha and ER-beta receptors, acts via both genomic and non-genomic mechanisms to modulate the pain response, neurotransmitter systems, and other modulatory systems [23, 24]. For AP development in all neurons, voltage-gated Na channels have a key role in response to rapid depolarization, eliciting an AP in TG neurons, which leads to pain perception. Voltage-gated Na channels Nav1.1 to 1.9 are expressed in TG neurons, including Nav1.7 (a tetrodotoxin-sensitive Na channel; TTX-S), Nav1.8 (a tetrodotoxin-resistant Na channel; TTX-R), and Nav1.9 (TTX-R), which can be stimulated to induce an AP [25, 26]. Our results demonstrated that AP threshold, rheobase and AP height were altered in the condition of high estrogen level at proestrus and estrus stages. These findings are consistent with a previous study demonstrating that high estrogen level increases the specific expression of Nav1.8 and Nav1.9, as well as the excitability of trigeminal ganglion neurons by reducing the AP threshold and rheobase and that estrogen also increases the height of the AP [19]. Nav1.7 and Nav1.9 activates at subthreshold resulting to shift of membrane potential closing to AP threshold. After membrane potential reaches AP threshold, opening of Nav1.8 produces AP upstroke in rapid depolarization phase [26, 27]. In addition, estrogen has been shown to increase the expression of ERK [15], which phosphorylates voltage-gated Na channels in TG neurons [28]. Thus, high estrogen level during the proestrus and estrus stages may increase the expression of Nav1.8, Nav1.9, and ERK, which enhances the excitability of TG neurons resulting in lower threshold, lower rheobase and higher AP height of AP development.

Additionally, voltage-gated K channels play a key role during the falling phase, undershoot phase and repolarization phases. Our findings demonstrated that AP falling time were reduced while AHP depth were increased in the condition that estrogen is elevated at proestrus and estrus stages. Previous research has demonstrated that estrogen alters the duration of an AP through the calcium-activated K channel (BK \( \text{Ca} \)) and changes the depth of AHP through the calcium-activated K channel (SK \( \text{Ca} \)) in the hippocampal pyramidal neurons [29]. Moreover, estrogen also activates L-type Ca channels to allow Ca\(^{2+}\) influx, which increases
intracellular Ca²⁺ and activates voltage-gated K channels [26, 28]. Consequently, a high level of estrogen at the proestrus and estrus stages may potentiate the K efflux during AP development in TG neurons, which presents as shorter AP falling time and deeper AHP depth.

Overall, our results indicated that increase of TG neurons excitability in the condition of high estrogen level during proestrus and estrus stages may involve migraine attacks during menstrual cycle. It has been suggested that activation of voltage-gated ion channels surrounding TG nociceptors induces AP development which increases TG neurons excitability and causes headache pain perception over the migraine attack [30]. Upregulation of voltage-gated ion channels following high level of estrogen may facilitate AP development in TG neurons likely reflects a raise in excitability of the trigeminal system to generate migraine attacks in menstrual migraine, as TG neurons increase their excitability before headache starts. Importantly, the influence of estrogen fluctuation on AP properties changes in TG neuronal excitability may help explain the profound headache observed in menstrual migraine as well as suggest a novel target for the treatment of this migraine condition.

Conclusion

Our study demonstrated that estrous cycle modulates AP properties of TG neurons in intact female rats. Interestingly, modification of AP development in TG neurons at four different stages of the estrous cycle may be induced by the cyclical fluctuation of estrogen levels, which may relate to modulation of voltage-gated ion channels. The results of this study reveal that the neuronal excitability of the trigeminal system increases during menstruation, which may be the fundamental mechanism underlying menstrual migraine.

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Conflict of interest  None

References


ABSTRACTS
KEYNOTE LECTURE

PLENARY LECTURES

MEMORIAL LECTURES
Keynote Lecture

Some observations on decision making in rodents

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It is estimated that humans make hundreds of decisions per day. To identify nerve cell circuits that mediate decision making we investigated, in rodents, those anatomical circuits in their cortex that contribute to decision making. We use a simple somatomotor behavioral task called the gap crossing task (“go” or “no go”). It is based on exploratory movement of facial vibrissae (whiskers) and object touch by whiskers. The cortical area in which whisker movement and touch are represented, by nerve cell activity, is the S1 vibrissal cortex. Physiological measurements indicated that two deep layer L5 pyramidal cell types are essential for this task (de Kock et al., 2007). To establish how these cells are embedded in the entire cortical circuitry we established the detailed cellular anatomy of nerve cell circuits in a single and in multiple cortical columns of S1. We use 3D reconstructions of dendrites and axons of physiologically characterized neurons and reverse engineering them into cortical columns in silico (Oberlaender et al., 2011). We discovered a new building block of circuits in S1, the intracortical unit (IC unit). It comprises connections within about the 9 columns that are activated when a whisker touches an object (Narayanan et al., 2015).

Currently we investigate quantitatively the projections of the deep layer pyramidal cells of S1 cortex to subcortical areas like thalamus and pons and also find a whisker specific projection pattern (map) of L5 pyramids in the target areas. Based on anatomical pathways the mechanism of decision making seems to depend on the somatosensory branch, primarily on signals generated by L5 cells. Due to their distributed projections to other brain areas, that however maintain a whisker map, it seems likely that the decision in the go / no go task is triggered by deep L5 cells and is eventually made by coincident activation of their target cells in subcortical nuclei.
PL 1
Short-Term Synaptic Plasticity: Inspired by Biophysics
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Synaptic Plasticity is held to be at the basis of most signal processing capabilities of the central nervous system. Long-term plasticity receives most attention by neuroscientists, since it underlies learning and memory. Short-term plasticity (STP), on the other hand, is not less important, since it mediates basic signal processing tasks, such as filtering, gain control, adaptation, and many more. My laboratory has studied STP at the Calyx of Held, a glutamatergic nerve terminal in the auditory pathway, which is large enough to be voltage-clamped in the ‘whole-cell mode’, using patch pipettes. STP is highly modulated by second messengers, such as Ca++ and diacylglycerol (DAG). We found that there is pronounced heterogeneity between individual synapses, some showing moderate to strong depression during stimulus trains of 100 Hz or 200 Hz, others displaying a sequence of facilitation and depression. After application of phorbol ester, mimicking the effect of DAG, EPSCs are enlarged and strong depression is observed only. Lee et al. 2013 (PNAS 110, 15079) showed that application of DAG accelerates a process, which they termed ‘superpriming’, a slow transition of release-ready vesicles from a ‘normally’ primed state to a faster, ‘superprimed’ one. Evidence will be shown, which suggests that the heterogeneity between synapses is due to different degrees of ‘superpriming’ at rest. Also, we find that the DAG-dependent process (mimicked by application of PdBu) is strongly Ca++ dependent. The joint modulation by both [Ca++] and PdBu can best be modeled by assuming a multiplicative effect on the rate-constant of superpriming. This, according to basic biophysical principles, is expected, if both modulators act on the energy barrier of the same rate-limiting step. No COI.

PL 2
Intracellular protein degradation: From basic mechanisms thru human diseases and on to drug targeting
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Between the 50s and 80s, most studies in biomedicine focused on the central dogma - the translation of the information coded by DNA to RNA and proteins. Protein degradation was a neglected area, considered to be a non-specific, dead-end process. While it was known that proteins do turn over, the high specificity of the process - where distinct proteins are degraded only at certain time points, or when they are not needed any more, or following denaturation/misfolding when their normal and active counterparts are spared - was not appreciated. The discovery of the lysosome by Christian de Duve did not significantly change this view, as it was clear that this organelle is involved mostly in the degradation of extracellular proteins, and their proteases cannot be substrate-specific. The discovery of the complex cascade of the ubiquitin solved the enigma. It is clear now that degradation of cellular proteins is a highly complex, temporally controlled, and tightly regulated process that plays major roles in a variety of basic cellular processes such as cell cycle and differentiation, communication of the cell with the extracellular environment and maintenance of the cellular quality control. With the multitude of substrates targeted and the myriad processes involved, it is not surprising that aberrations in the pathway have been implicated in the pathogenesis of many diseases, certain malignancies and neurodegeneration among them, and that the system has become a major platform for drug targeting. No COI.
Ouay Ketusingh Lecture

**Fibroblast growth factor 23 and vitamin D as feedback regulators in the bone-kidney-intestinal axis for calcium and phosphorus homeostasis**

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Fibroblast growth factor-23 (FGF-23), a hypophosphatemic hormone from osteoblasts and osteocytes in bone, is known to downregulate the renal tubular phosphate transporter expression, thereby promoting renal phosphate excretion. Its production and secretion are stimulated by high plasma levels of 1,25(OH)2D3. FGF-23, in turn, downregulates production of 1,25(OH)2D3 as well as the stimulatory effect of 1,25(OH)2D3 on renal reabsorption and intestinal absorption of phosphate, thus completing the bone-kidney-intestinal axis of phosphate regulation. Since there are strong links between 1,25(OH)2D3 and calcium-phosphate homeostasis and the inverse relationship between FGF-23 levels and 1,25(OH)2D3 reduction, it is possible that FGF-23 not only regulates phosphate metabolism, but may also modulate calcium metabolism by counteracting the effect of 1,25(OH)2D3 on intestinal absorption of calcium. In 2012, our laboratory demonstrated that FGF-23 did inhibit the 1,25(OH)2D3-enhanced calcium absorption in mouse duodenal epithelial cells by binding to FGF receptors in the basolateral membrane, and activating MAPK/ERK, p38MAPK, and PKC cascades. FGF-23 action prevented 1,25(OH)2D3-induced upregulation of TRPV5, TRPV6, and calbindin-D9k, thus diminishing calcium absorption. In 2013, we further elaborated the counterbalancing effect of FGF-23 on 1,25(OH)2D3-induced calcium absorption. Results from both murine duodenum and intestinal epithelium-like Caco-2 monolayer showed that FGF-23 directly inhibited the 1,25(OH)2D3-enhanced transcellular and paracellular calcium transport. Our findings, thus, provide strong support for the role of FGF-23 as a negative feedback hormone in the bone-kidney-intestinal axis of calcium and phosphate homeostasis. No COI.

Dithi Chungchroen Lecture

**Anti-diabetic drugs, Obesity, and Insulin resistance: The Good, the Bad, and the Ugly in the Heart**

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Cardiovascular diseases have been a major health burden in most countries around the world including Thailand. Despite the advancement in therapies in both pharmacological as well as device interventions, many critical problems regarding the health issues on the cardiovascular diseases still exist. Currently, obesity is also considered as an epidemic in which its prevalence is dramatically increased. In obese subjects, a number of serious cardiovascular risk factors already exist including insulin resistance, dyslipidemia, inflammation and oxidative stress. The risk of developing heart disease has been shown to be increased in obese subjects. In the past decades, pharmacological interventions have been used as therapeutic strategies in treating subjects with obese-insulin resistance. Although several drugs have been shown to exert a reliable glycemic control, some of these drugs also have serious adverse cardiovascular effects. Recently, several new anti-diabetic drugs have been shown to exert cardiovascular protective effects in addition to their glycemic control. Despite these reports, inconsistent findings exist. At this time, it is crucial that these anti-diabetic drugs be investigated for their mechanisms as well as possible beneficial/adverse effects on the heart. The knowledge obtained from these future studies will reassure the cardiovascular safety of these anti-diabetic drugs to be used in patients. No COI.
Memorial Lectures

Prasop Ratanakorn Lecture

Melatonin regulating neurogenesis and neurodegeneration

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Adult mammalian neurogenesis is an ongoing process in two significant brain regions, the subventricular zone, located laterally to the lateral ventricle and the subgranular zone in the dentate gyrus of the hippocampus. The prominence of adult neurogenesis lies in its roles in the replacement of dead neurons with functional neurons, learning and memory, and the prevention of neurodegeneration. An extensive range of factors, including trophic factors, transcription factors, neurotransmitters, hormones, epigenetic factors, glial cells, pathological manifestations, diet, exercise, drug abuse and aging can modulate these processes. Nevertheless, in this presentation, we will be converging on the neurogenic properties of melatonin, a major indoleamine of the pineal gland. It has been suggested in various recent papers that melatonin serves as a major contributor to the proliferation and survival of neural stem cells through its membrane bound G-protein coupled receptors. The secretion of melatonin by the pineal gland declines with age and contributes to a diminution in neurogenesis and an escalation in the probability of neurodegeneration. In this presentation, the stress-induced neurodegeneration and suppression of progenitor cell proliferation and diminished neurogenesis will be used as a study model. The interrelationship between melatonin and dexamethasone (a stress hormone) on the underlying mechanism of neural stem cell proliferation will be discussed. Hence, understanding and evaluating the neurogenic mechanisms of melatonin can serve as a crucial step to the development of therapies for neurodegenerative diseases.

Keywords: Neurogenesis, melatonin, aging, neurodegeneration

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John Young Lecture

Molecular identification and role in physiology of the volume-regulated anion channel VRAC

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Cells need to regulate their volume in response to osmotic stress, during cell division, growth and migration, and during apoptosis. A crucial player in regulatory volume decrease (RVD) is the volume-regulated anion channel VRAC that has been known from physiological and biophysical studies for more than two decades. VRAC is closed under resting conditions and can be opened by cell swelling or other stimuli, for instance those leading to apoptosis. Many of studies investigated mechanisms of VRAC activation, which remain, however, very poorly understood. Many ‘candidates’ have been proposed to embody VRAC, but all of them crashed and the proteins underlying VRAC were not known until 2014. Using a genome-wide siRNA screen that used YFP fluorescence quenching by hypotonicity-induced iodide influx as read-out, we and others recently identified LRRC8A as an essential VRAC component. We went on to show that LRRC8A is not sufficient to form VRAC, but needs heteromerization with at least one other LRRC8 subunit (B-E) for yielding swelling-activated anion currents. The specific LRRC8 subunit composition determines the inactivation properties of VRAC, demonstrating that LRRC8 protein directly participate in channel formation. Swelling-induced taurine and glutamate efflux also depend on LRRC8 heteromers, suggesting that a strict distinction of VRAC and VSOAC is not warranted. A weak homology of LRRC8 proteins with pannexins suggest that VRACs may be formed by hetero-hexamers. The molecular identification of VRAC-forming proteins now allows to rigorously address the many enigmas surrounding this important channel. Current studies, that focus on the role of different LRRC8 subunit in VRAC’s transport properties, its regulation, and physiological functions, will also be presented.
SPECIAL LECTURES

LUNCHEON LECTURES
SL 1
Myofilament Dysfunction in Heart Failure
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Heart failure is characterized by a progressive decline in pump function. A decline in cellular contractile function has been shown to be at the basis of this syndrome. The mechanisms underlying myocyte dysfunction are incompletely understood, which hampers the development of novel therapeutic strategies to combat morbidity and mortality. Numerous studies have shown that cardiac cellular calcium homeostasis is disturbed in both human and experimental heart failure, and this may be responsible for the reduction in contractile function of the cardiac myocyte. However, the function of the cardiac contractile apparatus has, until recently, received far less attention. Here we show that myofilament function is depressed in human and experimental heart failure. Moreover, depressed contractile protein function is not caused by altered protein isoform expression, but rather due to post-translational modifications, most likely maladapted contractile protein phosphorylation, in particular the troponins, myosin regulatory light chain, and myosin binding protein C. Our results show that contractile protein phosphorylation is an important (negative) regulator of cardiac myocyte contractile function and a potential target for therapeutic interventions. No COI.

SL 2
Circadian rhythms, molecular clock and skeletal muscle; why muscles need to keep time
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Disruption of circadian rhythms in humans and genetic models of circadian disruption in rodents have implicated a fundamental role for circadian rhythms in systems health including links to metabolic and cardiovascular diseases. These models also exhibit skeletal muscle weakness and altered metabolism. Here we show that disruption of the molecular clock in adult skeletal muscle is sufficient to disrupt maintenance of muscle function and phenotype as well as skeletal and cardiovascular homeostasis. Targeted deletion of the molecular clock gene, Bmal1 (iMSBmal1-/-) in skeletal muscle caused pathological changes including reductions in specific tension, altered gait, a shift toward a more oxidative fiber type, and muscle fibrosis. Surprisingly, the iMSBmal1-/- mice exhibited pathological changes in bone and cartilage along with cardiac hypertrophy and elevated blood pressure. We found that the Renin Angiotensin System (RAS) was altered with loss of muscle Bmal1 with increased expression of angiotensinogen and other RAS components in liver and skeletal muscle consistent with elevated blood pressure, changes in the skeletal system and muscle fibrosis. These findings highlight the critical function of the molecular clock in skeletal muscle and uncover the fundamental role that skeletal muscle, an organ system comprising approximately 40 percent total body mass, impacts systemic health. No COI.
Novel aspects of the autoregulation of blood flow: from isolated vessels to human

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Supply of the brain can be achieved only if the blood volume and consequently intracranial pressure is tightly controlled. Thus a very effective autoregulation must be present, which is an important feature of the cerebral circulation. This issue has been investigated by several previous studies and it was logically assumed that autoregulation of CBF is somehow coupled to changes in hemodynamic forces, such as pressure and shear stress.

**Pressure sensitive vasomotor response:** For many years, autoregulation of CBF has been primarily explained by the pressure-induced myogenic mechanism of cerebral vessels: the inherent property of vascular smooth muscle to dilate to a decrease and to constrict to an increase in intraluminal pressure. There are two critical downstream mechanisms are activated by increases in wall stress: Ca\(^{2+}\)-dependent and –independent, RhoA/Rho kinase pathway, sensitizing actin-myosin proteins to Ca\(^{2+}\), leading to the constriction.

**Flow sensitive vasomotor response:** Only very recently, it was shown in certain cerebral vessels, such as the middle cerebral artery a flow sensitive mechanism also exists. In contrast to peripheral arterial vessels, in the presence of constant pressure increases in flow elicit constrictions in this type of vessels. The constrictions are mediated by 20-HETE (20-hydroxyeicosatetraenoic acid, a coconstrictor metabolite of arachidonic acid synthesized by cytochrome P450 hydroxylases) and reactive oxygen species (ROS).

On the basis of above, it is plausible that in the cerebral vascular network, during increases in systemic blood pressure when both pressure and flow changes (for example during exercise) the flow-constriction is superimposed on the pressure-constriction resulting substantial constrictions of arterial vessels providing a relatively stable level of blood flow. These mechanisms are present in vivo protecting the capillaries from pressure and flow overload and the blood brain barrier (BBB). No COI.

The role of prostaglandin E\(_2\) in gastric mucosal inflammation and the significance of dysregulation of degradation pathway of prostaglandin E\(_2\) in gastric carcinogenesis

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Prostaglandin E\(_2\) (PGs), which are synthesized by cyclooxygenase (COX) from arachidonic acid liberated from membrane phospholipids, have a variety of biological effects. In physiological condition, PGE\(_2\) is produced by COX-1, which is regularly expressed late-limiting enzyme for synthesis of PGs to maintain gastric mucosal integrity and wound healing. PG is also produced by COX-2, an inducible isoform by inflammatory stimuli, mainly Helicobacter pylori infection in the stomach. Our previous study shows that PGE\(_2\) exert anti-inflammatory effect against Helicobacter pylori infection-induced proinflammatory responses in the stomach, which may suppress initial event of gastric carcinogenesis such as progression of cell turnover of gastric glands. However, as inflammatory change in gastric mucosa progresses, PGE\(_2\) promotes carcinogenic effect such as promotion of cell proliferation and tumor angiogenesis. Accumulating clinical and experimental evidence establish the involvement of PGE\(_2\) in a variety of cancer in the gastrointestinal tract. Although tissue levels of PGE\(_2\) stem from relative rates of biosynthesis and degradation, most of previous studies about gastrointestinal carcinogenesis have extensively focused on production system of PGE\(_2\), especially COX-2. In addition, recent studies indicate that degradation pathway of PGE\(_2\) which includes PG transporter and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) have tumor suppressor property. Our recent studies showed that both PGT and 15-PGDH are the prognostic factor for poor survival of gastric adenocarcinoma patients who underwent gastrectomy. Our studies demonstrate that reduced expression of PGT and 15-PGDH in gastric carcinogenesis is associated with tumor cell proliferation and tumor angiogenesis. Thus, our studies suggest that the maintenance and restoration of regulation system of PGE\(_2\) could be a key for prevention of gastric carcinogenesis. No COI.
SL 5

**Evolutionary and comparative cognitive neurobiology of human and non-human primates**

Atsushi Iriki

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Human evolution has involved a continuous process of addition of new kinds of cognitive capacity, including those relating to manufacture and use of tools and to the establishment of linguistic faculties. The dramatic expansion of their brains that accompanied additions of new functional areas would have supported such continuous evolution. Extended brain functions would have driven rapid and drastic changes in the ecological niche of human ancestors, which in turn demanded further brain resources to adapt to it. In this way, humans have constructed a novel niche in each of the ecological, cognitive and neural domains, whose interactions accelerated their individual evolution through a process – namely, “Triadic Niche Construction”. Thus emerged human intelligence should be structured to comprise a part of dynamic patterns of holistic terrestrial ecosystem. The primate brain’s functional characteristics seem to play a key role in this triadic interaction. We advance a speculative argument about the origins of its neurobiological mechanisms, as an extension with wider scope of the evolutionary principles of adaptive function in the nervous system of the non-human primates. Neurobiological mechanisms to acquire novel tool-use skills in non-human primates would shed light on such properties of human intelligence developed through the course of evolutionary processes. No COI.

SL 6

**Systems Biology of Aquaporin-2 Regulation in Kidney Collecting Duct**

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Vasopressin regulates water transport in the renal collecting duct epithelium in part by increasing abundance of the AQP2 water channel protein. We are using quantitative proteomics (LC-MS/MS) and transcriptomics to identify how vasopressin regulates gene expression in collecting duct cells. We used dynamic SILAC (LC-MS/MS) to quantify the effect of vasopressin on the steady-state translation and degradation rates of each protein in cultured mouse mpkCCD cells. Of the 4403 proteins quantified, vasopressin significantly altered protein half-life of <20 proteins. This included AQP2, whose protein half-life increased from 10 to 14 hrs. Many more proteins showed increases in translation rate including AQP2 (10 fold increase). Simultaneous global measurements of protein abundances (standard SILAC quantification) and transcript abundances (Affymetrix expression arrays) revealed that as many as 35% of proteins that undergo a change in abundance in response to vasopressin had no corresponding change in mRNA (despite sufficient statistical power), pointing to the possibility of extensive translational regulation. AQP2, however, showed an increase in mRNA roughly proportional to the measured increase in translation. A change in mRNA abundance could be due to a change in transcription rate or mRNA degradation rate. To begin to address the former, we carried out simultaneous RNA-seq and ChIP-seq profiling for RNA Polymerase II (Pol-II). These experiments showed a 6-fold increase in Pol-II binding to the Aqp2 gene with an accompanying 18-fold increase in mRNA. The increases for Aqp2 outstripped the vasopressin-induced changes in all other genes, pointing to a highly selective transcriptional network triggered by vasopressin signaling. No COI.
Small bowel injury caused by indomethacin in animals had been reported in 1960s. This phenomenon, however, has been ignored for 40 years in a clinical field until capsule endoscopy was available. NSIADs and/or low-dose aspirin are widely used for patients with chronic arthritis and/or ischemic vascular diseases respectively and now we have a lot of publications regarding NSAID-enteropathy. We have shown in the animal model that indomethacin increases gram-negative bacilli, stimulates proinflammatory cytokines, and neutrophil infiltration in the mucosa of small bowel. The enteropathy was inhibited by antibiotics specific for gram-negative bacilli, antibodies against TNFα and IL-1β, and antiserum against neutrophils. These results may indicate that gram-negative bacilli overgrown translocates through the mucosa weaken due to the insufficiency of prostaglandins, then the inflammatory system is stimulated, which triggers the injury. The inflammatory activation and injury were attenuated in TLR-4 knockout mice, whereas they were enhanced by treatment with TLR-4 ligands such as LPS and HMGB1, indicating the TLR-4-dependent pathway may play a key role. Therefore, this pathway may be the target of drug development for the prevention and treatment of NSAID-enteropathy. Recently we have found that the NLRP3 inflammasome/IL-1β axis could be another target. To prevent or treat NSAID-enteropathy, several small studies have done with available drugs and substances and have shown that misoprostol, a prostaglandin derivative, rebamipide, a mucoprotective drug which may affect intestinal microbiota, and probiotics may be promising. Further clinical studies are needed with possible drugs to inhibit the TLR-4-dependent pathway or the NLRP3 inflammasome/IL-1β axis. This study is supported by Otsuka Pharmaceutical Co., Ltd., which provided rebamipide and its placebo used.
TEACHING PHYSIOLOGY

KEYNOTE ADDRESS

LECTURES

SYMPOSIA
TPK 1

Understanding the changing learner: a critical success factor of higher education of the 21st century

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Exponential growth in information and technology together with rapid development and advancement in information technology make learners of higher education now different from those of the 20th century. They are multitaskers. They prefer learning from media with pictures, sound, and video than from conventional textbooks. Instead of learning in a linear, logical, and sequential pattern, they enjoy random access to content. Study and working in team make them better prepare for class and learn significantly more than independent and individual exercise. The learning environment should be designed to emphasize more on internal or intrinsic motivation than extrinsic one since internal motivation lead them to active engagement in learning, deeper understanding in content, and the desire to learn more. Moreover, they pay more attention to relevant, real world 21st century contexts than the classic theoretical simulation. Understanding the changing learners of this century is the crucial factor of the transformation from industrial model of pedagogy towards collaborative learning and collaborative knowledge production – the most likely successful model of higher education of the 21st century.

TPK 2

Learning from Productive Failure

Manu Kapur

*National Institute of Education, Singapore

In my talk, I will advance a theoretically- and empirically-grounded case for designing for and learning from failure, and instantiate it in a learning design I call Productive Failure (PF). I will describe the key mechanisms and the design principles of PF, and report findings from an on-going program of research in the real ecologies of Singapore Math classrooms. I will end by deriving implications for learning and the design of instruction. No COI.
TPL 1

**Teaching challenges in a modern university environment**

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Personalised Medicine will become increasingly important in all areas that are susceptible to pharmacological treatments. Understanding and application of personalised medicine requires detailed molecular, cellular and systemic knowledge that is difficult to achieve in current undergraduate teaching curricula. Class sizes have increased significantly and only a fraction of students will end up in research professions for which their training has been designed for. As a result universities have pushed for flexible degrees, reduction of prerequisites and broader skills sets. The challenge is how to combine these without reducing qualification and skills. Due to the enormous body of knowledge that has been accumulated in biology/medicine it is no longer possible to teach a comprehensive understanding of the field. However, due to the attractiveness of advanced knowledge understanding of basic concepts are often underrepresented as they are considered old and unattractive. Basic knowledge and basic concepts are particularly suitable to online delivery, but it must be ensured that content is not getting lost as a result of single exposure of the material during a degree. Repeated learning is a central part of knowledge acquisition. Online material also needs to be attractive to avoid learning fatigue. A combination of online delivery of basic content blended with tutorial modules of advanced content and problem based approaches appears to become a new teaching standard across many universities. No COI.

TPL 2

**Creating the Future: complementary approaches to transdisciplinary learning**

Philip Poronnik

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There are diversifying pressures on ensuring university science students are graduating with the capabilities and qualities that they require. The traditional attributes of subject knowledge, problem solving and team work skills, though essential, may well not be sufficient for our students to recognize their full potential. The broader workplace of the mid-21st century is looking for what some call “renaissance talent” in graduates. Many employers in the sciences are looking for students that have studied an arts subject as well as an ever-increasing emphasis on statistical analysis. There are also repeated calls, starting from primary school, for students to learn coding skills. At the same time we continue to observe declining lecture attendances and student engagement with their learning. We have long championed a more creative science curriculum, arguing that we should focus more on creating learning environments where students “learn to be scientific” rather than “learn about science” through identify-forming activities that aims in turn to increase engagement and learning outcomes. This involves designing learning environments in which students can use their command over subject content to draw the links between diverse disciplinary areas, react to new ideas and include elements of the unexpected in their work. In response to these challenges, we are investigating the use of creative coding strategies to provide both students and academic staff with platforms that can allow creative exploration and analysis of data sets and pathways. This talk will outline the new approaches and discuss progress to date. No COI.
TPS 1
Technology enabled strategies for student engagement in learning medical sciences
T Hinton\textsuperscript{1}, LM Tang\textsuperscript{2}, KA Bragg\textsuperscript{3}, P Poronnik\textsuperscript{4}, L Engelen\textsuperscript{4}, A Bauman\textsuperscript{4}, J Kay\textsuperscript{2}, ML Day\textsuperscript{3}
\textsuperscript{1}Discipline of Pharmacology, School of Medical Sciences, University of Sydney, Australia
\textsuperscript{2}School of Information Technology, University of Sydney, Australia
\textsuperscript{3}Discipline of Physiology, School of Medical Sciences, University of Sydney, Australia
\textsuperscript{4}School of Public Health, Sydney Medical School, University of Sydney, Australia

One of the key challenges in medical science education is developing experimental design and data analysis skills. The wide availability of wearable activity tracking devices provides an immersive opportunity for students to be active participants in these processes through personal engagement and to take part in a real research project.

We have developed a practical class for second year medical students (a cohort size of 250) in which they design an experiment for self-quantification of physical activity. Students are required to consider aspects of experimental design such as sample size, dependent and independent variables and hypothesis generation. Students were provided with a Fitbit zip wireless activity tracker at the beginning of semester and asked to wear the device for 6-8 weeks, except when asleep. Activity data was collected via the Fitbit website and analysis of daily activity levels and the time students wore the devices each day was performed. This practical not only engaged students in collection of personally relevant data around which they learnt elementary data presentation and statistical analysis but also helped to contextualise understanding of experimental design. This practical also provides an opportunity to enhance awareness of health and wellbeing of individuals and communities. Such projects highlight the potential for future opportunities to use personal activity monitoring for cloud-based global collaboration between large cohorts of students.

TPS 1
Engaging Students to Face the (Physiology) Book
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Teaching Physiology is an evolving art. If ‘the consumer is often right’, then our task as educators should be aimed at better transmission of Physiological information so that the students will ‘buy’ them. The hard copy texts are seldom carried under the arms of students with the availability of electronic versions. Nevertheless, a crowded medical curriculum easily discourages the less diligent student to read and think through chapters in Physiology books. An over-dependence on power point (Ppt) notes to pass assessments is a major factor in shallow, unintegrated understanding of Physiology. Students need to ‘think outside the Ppt Box’. We teach to engage and provoke our students to discover more. Posing unanswered Physiology questions during a lecture is one way to stimulate the indifferent audience in class. The use of Facebook Physiology interactive page for each medical class in Universiti Malaya has also help to enhance learning in Physiology. The decreasing number of small tutorials to discuss Physiology in integrated curriculum appears to have a negative impact on the depth of Physiology knowledge among students. Intra-Physiology integration between organ systems in our teaching might be the more useful theme to emphasize to help students conceptualize homeostatically and apply Physiology to the clinical situations.
TPS 1
Integration of Physical Examination with Student Practicum through case-based learning in a vertically integrated module “Cardiovascular System”
Mei-Ling Tsai
National Cheng Kung University, Taiwan

Self-directed learning is an important skill for lifelong learning. Although PBL is considered as a suitable pedagogy for cultivating self-directed learning skills, it is a highly touted Western instructional model but has not had the same success in East Asian institutions. Recent education research regarding Cognitive Load Theory has shown that PBL’s effectiveness as an instructional instrument requires participants possess certain skill sets to optimally interact with domain specific knowledge that our medical students, coming straight out high school, simply have not acquired. To address this, a modified Case Based Learning program (CBL+) was developed to provide instructional scaffolding to provide the necessary skill base for the students to effectively participate in the 3rd year cardiovascular system within the NCKU medical school. To integrate all clinical teachings with the case used in the block, case-specific content was integrated with that related to Physiology Practicum. Various class activities related to physical examination were implemented to enhance the skills related to self-directed learning including identifying questions, looking for resources, developing critical thinking skills, and constructing knowledge. Workbooks were used as portfolio to analyze how the questions are formed. Concept map was used to analyze the development of their thinking process. As our data suggested, integration of case-based learning and critical thinking into student practicum helps students identify their problems and search for the answers through a structured thinking process. The process lowers students’ anxiety in the first experience of self-directed learning.

TPS 1
Learning physiology: Integration, student engagement and collaboration
Dow Rhoon Koh
Yong Loo Lin School of Medicine (YLLSOM), National University of Singapore

At the YLLSOM, physiology is one of the key basic science topic taught in medicine year one along with anatomy and biochemistry. These topics are anchored by their respective departments, Physiology, Anatomy and Biochemistry. These topics were traditionally taught in a discipline-specific fashion until a major change to integrate horizontally the topics into systems biology, cell biology and human structure and function. This provided the framework to incorporate 21st century skills such as self-directed learning, life-long learning, collaborative and team learning, communication and professionalism early into the year one curriculum. Initially, the learning of Physiology became more integrated with that of anatomy and biochemistry at the organ system level. However, the increasing specialisation of the basic sciences and teachers made it increasingly a challenge to keep the perspective of the whole in Physiology. This provided a major impetus to further integrate and embed Physiology into the curriculum and engage an increasingly IT savvy and hands-on generation of students incorporating flipped classroom, cross discipline case-based learning, simulation learning and collaborative learning approaches. In addition, the challenge also falls on the teachers and faculty who need to respond appropriately to the needs of the curriculum and students by way of faculty development, cross disciplinary approaches to teaching and developing better ways to foster student learning in a relevant and contextual fashion. It is hoped that this will keep the teaching of Physiology in step with best practices and feedback driven quality improvement in the school.
TPS 2

Engaging Medical Students in Basic Principles of Cell Physiology Using “Hands-on” Practical Class Activities

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Active learning of basic principles of cell physiology in large Medical Schools such as UNSW Australia faces challenges arising from large student numbers, and the delicate experimental procedures and expensive equipment that can be needed to measure single cell properties. Valuing strongly the learning gained by experimentation in Physiology, we have developed a number of practical classes to illustrate basic cell physiology principles. In one of these, a cellular fluid homeostasis practical, we measure the haematocrit of human red blood cells to which we separately add the Na+ ionophore, monensin (10–20 µM, 0.1–0.2% DMSO), the potassium ionophore, valinomycin (10–20 mM, 0.1–0.2% DMSO), and a DMSO vehicle control. Students fill glass haematocrit tubes with each of the three samples, and then centrifuge the samples to separate the whole blood sample into plasma and red blood cells. The proportion of red blood cells (the haematocrit) is measured using a haematocrit reader (or accurate ruler). Addition of monensin increases the haematocrit by 4–7%, due to Na+ influx leading to H2O influx and cell swelling. Typical haematocrit values in the valinomycin samples are 2–5% lower than control, due to cell shrinkage secondary to K+ efflux. Although each batch of blood is slightly variable, the small drug-induced changes are consistently observed. This novel and simple experiment effectively demonstrates that fluid (H2O) moves across cell membranes due to osmotic forces induced by ionic fluxes. Students enjoy the experiential activities, get to appreciate variability and the importance of accurate experimental observations, and are further engaged by understanding that antibiotics such as valinomycin act by altering the ion selectivity of the membrane. No COI.

TPS 2

Physiology teaching, asian pacific perspective and new models

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Physiology teaching in the medical schools and other health science consists of giving lectures and experiments to the students and in the traditional curriculum the students get these after the anatomy and histology courses in the first year. This traditional way is a discipline based system and long hours of physiology lectures are given. After the era of the reform of medical education which PBL (Problem Based Learning) was introduced, physiology lectures is given in blocks and is an organ based system and integrated horizontal and vertical in the organ blocks. Many medical schools in the Asian Pacific region adopted the PBL system with many models of the hybrid PBL (I, II, III, IV) Examples of these new models will be discussed and examples will be given. No COI.
Unique Role of Physiology in an Integrated Curriculum

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The need for greater integration of subjects in the medical curriculum and even in life sciences is featured consistently in pedagogical literature. Approaching curriculum goals by employing curriculum design and teaching and learning strategies in an integrated manner is meant to achieve the goals more efficiently. Although, the discussion about integration often lead to polarization amongst teachers that argue for it emphatically and those that still insist for dominance of discipline-based teaching while some approach with intermediate steps between the two extremes. The question to be asked to teachers and curriculum planners is not whether they are for or against integration but rather where on the continuum between the two extremes should they place their teaching. Leaving teaching and learning strategies aside the most important fact is the place that discipline of physiology enjoys in an integrated physiology curriculum. Not only in curriculum for undergraduate program in medicine but also basic science curriculum at most life science institutions consist of courses (e.g. general anatomy, physiology, biochemistry, pharmacology etc) learning can be made more contextual and enjoyable by employing an integrated approach rather taught as stand-alone content domains. This review highlights that how truly and deeply place of discipline of physiology is recognized in an integrated curriculum. No COI.

Current trends in Physiology education in Japan - Physiology educator accreditation and government-supported MD-PhD course

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Physiological Society of Japan (PSJ) has provided opportunities to exchange skill, findings and knowledge in research. However, a systematic education program was not held until 2012. Education Committee of PSJ has started education program since 2013 to provide opportunities to learn teaching skills and to obtain up-to-date knowledge in Physiology. PSJ members who attend this course with teaching experiences of Physiology and/or publication of original article in Physiology can earn points for accreditation. After evaluation, PSJ gives accreditation to those who fulfill the criteria. After the start of the program, the number of PSJ members has increased. Thus, the education program has attracted many Physiologists and may have potential to improve Physiology teaching quality. Another serious problem is a decline in young Physiologist, particularly medical school graduates. This may be because 2 years’ internship has become obligatory, and because the time to study basic medicine has decreased under clinical-oriented curriculum. Through collaboration with Japan Society for Medical Education, Medical Societies in basic medicine including PSJ have proposed to government to support research-oriented medical students. The grant was approved and 10 schools were selected. In such schools, the education curriculum has been modified so that students can also take PhD curriculum as a MD-PhD student. They can keep their PhD student position during internship. After obtaining PhD, their faculty position is guaranteed. Interestingly, many universities that could not obtain this grant had also started similar programs. Thus, this program may contribute to increase the number of MD basic researchers. No COI.
TPS 3

Progressive development of scientific literacy through assessment in inquiry-based biomedical science curricula

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A key outcome of science education is the development of graduates’ scientific literacy, defined as “an individual’s scientific knowledge, and use of that knowledge to identify questions, to acquire new knowledge, to explain scientific phenomena, and to draw evidence-based conclusions…” (OECD 2010, pg 137). To progressively develop such advanced skills within a broad major like biomedical science, it is essential to guide students along critical learning pathways. We have designed a series of inquiry-based classes to scaffold the development of these skills and vertically-integrated these across the curriculum (Zimbardi et al. 2013). To facilitate skills development within these classes, students undertake increasingly complex assessment tasks as they progress through each course, requiring them to draw on their developing content knowledge to propose and undertake experiments, and to make conclusions based on their findings and evidence from scientific literature. Longitudinal analysis of a variety of assessment tasks from students across four semesters demonstrates the developmental trajectory of these skills. Specifically, they demonstrate increases in their ability to formulate testable hypotheses with measurable outcomes, their appreciation of cutting-edge methodologies and deeper understanding of the contestable nature of increasingly complex areas of scientific knowledge. This presentation reports on the design and use of these assessment tasks within the series of inquiry-based curricula, and their impact on the progression of student learning. No COI.

TPS 3

A strategy for student engagement in peer student presentations- assessing critical thinking skills

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In 3rd year Physiology at the University of Adelaide, students work in small groups (n=2 to 4) in a research laboratory throughout the year, and present their results as a group (10 minutes talk, 5 minutes of questions) to an audience consisting of their peers. A large problem has been a lack of engagement of the student audience with the process. Following the dictum that “assessment drives learning”, we therefore changed the format so that all students had to formulate questions to ask of the presenting group, and they were assessed on those questions. Each group presenting prepared a poster to illustrate their talk which was made available online a week before the presentations. Students (as groups) were assigned 3 of those posters to read and prepare questions. On the day of the presentations, they were assigned to ask questions to one of those 3. The marking rubric incorporated a section on “group asking questions”: criteria for marking the questions asked, as well as “group presenting”: the criteria for assessing the presentation poster. The presentation sessions are now much more interactive- the assessors now mostly only observe and assess, rather than having to initiate questions. Anecdotally, the students are engaging more in the critical appraisal of the work of their peers. In future to improve students’ critical skills, we intend to give prior guidance on how to formulate questions- open ended vs closed questions, questions which show some insight vs “generic” questions. No COI.
TPS 3

Enhancing student engagement and critical thinking in learning physiological concepts: A guided inquiry approach

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Lectures develop students’ physiology knowledge but not their communication, teamwork or critical thinking skills. The challenge is to modify lectures to develop these skills and to encourage student engagement and active learning. Using the theories of constructivism and peer learning we developed a guided inquiry (GI) approach to lectures. During GI lectures, relevant background is first presented by the lecturer, a GI worksheet in which students work in groups to interpret graphs, answer questions and solve problems follows. An interactive discussion about the GI concepts concludes the lecture. Our study consisted of two phases. In phase one, GI was trialled with radiography students (N=56). A survey was administered at the end of the lecture series (blood and respiration). Students indicated that the GI helped them to better understand the physiology concepts (100%) and that the discussion with their peers was an important part of their learning (86%). In the second phase of the study, GI was used in lectures to science students (N= 159) and diagnostic quizzes (pre and post-test) were used to evaluate student learning. The results showed a significant improvement in student performance between the pre and post-tests (\(P < 0.05\)). In a survey of student perceptions, students found that the GI lectures encouraged them to take notes during the lecture (55 responses, 82%) and to attend the lectures (75%). In conclusion, we found that a GI approach can be used to motivate active student learning, enhance student understanding of core physiology concepts and develop critical thinking skills. No COI.
Teaching Physiology SYMPOSIUM 4:
Learning physiology: the student’s voice

TPS 4
Learning Physiology: The Students’ Voice
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With an ongoing transformation of healthcare professional education, e.g. from a discipline-based to a competency-based curriculum, an explosion of knowledge and characteristics of the 21st century learner, teaching physiology has been transformed in many institutions in order to effectively facilitate students’ learning. Various active learning formats such as an interactive lecture, a flipped classroom, a simulation-based learning, or an edutainment have been introduced to help students learn challenging physiological concepts. Regarding students’ learning, well-written examination questions can evaluate learning as well as how effective and successful the learning activity was. Nevertheless, good physiology classes cultivate more than information and knowledge, including students’ enthusiasm for physiology. In this session, students from various institutions across FAOPS member countries will be invited to share experiences, thoughts and impressions regarding their perceptions on physiology education including physiological knowledge, skills, and attitude toward teaching and learning physiology in the 21st century. No COI.
SYMPOSIAS
Roles of lipid raft in epithelial Na\(^+\) channel (ENaC) function

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ENaC plays a critical role in homeostasis of fluid content and blood pressure by controlling Na\(^+\) transport in epithelial cells. The amount of ENaC-mediated Na\(^+\) transport is closely correlated to the activity (open probability; Po) of individual ENaC and the number (N) of ENaCs expressed on the apical membrane: these Po and N are regulated through post-translational processing such as proteolysis with GPI-anchored proteases and Nedd4-2-dependent ubiquitination. On the other hand, cholesterol- and sphingolipid-enriched lipid raft is a crucial platform for protein trafficking and signal transduction, and accumulates in the apical membrane more than in the basolateral membrane of epithelial cells. Consequently, lipid raft might be a membrane domain suitable for post-translational processing of ENaC. In this study, we elucidate the role of lipid raft in the apical trafficking of endogenously expressed ENaC and post-translational processing in renal epithelial A6 cells with or without stimulation of aldosterone, and present the importance of lipid raft for ENaC post-translational processing including trafficking.

No COI.

Short Palate Lung and Nasal Epithelial Clone 1 (SPLUNC1) dissociates and internalizes the Epithelial Sodium Channel (ENaC)

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αβγENaC regulates sodium and water absorption across airway epithelia. In cystic fibrosis airways, hyperactive ENaC dehydrates airway surfaces which results in mucus thickening and increased probability of infection. SPLUNC1 is a negative regulator of ENaC but its underlying mechanism of action is unknown. Here, we tested the hypothesis that SPLUNC1 works by internalizing ENaC. Surface biotinylation was performed in HEK293 and Human bronchial epithelial cells (HBECs) to investigate ENaC surface level. Immunoprecipitation, immunostaining and acceptor-photobleaching fluorescent resonance energy transfer (FRET) were performed in HEK293 to investigate ubiquitination, co-localization, and conformational change of ENaC respectively. A Nedd4-2 dominant-negative construct was a gift from Dr. Peter Snyder (UI). SPLUNC1 reduced the %FRET efficiency between βENaC-GFP and γENaC-mCherry from 9.8±1.4 to 5.0±1.1%. SPLUNC1 decreased plasma membrane αENaC by 6.7-fold in HEK293 cells and 2.6-fold in HBECs without affecting the plasma membrane βENaC. When αγENaC was co-expressed, SPLUNC1 did not affect plasma membrane αENaC. SPLUNC1 ubiquitinated αENaC by 4.5 fold, which was abolished when Nedd4-2 ubiquitin ligase function was blocked by αENaC PY-motif truncation or Nedd4-2 dominant negative transfection. Pre-treatment with chloroquine, a lysosome inhibitor, but not MG-115, a proteasome inhibitor, abolished intracellular αENaC degradation without affecting ENaC internalization. Internalized αγENaC by SPLUNC1 co-localized. In conclusion, upon the binding of SPLUNC1 to βENaC, SPLUNC1 allosterically triggers Nedd4-2 mediated αENaC ubiquitination that results in the dissociation of ENaC subunits, internalization and degradation of αγENaC but not βENaC via the lysosomal pathway. No COI.
Symposium 1 New insights into integral regulators of the epithelial ion transport in health and disease

S 1

CFTR chloride channels as promising therapeutic targets in multiple diseases
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Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated chloride channel expressed in luminal membrane of epithelial cells. Our laboratory has investigated roles of CFTR as drug targets for human diseases and identifies pharmacologically favorable CFTR inhibitors using cross-disciplinary approaches. Using cell-based assays of CFTR function, several novel types of CFTR inhibitors have been identified from collections of both synthetic small molecules and natural compounds including hydrazide-containing compounds, hydrolysable tannins, steviol derivatives, chalcones and xanthones. Furthermore, using the identified compounds to probe CFTR function, roles of CFTR as drug targets for cholera, polycystic kidney disease (PKD) and thalassemia have been validated. Interestingly, we recently demonstrated that existing drugs including diclofenac and pranlukast inhibit CFTR function and may be beneficial in the treatment of secretory diarrheas and PKD, respectively. In addition, our group showed that indirect inhibition of CFTR function by chitosan oligosaccharide, a biomaterial prepared from chitin, reduces intestinal fluid secretion in vivo via AMPK-dependent mechanism. Therefore, CFTR serves as a promising drug target whose functional inhibition, through either direct or indirect mechanism, may be of therapeutic benefit in secretory diarrheas, PKD and/or thalassemia. No COI.

S 1

Ca²⁺ signaling defects underlying exocrine dysfunction
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Radiotherapy for head and neck cancer induces significant acute and long-term by-stander effects on tissues within the treatment area, such as the salivary glands which undergo irreversible loss of fluid secretion. In patients this results in severe dry mouth conditions with debilitating effects. Surprisingly loss of function precedes fibrosis and destruction of glandular tissue, which is a much slower process. The mechanism underlying the loss of salivary gland function is not known. Our earlier studies have identified that the ROS-sensing Ca²⁺ channel, TRPM2, is activated as a consequence of irradiation (IR) and Ca²⁺ entry via this channel contributes to irreversible loss of salivary gland function. TRPM2⁻/⁻ mice display an initial loss of function that recovers to near normal levels within a month after IR while TRPM2⁺/⁺ mice show no such recovery. Our data showed that activation of TRPM2 by IR involves increase in PARP activity and treatment of TRPM2⁺/⁺ mice with PARP inhibitors prior to IR induced significant protection of salivary function. To understand the link between TRPM2 and loss of salivary gland function, we examined Ca²⁺ signaling in glands from IR-mice and in vitro in IR-cells. Our findings show that following IR, store-operated Ca²⁺ entry (SOCE), a critical requirement for fluid secretion, is irreversibly decreased in cells from TRPM2⁺/⁻ but not TRPM2⁻/⁻ mice. Importantly, IR leads to compromise of mitochondrial function, caspase activation, and caspase-dependent cleavage of critical proteins involved in SOCE. Together, our studies indicate a novel link between TRPM2 and mitochondria that can account for the loss of salivary fluid secretion. No COI.
Ubiquitylation is a multifaceted post-translational modification that ensures protein levels are subject to tight spatio-temporal control and is a key mechanism of cellular proteostasis. It is a hierarchical process that involves a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase (E3). There are over 600 known E3 ligases that are ultimately responsible for substrate specificity. The topology of the ubiquitin chains also determines outcome. Typically, chains linked via Lys48 on ubiquitin are directed to the proteasome, Lys63 chains modulate protein function while monoubiquitylation regulates endocytosis. Analogous to the regulation of protein phosphorylation by the opposing activities of kinases and phosphatases, ubiquitylation by E3 kinases is also balanced by deubiquitylating enzymes. In the brain, complex ubiquitin-dependent proteostatic networks regulate not only the normal function of the brain by tightly controlling protein levels but also to prevent the accumulation of toxic aggregates in various cellular compartments. The sheer complexity of the ubiquitin pathways has necessitated the development of new proteomic methods to allow an unprecedented view into the ubiquitin-modified proteome (ubiquitome). Mass spectrometry methods using various affinity matrices have started to look at the brain ubiquitome with upwards of 1000 ubiquitylated proteins reported. We have recently developed methods for ubiquitome analysis that use less than 500 ug of tissue. We have been able to detect over 1000 ubiquitylated proteins in embryonic mouse brain, expanding the known brain ubiquitome by ~15%. We have also performed studies in Nedd4−/− mice and have identified ~150 proteins with altered ubiquitylation profiles in the brains of Nedd4−/− embryos compared to wild-type. This presentation will highlight our new data from brain and...
The KLHL proteins, the WNKs, and renal Na⁺ transport
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Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by salt-sensitive hypertension, hyperkalemia and thiazide sensitivity. Mutations in with-no-lysine kinase 1 (WNK1) and WNK4 genes were reported to cause PHAII in 2001. Since then, we have identified and characterized novel WNK kinase signaling regulating renal salt handling and vascular tonus. WNK kinases constitute a signaling cascade with oxidative stress-responsive gene 1 (OSR1), Ste20-related proline-alanine-rich kinase (SPAK) and the solute carrier family 12a (SLC12a) transporter, including thiazide-sensitive NaCl cotransporter (NCC). However, although it was clear that the abnormal activation of this signaling cascade is the molecular basis of PHAII, the pathogenic effect of WNK4 mutations in PHAII and the regulator mechanisms of WNK signaling by various hormonal and dietary factors were poorly understood. Recently, two additional genes, Kelch-like 3 (KLHL3) and Cullin 3 (CUL3), were identified as responsible for PHAII. We and others found that KLHL3 forms an E3-ligase with Cullin3 which ubiquitinates WNK kinases. In PHAII, the disease-causing mutations of WNK4 and KLHL3 affect their interaction, thereby decreasing ubiquitination of WNK4 and leading to the increased levels of WNK4, which strongly activates NCC. We also discuss the involvement of this novel mechanism of WNK regulation by ubiquitination in various pathophysiological situations other than PHAII. No COI.

IRBIT-mediated synergism in epithelial transport
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Epithelial fluid and HCO₃⁻ secretion is mediated by basolateral HCO₃⁻ entry mediated by the 2Na⁺-1HCO₃⁻ cotransporter NBCe1-B, and luminal HCO₃⁻ exit mediated by the concerted activities of the Cl⁻ channel CFTR and the 1Cl⁻/2HCO₃⁻ exchanger Slc26a6. Hence, a key step in fluid and HCO₃⁻ secretion is luminal Cl⁻ efflux that fuels the Cl⁻/HCO₃⁻ exchange. The two prominent luminal Cl⁻ channels are CFTR and Ca²⁺-activated Cl⁻ channel (CaCC). In secretory epithelia the Ca²⁺ and cAMP signaling synergize to regulate their major function of protein and fluid and electrolyte secretion. In fact, hormonal synergism has been known for more than 80 years, yet we do not know much about the molecular mechanism of synergism. This began to change with the finding of the role of IRBIT (IP3 receptors binding protein released with IP3) in secretory ducts fluid and HCO₃⁻ secretion. In this presentation, the molecular mechanism of synergism in epithelial transport will be discussed in the context of regulatory processes of Cl⁻ channel and transporters located in apical membrane of ducts by IRBIT, focused on the IP3 receptor phosphorylation and microdomains. No COI.
Human pancreas secretes pancreatic juice which contains as much as 140 mM bicarbonate (HCO$_3^-$). Recently, we have shown that [Cl$^-$]-sensitive activation of WNK1-OSR1/SPAK pathway plays a critical role in pancreatic HCO$_3^-$ secretion by increasing the bicarbonate permeability (PHCO$_3^-$/PCl) of CFTR1). However, how [Cl$^-$]-sensitive kinases modulate PHCO$_3^-$/PCl of CFTR remains elusive. In the present study, we investigated molecular mechanisms that underlie the WNK1-OSR1/SPAK-induced regulation of CFTR anion selectivity. Overexpression and knockdown of each kinase in HEK 293 and epithelial cells revealed that WNK1 is the key molecule that governs overall effect of [Cl$^-$]-sensitive kinases on the CFTR bicarbonate permeability. Furthermore, experiments with truncated WNK1 indicated that N-terminal parts of WNK1 are required to regulate PHCO$_3^-$/PCl of CFTR. Interestingly, WNK1 affects permeability of other anions as well as bicarbonate in patch clamp recordings. Especially, the interval of relative permeabilities (Px/PCl) between each anion was greatly narrowed by WNK1. These findings suggest that WNK1 increases the bicarbonate permeability of CFTR by modulating the polarizability of anion selectivity filter and provide insight into the fundamental question of how ion selectivity of anion channels can be regulated by cytosolic signaling at the molecular level. No COI.
How are zinc-requiring enzymes activated by zinc transporters in the early secretory pathway?

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Zinc homeostasis in cells is tightly controlled within narrow boundaries through the highly integrated processes of zinc uptake, sequestration and efflux across the cell membrane. Thus, zinc transport proteins are essential to these processes. In general, two SLC transporters, Zn transporter (ZnT) and Zrt, Irt-like protein (ZIP), primarily regulate zinc transport, which enable a variety of zinc-dependent proteins and enzymes to play pivotal roles in numerous and varied biological responses. Of these enzymes, zinc-dependent ectoenzymes receive much attention, because defects of their activities are involved in diseases pathogenesis. Zinc-dependent ectoenzymes are thought to be activated via coordination of zinc during the secretory process, and thus zinc transporters localized to the secretory pathway are indispensable for their activation. However, very little molecular information is available. We have shown that ZnT5-ZnT6 heterodimers and ZnT7 homodimers supply zinc into the early secretory pathway, and activate tissue non-specific alkaline phosphatase (TNA P) in an elaborate two-step mechanism via enzyme protein stabilization followed by enzyme conversion from the apo- to the holo-form. Moreover, we have shown that cooperative functions of ZnT1, ZnT4 and metallothionein (MT) contribute to the activation process of TNAP by regulating cytosolic zinc transfer to ZnT5-ZnT6 and ZnT7 complexes. Here, I will discuss the possibility that cytosolic or luminal zinc chaperone proteins may operate in this enzyme activation process, which will provide insights into the molecular mechanism of the activation of many zinc-requiring enzymes in the cells. No COI.

Possible role for zinc transporter 3 (ZnT3) in brain developmental disorders

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Zinc deficiency may cause abnormal brain development (Sanstead et al., J Nutr, 2000). However, it is unknown whether zinc transporter 3, a protein necessary for the development of synaptic zinc, has a role in brain development. In the present study, we examined whether ZnT3 null mice exhibit abnormalities in the brain development, especially those associated with autism. We examined social behavior, brain size, cytoarchitecture, matrix metalloproteinases (MMP) activities, brain-derived neurotrophic factor (BDNF) expression, and possible relationships among these in Zinc transporter 3 (ZnT3) null mice. At 4-5 weeks of age, compared with wild-type (WT) control mice, male ZnT3 null mice, but not females ones, exhibited autistic behaviors in 3-chamber sociability and social novelty tests as well as marble burying and open field tests. At 5 weeks of age, the size of the frontoparietal cortex and neurite density of ZnT3 null mice, were significantly greater than that of WT mice. Consistent with enhanced neurotrophic stimuli in ZnT3 null mice, the level of BDNF and TrkB in neurons and astrocytes was increased in male ZnT3 null mouse brains. Moreover, activities of matrix MMP 2 and MMP 9 were also increased. Consistent with the role for MMP in BDNF upregulation, megalencephaly, and, autism phenotype, treatment with minocycline, an MMP inhibitor, for 2.5 weeks, significantly attenuated all the above changes seen in ZnT3 null mice. Contrary to our expectation that the ZnT3 null state would reduce free zinc levels, it paradoxically increased free zinc levels in brain cells, which might cause increases in MMP activities and BDNF levels, and induce megalencephaly in male mice. Hence, neuronal zinc dyshomeostasis, rather than synaptic zinc deficiency, during the critical period of brain development, may be an underlying mechanism for the present phenomenon in zinc transporter 3 null mice. No COI.
Properties and function of the Endolysosomal Two pore channels
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In addition to the ER, acidic organelles such as lysosomes and endosomes store and release Ca²⁺. Endolysosomal Ca²⁺ homeostasis has several cellular functions; among them are apoptosis, trafficking, energy metabolism and fusion/fission events. The messenger NAADP appears particularly important in mobilizing acidic Ca²⁺ stores and in many cases NAADP-evoked signals are amplified by Ca²⁺ channels on the ER. The molecular basis for triggering of Ca²⁺ release from acidic organelles by NAADP however is unclear. Two endolysosomal channels have been implicated in NAADP-mediated Ca²⁺ release, the two pore channels (TPCs) TPC1 and TPC2. However, properties and function of the TPCs in NAADP-mediated Ca²⁺ release has been controversial and are not fully understood. This presentation will discuss properties of the TPCs channels and their role in the response to NAADP. Both TPC1 and TPC2 are similarly activated by the organellar-enriched lipid PI(3,5)P₂. Endolysosomal proteins that interact with PI(3,5)P₂ or affect its availability affect channel activity. Both channels are also activated by NAADP. In the case of TPC1 activation by NAADP and PI(3,5)P₂ appears to be complementary. Interestingly, although activation of the channels by NAADP does not show any desensitization as was concluded from indirect studies, the concentration dependence for activation of TPC1, but not of TPC2, by NAADP followed a bell-shaped curve that is described well by interaction of NAADP with high affinity activatory site and low affinity inhibitory site. TPC2, but not TPC1, acts as a Mg²⁺ sensor and its activity is modulated by changes in intracellular Mg²⁺ at the physiological range. These findings provide basic information of the properties of TPC1 and TPC2 that should facilitate understanding of their physiological role. No COI.

Alterations of lysosomal homeostasis in TRPML1 knock-out mice
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In addition to Ca²⁺ stored in the ER, Ca²⁺ storage and release from acidic intracellular organelles affect the overall Ca²⁺ signal and membrane trafficking. TRPML1 is expressed in late endosomes and lysosomes. Mutations in TRPML1 cause mucolipidosis type IV (MLIV) that is characterized by a psychomotor retardation, corneal opacity, retinal degeneration, and achlorhydia. In vitro studies of constitutive membrane trafficking concluded that TRPML1 plays a role in delivery or fusion of late endosomes and lysosomes, resulting in accumulation of material in the lysosomes and induction of autophagy. However, the role of TRPML1 in regulated exocytosis is not known. In the present study, we found enhanced amylase secretion in response to high agonist stimulation, even though there are no apparent effect on saliva fluid secretion. Interestingly, TEM images both of pancreas and parotid gland show enlarged vesicles. Some of the enlarged vesicle shows fusion of the lysosome and vesicles. Moreover, acid phosphatase activity was increased in the whole saliva and pancreatic fluids, and also Trpml1⁻/⁻ neurons maintain high basal and stimulated exocytosis of the neurotransmitter and neurotoxin glutamate. These features are unique to MLIV and were not observed in another lysosomal storage disease, Niemann-Pick type C1. From these results, we suggest that Trpml1⁻/⁻ deletion may relate with exocytosis of the secreting vesicles or related with fusion of the vesicles with the lysosome. This would be useful to show new function of Trpml1⁻/⁻ in secreting cells. No COI.
Vesicular CLC chloride-proton exchangers: Roles in physiology and disease

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The CLC family of anion transporters comprises both plasma membrane Cl⁻ channels and vesicular 2Cl⁻/H⁺-exchangers that are differentially expressed along the endosomal-lysosomal pathway. Their physiological and medical importance became apparent from human genetic disease and mouse models. CIC-5 is expressed on endosomes, mainly in epithelia. Its mutation in human Dent’s disease leads to proteinuria and kidney stones. Our KO mouse model revealed that CIC-5 is important for proximal tubular endocytosis. It disruption leads to hypercalciuria and kidney stones because of defective endocytosis and processing of calcitropic hormones. CIC-7, together with its beta-subunit Ostm1, resides on lysosomes. Disruption of either subunit leads to lysosomal storage, neurodegeneration and osteopetrosis in mice and men. CIC-4 mutations lead to human mental retardation, while disruption of CIC-3 and CIC-6 in mice entail neurodegeneration. The role of these transporters in endosomal/lysosomal function was previously attributed exclusively to impaired vesicular acidification, as these transporters may provide a shunt for the vesicular proton ATPase. While a role of CIC-5 in endosomal acidification has been ascertained, the lysosomal pH of CIC-7 KO mouse is, however, unchanged owing to a parallel cation conductance. We were puzzled by the fact that the vesicular CLCs are 2Cl⁻/H⁺-exchangers rather than Cl⁻ channels. Both are suited, in principle, as shunts for proton pumping. We asked whether chloride/proton exchange is essential for their function and converted CIC-3, CIC-5 and CIC-7 into pure chloride conductances in KI mice. This is possible by single point mutations. Surprisingly, these mice revealed that these mice have almost identical phenotypes as the respective KO mice, suggesting an important role for H⁺-exchange dependent vesicular Cl⁻ accumulation or changes in vesicular voltage. Another CIC-7 mouse model, in which we disrupted its ion transport totally without affecting the expression of the protein, furthermore indicated that the loss of protein-protein interactions explains some aspects of the CIC-7 KO mouse. No COI.
Vitamin D supplementation and sunlight exposure for the prevention of osteoporosis

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Dermal synthesis of vitamin D after sun exposure is a major source of vitamin D in humans. Recent studies have documented high prevalence of vitamin D deficiency even in tropical countries with abundant sunshine. It is likely that lifestyle and social factors may preclude adequate outdoor sun exposure and bringing about inadequate vitamin D status, especially in urban residents. In addition, air pollution may have a contributory role. Tropospheric ozone is a common urban air pollutant and an efficient absorber of UVB. The phenomenon is likely to be more marked in big cities, and may partially explain the high prevalence of vitamin D deficiency in these areas. Because routine measurement of 25-hydroxyvitamin D [25(OH)D] is not recommended, suggestion on vitamin D intake is provided for achieving desirable 25(OH)D levels (≥ 20 ng/mL in general) in over 97.5% of the population. Available recommendations vary from 200 IU/day to 2,000 IU/day. In the elderly with osteoporosis, higher 25(OH)D levels are warranted to improve muscle function and reduce fracture risk. Adequate vitamin D status in this particular population can achieved by at least 800 IU/day of vitamin D intake. Recommending increased intake of naturally occurring vitamin D-rich food is the ineffective strategy to overcome vitamin D deficiency since there are few food sources that are rich in vitamin D. Frequently, dermal synthesis of vitamin D is unpredictable. Race, calcium intake, renal function, body mass index and polymorphisms in key protein/enzymes involved in the vitamin D metabolism and action can all influence the variability in the increment of 25(OH)D levels after vitamin D supplementation. No COI.

Periodontal stem cells: molecular aspect and application

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Periodontal ligament (PDL) cells and tissue, located between tooth and alveolar bone, plays roles in tissue connection, distribute and withstand the masticatory force and help maintaining the homeostasis of the periodontal tissue. PDL cells possess the mesenchymal stem cell characteristics. They express several mesenchymal stem cell markers such as CD44, CD73, CD90 and CD105 as well as the embryonic stem cell markers including Rex-1, Oct4 and nanog. Under appropriate condition, PDL cells can differentiate into osteogenic, adipogenic and neurogenic lineages, suggesting the role of PDL cells in periodontal tissue repair and regeneration. Our previous results demonstrated the involvement of Notch signalling pathway in the modulation of differentiation fate of PDL cells in vitro. Moreover, we also found that PDL cells could also be induced to differentiate into insulin secreting cells under the influence of Notch signalling pathway. This ability of PDL cells suggests the high plasticity of PDL cells that might be suitable for stem cell therapy. Beside the role in repair and regeneration, PDL cells could also modulate the immune system through secret interferon gamma and IDO when activating with inflammatory cytokines such as IL-12 or IL-6, indicating the immunosuppressive property of PDL cells. Taken together, PDL cells are one of the potential sources of stem cell for stem cell therapy and tissue engineering. These works were supported by the Research Chair Grant, National Science and Technology Development Agency, Thailand. No COI.
Symposium 6  Cutting-edge research in bone and calcium metabolism in Thailand

S 6
Advance in pathophysiology of diabetes mellitus-induced osteoporosis
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How diabetes mellitus (DM) affects bone metabolism remains controversial for decades. It was previously believed that type 1 DM led to osteopenia, whereas type 2 DM was associated with increased bone density, suggesting that it might be protective against fragility fracture. However, several recent lines of evidence in both humans and rodents did not support the positive effect of type 2 DM on bone. Both type 1 and 2 DM indeed induce aberrant bone cell function (cellular complication) and abnormal extracellular matrix properties (matrix complication), thereby leading to impaired osteoblast-mediated bone formation, accelerated osteoclast-mediated bone resorption, and poor bone quality. Cellular complications are often caused by insulin resistance and hyperglycemia, while matrix complications, including abnormal structure and alignment of collagen, predominantly result from advanced glycation end products (AGEs). The DM-associated inflammation and pro-inflammatory cytokine release as well as impaired intestinal calcium absorption may also aggravate osteoporosis in diabetic patients, but their underlying mechanisms remain unknown. No COI.

Symposium 7  Muscle protein functions

S 7
Myofilament Length Dependent Activation and the Frank-Starling Law of the Heart
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The cellular basis of the Frank-Starling mechanism is sarcomere length (SL) modulation of myofilament Ca2+ sensitivity (LDA). The mechanisms that underlie LDA are unknown, but recent evidence has implicated the giant protein titin as a possible sarcomeric strain sensor. The aim our study was to elucidate the impact of SL on LDA and sarcomere structure in isolated rat myocardium from either wild-type or mutant rats expressing a giant splice isoform of titin. At stretch, wild-type muscles showed reduced increase in passive tension and twitch force, and blunted LDA. Time-resolved small angle x-ray diffraction of intact twitching muscles during diastole revealed at stretch a significant increase in intensity and spacing of myosin M2, troponin T3, and myosin binding protein C C2. These SL dependent changes in sarcomere structure were absent in titin mutant muscles. Cross-bridge radial spacing was significantly reduced upon stretch in wild-type, but not mutant muscles. Equatorial spacings and intensity ratios were similar in both groups of muscles. Electron density reconstruction revealed, only in wild-type, increased mass in both thick and thin filament, and the appearance of an as of yet unidentified moiety spanning the space between the thick and thin filaments at stretch. These results were independently confirmed in skinned myocyte fragments using a fluorescent probe. We conclude that stretch induces structural changes in both thick and thin filaments mediated by titin strain. Moreover, MyoBPC may interact with actin to mediate LDA.
Symposium 7  Muscle protein functions

Disrupt the muscle clock and alter metabolism and fiber type
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Skeletal muscle is a major contributor to whole-body metabolism as it serves as a depot for both glucose and amino acids, and is a highly metabolically active tissue. Within skeletal muscle exists an intrinsic molecular clock mechanism that regulates the timing of physiological processes. A key function of the clock is to regulate the timing of metabolic processes to anticipate time of day changes in environmental conditions. We developed an inducible skeletal muscle-specific Bmal1 knockout mouse model (iMS-Bmal1−/−) to test loss of clock function only in skeletal muscle. The results of this study show that skeletal muscle circadian transcriptome was highly enriched for metabolic processes and phase analysis revealed a temporal separation of genes involved in substrate utilization and storage over a 24-h period. Muscle specific loss of Bmal1 resulted in a significant change in many metabolic genes. This was associated with decreased glucose uptake, decreased expression of rate limiting enzymes in glycolysis and subsequent loss of body fat. In addition we also observed a gene signature indicative of a fast to slow fiber-type shift and a more oxidative skeletal muscle in the iMS-Bmal1−/− model. These data provide evidence that the intrinsic molecular clock in skeletal muscle temporally regulates genes involved in the utilization and storage of substrates independent of feeding and cage activity. Disruption of this mechanism caused by phase shifts (that is, jetlag) or night eating may ultimately diminish skeletal muscle’s ability to efficiently maintain metabolic homeostasis over a 24-h period. No COI.

Cardiac contractile activation: sex matters & exercise helps
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Heart failure is the common outcome of many cardiovascular diseases. Despite the many known risk factors of the disease, the underlying mechanisms are incompletely understood and the therapeutic strategies are still on trials and comparisons. A better survival from advanced heart failure in women than in men suggests a sex-specific adaptation of the heart to stress. The rationale of our studies has been based on the well recognizable sex differences in the incidence of cardiovascular diseases which suggest that female sex hormones may exert a protective adaptation but male sex hormones may add a risky effect. We aim to understand the nature, sites, and mechanisms of how sex hormones regulate cardiac muscle physiology especially the cardiac adaptations induced after hormone deprivation or exogenous loading. Our results show that sex matters on cardiac contractile activation through differential responses of both the activity of myofilaments and the handling of intracellular calcium. Significantly, in sex hormone-deprived rat models after gonadectomy regular exercise is able to serve as an alternative to hormone replacement therapy in preventing cardiac contractile dysfunction. No COI.
Symposium 8  **New approaches to the screening of cardiovascular function**

**S 8**  

**Significance of central aortic pressure and arterial stiffness in the assessment of arterial function**  
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In the circulatory system, large arteries function as conduits for the distribution of blood and cushions to dampen the pulsatile effects of ventricular ejection. Change in dimensions and mechanical properties along the length of the aortic trunk and peripheral branches affect the propagation of the pressure pulse generated in the ascending aorta, such that the pressure pulse increases in amplitude between the central aorta and peripheral sites. Mathematical representation of the transmission characteristics has enabled central aortic pressure to be derived non-invasively from conventional cuff sphygmomanometric measurements and the peripheral pulse. These techniques have shown that central aortic pressure has the potential to discriminate the effects of pharmacological agents that change blood pressure and heart rate on cardiac function. Wave reflection indices determined from the central aortic pressure wave have also been shown to have predictive power for cardiovascular risk. Arterial stiffness is a fundamental parameter that characterizes wave propagation phenomena. Arterial stiffness, as measured non-invasively by aortic pulse wave velocity is a significant predictor of cardiovascular risk and is a major determinant of increased systolic pressure in the elderly. Age-related changes in arterial stiffness occurring predominantly in the central aorta and large arteries have the effect of reducing the arterial elastic non-uniformity between central and peripheral arteries such that the difference between central and peripheral pulse pressure becomes less with age. Non-invasive measurements of central aortic pressure and arterial stiffness have the potential for improved characterization of arterial function and better stratification of cardiovascular risk. No COI.

**S 8**  

**Optical sensors for the assessment of vascular biomechanics**  
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In order to further understand the contribution of venous and arterial effects blood volume to the photoplethysmographic (PPG) signal, recordings were made from twenty healthy volunteer subjects during an exercise in which the right hand was raised and lowered with reference to heart level. Red (R) and infrared (IR) PPG signals were obtained from the right index finger using a custom-made PPG processing system. Laser Doppler flowmetry (LDF) signals were also recorded from an adjacent fingertip. The signals were compared with simultaneous PPG signals obtained from the left index finger. On lowering the hand to 50 cm below heart level, both ac and dc PPG amplitudes from the finger decreased (e.g. 18.70% and 63.15% decrease in infrared dc and ac signals respectively). The decrease in dc amplitude most likely corresponded to increased venous volume, while the decrease in ac PPG amplitude was due to autoregulatory adjustments on the arterial side in response to venous distension. Conversely, ac and dc PPG amplitudes increased on raising the arm above heart level. Morphological changes in the ac PPG signal are thought to be due to vascular resistance changes, predominately venous, as the hand position is changed. No COI.
A novel method for the assessment of vascular endothelial function

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The gold standard for the non-invasive assessment of endothelial function remains the ultrasonic detection of flow mediated vasodilation (USFMD) but technical difficulties confine this approach to specialist centres, so there is need of a simple and robust method suitable for patient screening. Naka et al. (European Heart J., 2006. 27,302-309.) have shown that the increase in radial artery pulse wave velocity (PWV) following release of an occluding cuff is due to relaxation of vascular smooth muscle and have validated this against USFMD. However, current PWV measurements are technically demanding and prone to movement artefacts. To minimise these problems we have designed a pulse transit-time measurement system using clip-on photoplethysmographic finger probes which detect the pulse arrival time difference (ΔPAT) at the ring finger of each hand. As a preliminary validation we have compared brachial-radial PWV changes during hyperaemic flow with simultaneously acquired ΔPAT values in 48 healthy volunteers and found a significant correlation between the two methods in the maximum hyperaemic response and the time taken to return to baseline. Secondly, we have compared ΔPAT with USFMD in 28 subjects with reasonable agreement in maximum hyperaemic response, although much scatter in the USFMD results. We are currently setting up a more rigorous validation at a centre specialising in USFMD measurements in which subjects will be infused with L-NMMA (a NO synthase blocker) and its inhibitory effect will be measured, assessing if the changes in ΔPAT are endothelially dependent. Ideally measurements of PWV and endothelial function should be non-contact and, to this end preliminary measurements of pulse transit time in the arm using a fast infra-red camera will also be described. No COI.
Immunity and sympathetic nervous system in hypertension
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The central nervous system determines sympathetic outflow and influenced by various peripheral inputs that are neural, humoral, and immune-inflammatory changes. Recently, we found that inflammatory changes reflected by nuclear factor kappa B in the brain are increased in heart failure associated with sympathoexcitation. We found that toll-like receptor 4 (TLR4) and its adaptor protein, myeloid differentiation primary response protein 88 (MyD88), expression levels are increased in heart failure. Central administration of an AT1R blocker attenuated them. These findings suggest that TLR4/MyD88 pathway as innate immunity is activated in heart failure thereby causing inflammatory responses leading to sympathoexcitation. Activation of TLR4 in the brain in addition to vasculatures has been shown in spontaneously hypertensive rats as well as angiotensin II-induced hypertensive rats from other laboratories. Interestingly, activation of this pathway is involved in cardiac hypertrophy process. Finally, we demonstrated that a decrease in regulatory T cells (CD4+CD8+Foxp3+ cells) proportion is crucial for the development of hypertension and cardiac hypertrophy in genetic hypertensive rats, which is involved in sympathetic neural input to the spleen. Taken together, innate and acquired immunity is closely communicated with the sympathetic nervous system via inflammatory response and play an important role in the pathogenesis of hypertension. No COI.

Visualizing baroreflex dysfunction by MRI/DTI in animal models
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The baroreflex represents the most fundamental mechanism of cardiovascular regulation, and is responsible for maintaining stable blood pressure and heart rate. Impairment of baroreflex inevitably results in a hypertensive or hypotensive state, and death in extreme case. Based on tractographic evaluations using magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) of the medulla oblongata, our group has successfully visualized baroreflex at work under normal and disease conditions. We found that the connectivity between the nucleus tractus solitarii (NTS) and nucleus ambiguus (NA) is disrupted in an experimental model of neurogenic hypertension, concurrent with impairment of the cardiac vagal baroreflex as detected by radiotelemetry. We further found that the disrupted NTS-NA connectivity is reversible, and is related to oxidative stress induced by augmented levels of NADPH oxidase-generated superoxide in the NTS. In an experimental model of hepatic encephalopathy, we found that the progressive hypotension and loss of baroreflex-mediated sympathetic vasomotor tone indicative of brain death was accompanied by an irreversible loss of the connectivity between NTS and rostral ventrolateral medulla. Intriguingly, the loss of connectivity between NTS and NA that accompanied defunct cardiac vagal baroreflex and bradycardia only occurred before cardiac death. We conclude that tractographic analysis is a new research tool for functional examination of changes associated with dynamic alterations in the connectivity between key neural substrates in the baroreflex circuitry. Our results further suggested that whether the disrupted connectivity is reversible determines whether such changes are pathophysiological or pathological and hence treatable or untreatable. No COI.
Blood pressure variability in diabetes: from the pig to humans

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Dysfunction of the autonomic nervous system is a common complication in diabetes mellitus. Cardiovascular autonomic neuropathy (CAN) may carry an increased risk of mortality. A model of experimental diabetes could be useful to evaluate. Yucatan miniature pigs were equipped with an arterial catheter for telemetric blood pressure (BP) analysis, and with a venous access. BP and heart rate (HR) oscillated at the respiratory range. Spectral analysis showed this respiratory component was the main determinant of the short-term variability of BP and HR. Atropine increased HR and BP and abolished the respiratory sinus arrhythmia. Propranolol diminished HR and the respiratory peak of HR. Baroreceptor-HR reflex was estimated using injections of phenylephrine and nitroprusside, and by cross-spectral analysis between BP and HR. The data in diabetic pigs demonstrate the dual (vagal and sympathetic) control of HR and the dominant role of respiration in the genesis of oscillations. The spectral and cross-spectral analysis of BP and HR were altered after 3 months of diabetes. The aim of the second study was to estimate the accuracy of a battery of BP and HR variability indexes obtained in different subgroups of diabetic subjects classified according to the conventional laboratory autonomic function tests (Ewing scores). BP was measured continuously at the finger level using a Finapres monitor in the supine position and during standing. The estimates of baroreceptor-HR function provided a powerful tool for assessing CAN at any stage of CAN including the early stage which was not detected by the conventional tests. No COI.
Symposium 10  **Hemodynamic and microvascular changes associated with toxins and endotoxins**

**S 10**

**Physiology of animal toxins : effects on renal ion transport**

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Animal toxins compose of enzymes, polypeptides, proteins are chemicals. Injury induced by animal toxins in majority is through hemodynamic changes leading to renal ischemia and partly through direct toxicity. Hemodynamic changes triggered by toxin and vascular inflammatory mediators are integral to injury and do not differ from those observed in sepsis and are characterized by decreased blood pressure, increased renal and systemic vascular resistance with decreased glomerular filtration rate and renal blood flow. By isolated renal perfusion which reflect direct toxin effects on vascular ion channels renal vascular resistances is either increased or decreased. Fractional excretion of Na is increased. The role of vascular ion channels including DEG/ASIC/ENac, Ca and K channels are considered. Renal tubular ion channels and transporters targeted by animal toxins are reviewed, focusing on Na and K transport. In most cases due to blockage of single channel clinical manifestation related to renal ion channel block may not be apparent. The effects are of only physiologic interest. At the clinical level, scorpion and bee envenoming with blockage of multiple channels or transporters are good models. No COI.

**S 10**

**On the role and mechanism of action by which Russell's viper venom induces acute renal failure**

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Russell's viper (Daboia russellii siamensis) envenoming is well known as a cause of an acute kidney injury. The pathophysiological mechanism for the acute effect of Russell's viper venom (RVV) on renal function has not been fully elucidated. The studies in vivo in experimental animals for the mechanisms of venom action on renal functions have been clarified in relation to changes in either extrarenal factors or/and intrarenal factors. Experimental animals injected with crude RVV and a major class of venom fractions especially phospholipase A2 (PLA2) and metalloprotease (MP) show changes in the cardiovascular system and renal hemodynamics. The initial drops in blood pressure and bradycardia are consistent with a tendency to increased total peripheral resistance and renal vascular resistance and restoration of blood pressure following these transient decreases are compatible with vasoconstrictor hormones stimulation as a compensatory mechanism. An increase in renal vascular resistance and decreases in renal blood flow and glomerular filtration rate are still apparent after envenomation. An in vivo study is complicated by the presence of proinflammatory cytokines and vasoactive mediators from the host during envenomation. *In vitro* studies can be evaluated the direct effect of venom action on changes in renal physiology without the influence of extra renal factors. A number of studies for the direct action of RVV have been performed e.g. in the isolated perfused kidney, changes in the characteristic polarization of the renal tubular cell membrane, changes in mitochondria activity and changes in Na,K-ATPase activity of the renal tissues after envenomation. Accumulating evidence demonstrates that a major class of enzymatic activities of venom fractions for PLA2 and MP perform main functions as a cause of an acute kidney injury, but their precise roles and mechanisms of action on renal hemodynamic and renal tissues have to further elucidate. The true effects of, PLA2 and MP should be evaluated by isolated renal perfusion study which will allow insight into how RVV are involved in the pathophysiology of acute renal failure. The recent studies in isolated perfused kidney will be described on how crude venom and its fractions act directly on the kidney function without extra-renal factors. The possible ion channel inhibitors/blockers are used to block some of the renal effects caused by the RVV venom and its fractions in isolated perfused kidney. No COI.
Microvascular changes associated to LPS and possible treatments
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Among various indications of intensive care unit hospitalization, sepsis is highlighted by its high incidence, morbidity, mortality, and cost to healthcare system. In sepsis syndrome, inflammatory response is associated with a state of oxidative stress that results in impairment of microcirculatory function. Several factors are related to this impairment, such as systemic hypotension, vasoconstriction, stiffness of red blood cells, increased leukocyte-endothelium interactions (adhesion and aggregation), and platelet/fibrin clot formation (leading to microthrombosis). Ischemia, impaired tissue perfusion, organ dysfunction, and death may occur depending on the severity and duration of microcirculatory dysfunction. Accordingly, drugs that assist in the reversal of microcirculatory changes could be decisive in sepsis treatment. Due to limitations involving the study of these drugs in human subjects, the development and evaluation of new treatments requires the use of reliable experimental models. Although no model is capable of reproducing in its entirety the complexity of sepsis in humans, LPS-induced endotoxemia is a well-established experimental model that reproduces many of the clinical features of sepsis syndrome and allows homogeneous and reproducible *in vivo* studies of the microvascular function. In fact, using this model, we have already tested several drugs with microvascular action, such as inotropics, sedatives, and anticoagulants, achieving promising results. Thus, the aim of my lecture is to present the microvascular changes associated with the LPS-induced endotoxemia model and our experience with possible treatments. No COI.
Flow-mediated the activation of integrin α5 requires its translocation to membrane lipid rafts in vascular endothelial cells

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Focal distribution of atherosclerotic lesions reflects a significant role of local hemodynamic forces in atherogenesis. Sensing mechanical forces, membrane lipid rafts are crucial for maintaining endothelial function. To investigate whether lipid rafts mediate the effects of different shear stress on endothelial cells (ECs), we compared translocation of proteins in lipid rafts under laminar (LSS) and oscillatory shear stress (OSS), and further investigated the contribution of these proteins in the development of atherosclerosis. After isolation of lipid rafts with sucrose density gradient centrifugation, quantitative proteomics and bioinformatics analysis revealed that more than 100 proteins redistributed in lipid rafts under different flows, among which integrin α5 was significantly elevated in the lipid rafts of ECs exposed to atheroprone OSS (4+/−0.5 dyne/cm² for 2h) than atheroprotective LSS (12+/−0.5 dyne/cm² for 2h). Moreover, western blot showed that OSS increased integrin α5 activity. Both knockdown caveolin-1 and disruption of cytoskeleton prevented disturbed flow-induced integrin α5 translocation, suggesting that integrin α5 translocated depending on caveolin-1 and cytoskeleton. Further, OSS activated integrin α5 could induce NLRP3 inflammasome in ECs, which, in turn, caused endothelial activation. In vivo, integrin α5 activation, ICAM-1 and VCAM-1 expression were observed in the atheroprone aortic areas and in partially ligated carotid arteries of LDLR−/− mice. As well, interference by shRNA adenovirus of integrin α5 greatly retarded the EC activation at OSS zones of partially LDLR−/− mice. In conclusion, proteins translocation in lipid rafts causally contributes to distinct features of ECs under different flows. Atheroprone flow induces integrin α5 activation through lipid rafts anchoring and activates NLRP3 inflammasome in ECs, which revealed a novel mechanism for endothelial activation. No COI.

Mechanical regulation of histone deacetylases and microRNAs in vascular endothelial pathophysiology in response to disturbed flow

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In atherosclerotic lesions, synthetic smooth muscle cells (sSMCs) induce aberrant microRNA (miR) profiles in endothelial cells (ECs) under flow stagnation. Increase in shear stress induces favorable miR modulation to mitigate sSMC-induced inflammation. We addressed the role of miRs in sSMC-induced EC inflammation and its inhibition by shear stress. Coculturing ECs with sSMCs under static condition causes transient increases of 4 anti-inflammatory miRs (146a/708/451/98) in ECs. Shear stress (12 dynes/cm²) to cocultured ECs for 24 hours augments these 4 miR expressions. In vivo, these 4 miRs are highly expressed in neointimal ECs in injured arteries under physiological levels of flow, but not expressed under flow stagnation. Silencing either Nrf-2 or miR-146a led to increased neointima formation of injured rat carotid artery under physiological levels of flow. Overexpressing miR-146a inhibits neointima formation of rat or mouse carotid artery induced by injury or flow cessation. In addition, application of oscillatory shear stress (OSS, 0.5 ± 4 dynes/cm²) to cultured ECs sustainably up-regulated class I and II HDACs class I and II histone deacetylases (HDAC-1/2/3 and HDAC-5/7, respectively) and their nuclear accumulation, whereas pulsatile shear stress (PSS, 12 ± 4 dynes/cm²) induced phosphorylation-dependent nuclear export of class II HDACs. Intraperitoneal administration of the class I-specific HDAC inhibitor valproic acid into bromodeoxyuridine (BrdU)-infused rats inhibited the increased EC uptake of BrdU at poststenotic sites. Our findings demonstrate the important roles of different groups of HDACs and miRs in regulating the oxidative, inflammatory, and proliferative responses of ECs to different patterns of flow. No COI.
Shear Regulation of MicroRNA Transportation and Targeting in Vascular Homeostasis

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The local flow patterns acting on the arterial wall play a crucial role in determining whether the endothelium is protected from or predisposed to atherosclerosis. Recent research demonstrated that microRNAs (miRs) originated from vascular endothelial cells (ECs) mediate extracellular communication via transportation to the underlying smooth muscle cells (SMCs). In this study, miR profiling was performed both in ECs in response to oscillatory shear (OS, the main feature of atheroprone flow) vs. pulsatile shear (PS, the main features of atheroprotective flow) and in the perfusates. The expressions of endothelial-enriched miR-126 inside the cells were quite stable between OS and PS; however, the levels of miR-126 in the perfusates differ. Coculture of SMCs with ECs or treatment of SMCs with conditioned media from static ECs (EC-CM) increased SMC miR-126 level and SMC turnover; these effects were abolished by inhibition of endo-miR-126 and by the application of PS to ECs. RNA sequencing assays in sheared ECs indicated that the expressions of Soluble NSF Attachment Protein Receptor (SNARE)-associated proteins were differentially regulated by PS vs. OS. SMC miR-126 did not increase when cocultured with ECs subjected to inhibition of exocytosis/SNAREs. Endo-miR-126 represses its target mRNAs in the cocultured SMCs, indicating the signaling and functional roles of the transmitted endo-miRs. Systemic depletion of miR-126 in mice or pharmaceutical inhibition of endocytosis in the arterial smooth muscle inhibited neointimal lesion formation of carotid arteries induced by interference of blood flow. Our study suggests that atheroprotective and atheroprone flows modulate distinct miR transportation and targeting to result in beneficial or detrimental outcomes for the vasculature. No COI.
Hepatitis B virus (HBV) infection is a major public health problem, with more than 350 million carriers estimated worldwide. HBV, a member of the family Hepadnaviridae, is a relaxed circular double-stranded DNA virus that shows remarkable genetic variability. Chronic HBV infection is associated with a diverse clinical spectrum of liver disease ranging from asymptomatic carriers, chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Current data have suggested that host and viral factors may have important influences on the clinical outcome and treatment response. Universal vaccination against HBV leads to a marked decrease in the incidence of HCC. In addition, reduction of HBV-associated complications can be achieved by antiviral therapy, which includes nucleoside/nucleotide analogues (NA) and pegylated interferon-alpha (PEG-IFN). However, current therapies have some limitations and HBV cure is rarely achieved. NA therapy is generally well tolerated but prolonged or indefinite duration of treatment is needed. In contrast, PEG-IFN therapy is accompanied by a higher rate of sustained response but its use is compromised by frequent side-effects. Recent efforts have been focused on developing strategies to eliminate the virus and improving the cure rate for infected individuals.
Epigenetic mechanisms of visceral hypersensitivity in irritable bowel syndrome
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Irritable bowel syndrome (IBS), characterized by recurrent abdominal pain with altered bowel movement in the absence of an overt pathology, is one of the most common functional gastrointestinal disorders. The pathophysiology of visceral pain in IBS remains unknown. Hydrogen sulfide (H2S), a third gaseous modulator/mediator, has become recognized as an important endogenous molecular. H2S is synthesized from L-cysteine primarily via cystathionine-β-synthetase (CBS) and cystathionine-γ-lyase, and has been reported to be involved in nociceptive signaling and inflammation. However, the molecular and epigenetic mechanisms of CBS-H2S signaling in visceral nociceptive processing are not fully understood. Here we showed that neonatal colonic inflammation produced a significant upregulation of CBS expression in dorsal root ganglia (DRG). Intraperitoneal administration of CBS antagonist aminooxyacetic acid (AOAA) attenuated visceral hypersensitivity. In addition, application of AOAA reversed the hyperexcitability and decreased total sodium currents of DRG neurons innervating the colon, and reduced upregulation of voltage-gated sodium channel NaV1.7 and NaV1.8 in DRGs rats with visceral hypersensitivity. Methylation specific PCR and bisulfate sequence analysis demonstrated that promoter region of cbs gene was less methylated in DRG samples from rats with visceral hypersensitivity than that from controls. The expression of thymine DNA glycosylase was significantly upregulated in DRGs from IBS-like rats while the expression of DNA methyltransferases was not greatly altered in DRGs from IBS-like rats. Our results suggest that epigenetic regulation of CBS gene may contribute to the visceral hypersensitivity, thus identifying a potential therapeutic target for the treatment of chronic visceral pain in patients with IBS. No COI.

Pancreatic dopamine-evoked somatostatin release and blood glucose increase was cAMP-dependent and dopamine receptor 2 mediated
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Pancreas can produce dopamine which has been measured in the pancreatic juice. Dopamine receptor 2 (D2R) is able to regulate the level of blood glucose. But the mechanism is unclear. We have reported that D2Rs were constant expressed in the somatostatin secreting δ cells, but not in the insulin secreting β cells in rat and human islets. We hypothesized that dopamine may regulate insulin secretion through regulating somatostatin release from δ cells by binding with D2Rs. Immunofluorescence, HPLC/MS, radioimmunoassay, ELISA, pancreatic tissue incubation, and islets isolation etc. were employed. The results indicated that dopamine was abundantly existed in the pancreatic tissue, D2Rs and somatostatin receptor 2 (SSTR2) were respectively distributed in the δ and β cells in rat and human islets. In in-vivo study, activating D2Rs increased blood glucose, decreased insulin in serum and pancreatic tissue, and increased pancreatic somatostatin. In the isolated pancreatic tissue and islets, activating D2Rs inhibited insulin secretion, promoted somatostatin secretion and elevated intracellular cAMP level. The D2 agonist-induced inhibition of insulin secretion was blocked by SSTR2 antagonist. This study demonstrates that pancreatic dopamine is able to elicit a somatostatin-dependent inhibition of insulin secretion through activating D2Rs and intracellular cAMP pathway. Key Words: D2 receptors, somatostatin, insulin secretion No COI.
The inhibitory effect of oxytocin on mast cell degranulation mediated by Ca\(^{2+}\)-NOS-NO pathway

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To investigate the effects of OT on visceral hypersensitivity/pain and mast cells degranulation and the underlying mechanisms. The expression of oxytocin receptor (OTR) on mast cells was assessed with immunofluorescence assays and Western blot. The colon perfusion of TNBS was used to induce visceral hypersensitivity. The extent of visceral hypersensitivity was quantified by abdominal withdrawal reflex (AWR) scores of colorectal distension. Tissue sections were stained by toluidine blue for mast cell counting. The levels of histamine were measured by a commercial ELISA kit. The electrical properties of mast cells were tested by whole-cell patch clamp recording. The intracellular Ca\(^{2+}\) was determined by fluorescent microscopy. Nitric oxide (NO) was detected with the fluorescent nitric oxide probe DAF-FM diacetate. OTR was expressed in colonic mast cells in human and rat, as well as in human and mouse mast cell lines, HMC-1 and P815. OT decreased TNBS-induced visceral hypersensitivity, colonic mast cell degranulation and histamine release in rats and attenuated the C48/80-evoked histamine release and inward currents in HMC-1 and P815 cells. Pretreatment of OTR antagonist atosiban significantly reversed the inhibitory effect of OT on TNBS-induced visceral hypersensitivity in rats. Pretreatment of atosiban or L-NMMA significantly attenuated the inhibitory effect of OT on C48/80-evoked histamine release and inward currents. OT produced a concentration-dependent increase in intracellular Ca\(^{2+}\) in HMC-1 and P815 cells. OT increased the production of NO in HMC-1 cells. In conclusion, OT might exert the antinociceptive effect on colonic hypersensitivity through inhibition of mast cell degranulation via Ca\(^{2+}\)-NOS-NO pathway. No COI.
Essential role of nuclear receptors in metabolic and fluid regulation

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Nuclear receptors are ligand-activated transcriptional regulators of many physiological and pathophysiological processes. A large body of evidence demonstrates that nuclear receptors control a large variety of metabolic processes including lipid metabolism, adipogenesis, drug disposition, bile acid homeostasis, insulin sensitivity, blood pressure regulation as well as inflammation, fibrosis, cell differentiation, and tumor formation. Among dozens of nuclear receptors, peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs) and farnesoid X receptors (FXRs) have been identified and attracted enormous attention due to the key role these receptors play in metabolic regulation. Dysfunction of these nuclear receptors and genetic variants may contribute to the pathogenesis and progression of the metabolic syndrome, including insulin resistance, glucose intolerance or type 2 diabetes, obesity, dyslipidemia, hypertension, and albuminuria. This places PPARs, LXRs and FXRs into the frontline for novel therapeutic approaches for a broad range of metabolic disorders and diseases. For example, PPARγ agonists including antidiabetic thiazolidinediones (TZDs) and PPARδ clinically proved to be effective for improving insulin resistance and hyperlipidemia, respectively. In addition, it has been recently shown that some of these nuclear receptors are also essential in renal physiology and play important roles in the maintenance of fluid homeostasis. We will discuss the physiological and pathophysiological roles of PPARs, LXRs and FXRs with particular emphasis on the therapeutic potential of their ligands in the metabolic syndrome and disorders associated with water metabolism. No COI.

PPARγ Integrates energy metabolism, fluid metabolism, and cardiovascular function

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Peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear hormone receptor and PPARγ is a ligand-activated transcription factor promoting adipogenesis and energy storage, representing a novel target for antidiabetic therapy due to its role in sensitizing the action of insulin. Accumulating evidence shows that PPARγ is profoundly involved in water and sodium metabolism in the kidney. Activation of PPARγ in the kidney promotes Na+ reabsorption and fluid retention by upregulating expression of aquaporin 2 (AQP2) and epithelial sodium channels, two primary targets for vasopressin (AVP) regulation of collecting duct water permeability and sodium reabsorption. Systemic knockout of PPARγ in mice causes polyuria and urine concentrating defect with blunted response to AVP but normal AVP levels. Interestingly, protein expression of AQP2 and phosphorylated-AQP2 in the collecting ducts is unchanged in these mice, indicating a unique pathway independent of AVP by which PPARγ regulates water and sodium transport in the kidney. In addition, emerging evidence suggests that PPARγ in the vascular endothelial cells control adipose-specific capillary permeability. Paradoxically, activation of PPARγ lowers blood pressure likely via suppression of sympathetic activity. Taken together, PPARγ appears to play an important role in integrating energy metabolism, fluid metabolism, and cardiovascular function. No COI.
miRNAs in Nfat5 signaling, osmoregulation and urine concentration

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Nuclear factor of activated T cell-5 (also called tonicity response element binding protein or osmotic responsive element binding protein, Nfat5/TonEBP/OREBP) is a transcriptional factor playing important roles in renal osmoregulation as well as inflammatory responses. We have explored epigenetic mechanisms especially miRNAs in Nfat5 signaling, osmoregulation and urine concentration. Following a high NaCl hypertonicity (550 mOsmol/kg H2O) exposure for 2 h in mIMCD3 cells, 21 miRNAs were significantly up-regulated and 12 miRNAs were down-regulated by at least 2 folds, which included the significantly downregulated miR-200b-3p and miR-717 and significantly up-regulated miR-466(a/e)-3p and their close relatives. Transfection studies indicated miR-200b-3p and miR-717 were capable of targeting Nfat5 post-transcriptionally, thereby contributing significantly to hypertonicity-induced induction of Nfat5. Although in response to hypertonicity exposure miR-466(a/e)-3p were upregulated in vitro in cultured mIMCD3 cells, these miRNAs were found to be significantly down-regulated when mIMCD3 cells were cultured in the presence of arginine vasopressin or in vivo in renal tissues of water deprived mice. Sfmbt2-hosted miR-466(a/e)-3p were also found to be capable of silencing Nfat5 post-transcriptionally. In vivo, transgenic overexpression of miR-466a-3p was found to be associated with significant down-regulation of renal Nfat5 and other osmoregulation-related genes. On the other hand, sustained transgenic overexpression of miR-466a-3p was shown to cause significantly altered renal morphology, the development of polyuria and polydipsia, and disturbed ion homeostasis in mice. In conclusions, miRNAs such as miR-200b-3p, miR-717 and miR-466(a/e)-3p are important epigenetic regulators of Nfat5 signaling, osmoregulation and urine concentration. No COI.

High salt and chronic renal diseases

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High salt is a primary cause of hypertension. Immune cells especially T cells and monocytes/ macrophages have recently been identified to be an important sensor of high salt and may act via production of inflammatory and vasoactive mediators to disrupt the functional and structural homeostasis of kidney and vasculature, subseqeuently leading to hypertension and the progression of chronic kidney diseases (CKD). Glomerular diseases, characterized clinically by proteinuria and hypertension are major cause of CKD. Both hypertension and proteinuria are key players in CKD progression. Our current findings indicate that high salt may not only contribute to hypertension but also increases proteinuria. High salt loading rapidly elevated proteinuria in CKD humans and animals but not in normal controls. Inflammatory mediators including IL-13 were induced by high salt in T cells and glomerular cells and may play a role in proteinuria. Suppression of inflammation by calcineurin inhibitor largely prevented high salt induced proteinuria, whereas suppression of renin-angiotensin system had minimal effect. NFAT5/TonEBP, a transcription factor that senses high osmolarity was involved in high salt induced inflammatory mediators production and proteinuria. Moreover, high salt caused a stress response in cells especially the glomerular podocytes that may alter organization of cytoskeletons and microtubules in cells, resulting in inflammation and proteinuria. Further studies are needed to explore the underlying cellular and molecular mechanism(s) for high salt mediated proteinuria. Nevertheless, since salt intake is a modifiable factor for blood pressure and proteinuria control, clincial practice may proceed from now. No COI.
Development and regulation of gonadotropin-releasing hormone (GnRH) release during the prepubertal period

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Pulsatile GnRH release is the ultimate signal for central neuronal control of pituitary gonadotropin release and thereby fertility. Based on gonadotropin release, GnRH release before puberty is thought to be low, but actual measurements of GnRH release have not been made during the neonatal and early prepubertal period. We used the electrochemical method fast-scan cyclic voltammetry (FSCV) to monitor GnRH release in the median eminence (ME) in brain slices from gonad-intact male mice. In adults, the frequency of GnRH release in this preparation is similar to the luteinizing hormone (LH) pulse pattern in vivo. High-frequency GnRH release in ME occurred as early as embryonic day 18, peaked on the day of birth and remained elevated through 7-9d postnatal. By 2wks of age, release was minimal. We studied the regulation of GnRH release frequency at 1wk of age. GnRH release persisted in kisspeptin knockout mice, thus release at this age is kisspeptin independent. Testosterone given in vivo 4hr before slice preparation reduced spontaneous GnRH release frequency at 1wk, as did GnIH applied via the bath during recording. Blocking the GnIH receptor (GPR147) reversed the inhibitory effect of testosterone. Blocking GPR147 also increased GnRH release at 2wk of age. Exogenous GnRH failed to increase serum LH in 1wk old mice, when endogenous GnRH release was high frequency, but did in mice aged ≥2wk, after frequency was reduced. These data indicate high frequency GnRH release in early prepubertal male mice may result from lack of endogenous inhibition of the GnRH network. We postulate high-frequency GnRH release is needed for development of appropriate synaptic inputs, and that pituitary non-responsiveness at 1wk of age protects the reproductive system from premature activation. No COI.

Functional and Molecular Evolution of Kisspeptin Systems in Social Behaviours

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An evolutionarily conserved hypothalamic neuropeptide, kisspeptin (encoded by Kiss1 gene) and its receptor (Kiss-R=GPR54) signal a key role in vertebrate reproduction and puberty. In non-mammalian vertebrates, Kiss1 and its isoform Kiss2, and two Kiss-R types have been identified. Kiss2 gene appears to have been lost in the mammalian lineage. Recent studies in mammalian and non-mammalian vertebrates have implicated potential additional roles for kisspeptin based on its expression in brain regions such as the medial amygdala, hippocampus and the habenula. Recently, we have shown that kisspeptin modulates the release of 5-HT to inhibit alarm substance-evoked fear response in the zebrafish. These results suggest that kisspeptin may subserve an additional role in social behaviours to maintain emotional aspects of reproductive capability such as sexual motivation and arousal. No COI.
Animals living in temperate zones use changes in day length to adapt to seasonal changes in environment, but mechanisms underlying seasonal (photoperiodic) time measurement are not fully understood. Japanese quail is an excellent model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. We have demonstrated that local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating enzyme (type 2 deiodinase: DIO2) regulates photoperiodism. Functional genomics analysis in quail demonstrated that long day stimulus induces thyrotropin (thyroid stimulating hormone: TSH) production in the pars tuberalis (PT) of the pituitary gland, which triggers DIO2 expression in the ependymal cells of the MBH. In mammals, nocturnal melatonin secretion provides an endocrine signal of the photoperiod to the PT that contains melatonin receptors in high density. We have also demonstrated the involvement of TSH signaling pathway in mammals by using the TSH receptor null mice. Well known function of TSH derived from pars distalis (PD) of the pituitary gland is stimulation of thyroid gland. However, the mechanisms by which PT- and PD-TSH exert distinct functions within the body remained mystery. We found TSHs from two anatomical sources undergo different glycosylation and this tissue-specific glycosylation imparts different functions on a single hormone. No COI.
**Tonic inhibition of locus coeruleus neurons by GABAB receptors**

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The locus coeruleus (LC) nucleus contains noradrenergic (NAergic) neurons that provide the major norepinephrine (NE) supply to the forebrain. It has been shown that the release rate of NE in forebrain directly correlates to the discharging rate of NAergic LC neurons; accordingly, exploring how LC neuron firing rate is regulated is essential to understand the operation of brain functions associated with LC-NE system, such as the regulation of sleep-wakefulness cycle. Here we focus on the role of GABAB receptor (GABABR), a G-protein-coupled receptor. Our recent electron microscopy observations show that in LC most of the postsynaptic GABABRs locate at extrasynaptic sites. These extrasynaptic GABABRs are continuously activated by ambient GABA, resulting in tonic current of ~10 pA, which accounts for about 13% of total GABABR-mediated whole-cell current in LC NAergic neurons. These results suggest that there is still plenty of room for GABABR to regulate the excitability of LC NAergic neurons through manipulating ambient GABA. In support of this speculation, inhibiting GABA reuptake can effectively tune the firing rate of LC neurons. The GABABR-mediated tonic inhibition is not unique to LC NAergic neurons, it also occurs in NAergic A7 neurons. Most importantly, it is not only exists in brain slice preparation, but also in urethane anaesthetized condition. Since ambient GABA is reported to be higher in sleep than in waking condition using microdialysis method, our results suggest that GABABR-mediated tonic inhibition of LC neurons might be a molecular mechanism underlying higher LC neuronal activity in waking than in sleep condition (Supported by research grants from MOST, Taiwan).

**Orexinergic mechanisms in drug addiction and pain modulation**

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Orexin neuropeptides have a remarkable role in drug addiction and pain modulation. The locus coeruleus (LC) receives dense orexinergic fibers and expresses mainly the orexin receptors type-1 (OXR1) and is involved in opioid tolerance and dependence as well as pain modulation. However, the role of orexinergic transmission at the LC nucleus in these situations is unclear. Central administration of OXR1 antagonist (SB-334867) inhibits the development of morphine analgesic tolerance. In other words, OXR1 blockade by SB-334867 prevents the development of morphine analgesic tolerance. Moreover, the results indicate that intra LC microinjection of SB-334867 prior to each morphine injection or prior to naloxone administration reduce the severity of naloxone-induced morphine withdrawal symptoms. The whole-cell patch clamp recording results showed that in vitro application of orexin-A increases LC spontaneous firing rate and paired-pulse ratio (PPR). It also decreases spontaneous excitatory postsynaptic currents (sEPSCs) frequency of LC neurons, but did not change the sEPSCs amplitude. Our electrophysiological data indicate that orexin-A application decrease evoked excitatory postsynaptic currents (eEPSCs) and evoke inhibitory postsynaptic currents (eIPSCs) in LC neurons synapses. It is concluded that orexinergic transmission in the locus coeruleus appears to be involved in the drug addiction and pain modulation. Also, the in vitro results provide supporting evidences for a critical role of orexin signaling in LC neurons. It can be deduced that these changes in excitatory synaptic transmission may be elicited by presynaptic rather than presynaptic mechanisms. No COI.
Excitation of LC neurons by anesthetics

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The locus coeruleus (LC) regulates the activity of widely distributed cerebral networks through release of noradrenaline and other catecholamines in a one-to-many manner. Such “global” regulation is of particular importance in many physiological situations in which coordinated control of various functions implemented in many distributed networks is essential. Arousal and nociception are among such functions. This feature of the LC is also important in understanding mechanism of drugs. For example, dexmedetomidine, an alpha2-adrenoceptor agonist, exerts sedative effects by reducing conscious level and elevating nociception thresholds through suppressing LC neuron activities. General anesthetics are the most frequently and widely used central acting drugs that exert robust inhibitory effects in human and animals. However, sevoflurane, one of the most frequently used volatile anesthetics, has been shown to cause aberrant agitation in human patients, especially in children and at the emergence. As this “emergence agitation” is prevented by dexmedetomidine, we hypothesized that LC plays a key role in this sevoflurane-induced aberrant excitation. In brain slices from young rats, 5% sevoflurane induced an early-phase inward current in most of LC neurons even in the absence of synaptic inputs, in a manner inhibited by a gap junction inhibitor. Such excitatory current was not observed with nonvolatile general anesthetics and in non-LC neurons examined. It is thus expected that a potent drug effect on arousal and nociception would involve its direct effects on LC neuron excitability. In collaboration with Yutaka Yasui and Eiji Masaki. No COI.
Empathy for pain in rodents
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Empathy, a basic prosocial behavior, is referred to as an ability to understand and share other’s emotional state. Empathy is also a basis of altruism. In contrast, anti-empathy may be associated with autism, narcissism, alexithymia, personalality disorders, schizophrenia and depression. Thus study of the brain mechanisms of empathy has great importance to both scientific and clinical advances. However, less is known about the molecular and cellular mechanisms of empathy due to lack of laboratory animal models in that only human and non-human primates have been considered to have such unique emotional sharing. Recently, a group from McGill university and we independently found that both mice and rats have empathy for pain. We also mapped out that the medial prefrontal cortex including the anterior cingulate cortex, prelimbic cortex and infralimbic cortex is involved in pain empathy in rats, suggesting existence of a neural network associated with development of pain empathy in the CNS. In the present lecture, I would like to give a brief outline of the advances in study of empathy for pain in rodents, try to provide with a bio-psychosocial-behavioral model for studying pain and its emotional comorbidity with anxiety and depression. No COI.

Involvement of the anterior cingulate cortex in pain-related negative emotion
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The anterior cingulate cortex (ACC) is thought to be key neural substrates underlying emotional responses. Using pain-related conditioned place avoidance models (CPA), we observed that bilateral excitotoxic lesions of the ACC suppressed the formalin-induced CPA (F-CPA). Intra-ACC NMDA receptors antagonist, AP5, but not AMPA/KAR receptors antagonist DNQX, F-CPA was effectively eliminated. NMDA receptor activation induced pPKA, pERK and pCREB in rACC slices. Blockade of PKA and ERK activation in the rACC prevented the induction of F-CPA. Thus, NMDA-PKA-ERK-CREB pathway activation in the rACC is required for the induction and expression of pain-related negative affect. Estrogen has been reported to enhance NMDA-mediated synaptic activity in the hippocampus and cortex. We demonstrated that estrogen acutely enhanced the EPSCs in rACC slices by increasing the ratio of NMDA–EPSCs to AMPA–EPSCs and presynaptic glutamate release. Intra-rACC injection of estrogen receptor (ER) inhibitor ICI 182, 780 or inhibitor of aromatase androstatrienedione completely blocked F-CPA, suggesting that estrogen in the rACC facilitated NMDA receptor-mediated synaptic transmission to drive pain-related negative emotion. Using a chronic constriction injury (CCI) model of neuropathic pain, we profiled gene expression in the rat brain and identified sip30, which was upregulated in the rACC after CCI. Knockdown of SIP30 by intra-rACC injection of shRNA targeting the rat sip30 gene suppressed the frequency of mEPSCs in rACC slices. Inhibition of CCI-mediated induction of SIP30 reduced neuropathic pain-evoked place escape/avoidance paradigm (PEAP), suggesting that SIP30 in the rACC mediates neuropathic pain-evoked negative emotion via modulation of glutamate release and excitatory synaptic transmission. No COI.
Symposium 18  Synapses and circuits: From formation to disorder

S 18
Subcellular Purkinje neuron translatome at rest and during plasticity
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Two major networks underlay brain complexity: neuronal connections and molecular signaling pathways. Until recently, the diversity and complexity of the spatially intermingled neurons posed a serious challenge to the exhaustive identification and quantification of components, in a neuron-type specific manner. This is an obstacle especially for our understanding of the protein-synthesis dependent late phase of synaptic plasticity. To address this problem, we present a novel approach to identify ribosome-associated translating mRNAs, from selected sub-neuronal domains of Purkinje cells (PC) in the rat cerebellum. We combined microdissection, Translating Ribosome Affinity Purification (TRAP) and quantitative nanoCAGE RNA deep-sequencing, we obtained snapshots of RNAs bound to cytoplasmic or rough endoplasmic reticulum (rER)-associated ribosomes, in the PC and its dendrites, at different time points following plasticity induction. We show that the approach yield selective enrichment in PC markers, identifies transcripts not previously known to be enriched in PCs and some hitherto uncharacterized mRNAs. We introduced an improved detection of translating mRNAs for membrane receptors and ion channels, allowing estimate of relative synthesis rate. The late phase of plasticity was specifically correlated with altered translation of an mRNA subset, associated with markers of synaptic plasticity and cell signaling. No COI.

S 18
Developmental plasticity in spatial coding impacts on navigational behaviour
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We asked if synaptic plasticity of excitatory and inhibitory transmission in the developing circuitry of the vestibular nucleus (VN) impacts on behavioural outcome. We demonstrated that LTP of glutamatergic synapses at interneurons in the VN enabled postnatal emergence of graviceptive behaviour in rats. Furthermore, GABAergic transmission in the VN was excitatory in the first postnatal week but switched to inhibitory in the second postnatal week. During this period, LTD of GABA transmission in the VN could be modulated by neuromodulators such as endocannabinoid or BDNF. With neonatal administration of GABA-A receptor agonist to the VN, we found that the proportion of LTD-expression neurons was reduced and the emergence of graviceptive behaviour was advanced. Perturbation of synaptic plasticity in the postnatal VN impacted on spatial map formation in the adult brain with effects on spatial navigation. Taken together, tuning the vestibular circuitry for spatial coding during a postnatal period of plasticity is critical to the recruitment of effectors for orienting behaviours in the adult animal. (HKRGC-GRF 761711, 761812, 762313, N_HKU735/14). No COI.
Multiple actions of endocrine disrupting chemicals on thyroid hormone-mediated brain development

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Thyroid hormone (TH) plays a critical role in brain development. Deficiency of TH during the perinatal period causes abnormal brain development known as cretinism in humans. On the other hand, there is a potential risk of exposure to toxic substances through food during the perinatal period. In fact, several chemicals disrupt TH-action in the developing brain. Such chemicals are known as endocrine disrupting chemicals (EDCs). They affect TH systems through different mechanisms. For example, although hydroxylated polychlorinated biphenyl (PCB), polybrominated diphenyl ether (PBDE) and perfluoro-octanesulfonate (PFOS) suppress TH-mediated dendrite arborization of cerebellar Purkinje cell in culture, PCB and PBDE suppress TH-mediated transcription by dissociating TR from TH response element, whereas PFOS suppresses deiodination of thyroxine (T4) by inhibiting type 2 iodothyronine deiodinase activity. On the other hand, several isoflavones such as genistein and daizein activate TH-mediated dendrite arborization by augmenting TH-mediated transcription through recruiting coactivators. It should be noted that the effects may be induced at low dose exposure. Perinatal administration of PCB or PFOS during pregnancy or lactating period induces hyperactivity in the open field, motor coordination defect on the rotarod in their offsprings. These results indicate that various EDCs affect brain development at least in part through modulating thyroid hormone action. Thus, care must be taken not to intake synthetic chemicals particularly during pregnancy and lactating period. No COI.
Symposium 20  Leptin: A friend and a foe

Leptin's role in cardiorenal function

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Adipocytes secrete a number of bioactive molecules that maintain homeostasis via the activation of key cellular signalling pathways. The adipokine leptin signals via the leptin receptor and megalin in the hypothalamus, adipose tissue, liver, kidney, heart and placenta via a tissue-specific and receptor-specific manner. Most of the current knowledge concerning the role of leptin in cardiorenal function is focused on studies where plasma leptin concentrations are elevated to mimic the obese state. Chronic leptin exposure in animals via intravenous infusion results in increased arterial pressure and heart rate, while hyperleptinemia in humans has been shown to be associated with hypertension via the modulation of both systolic and diastolic blood pressure. Recent research has also demonstrated that leptin controls neuronal circuits specifically in the dorsomedial hypothalamus to modulate blood pressure. Further, the link between leptin and blood pressure may in part be controlled by the kidney. Acute exposure to leptin increases sodium excretion and urinary output in animal models. In addition, in isolated glomerular mesangial cells, leptin induces hypertrophy via the activation of phosphoinositide 3-kinase and mitogen-activated protein kinases. This in turn increases the amount of filtered protein and albumin reaching the tubules, which activates profibrotic and inflammatory pathways. Recently, we have established that exposure to elevated leptin in vitro increases the expression of a number of downstream signalling targets which modulates metabolic activity and increases fibrotic mediators which leads to a reduction in albumin handling by the proximal tubules. Thus leptin appears to play multiple roles in the modulation of cardiorenal function. No COI.

Adverse effects of leptin on sperm parameters

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Leptin, a 167 amino-acid product of the ob gene, was first identified in 1991 after a 40-year search that began following the emergence of a mutant strain of mice with hyperphagia, early-onset obesity, and delayed sexual maturation. Since then, leptin has been shown to be involved in the regulation of appetite and body weight, immune and reproductive functions, CNS development during the neonatal period, bone growth and development. Although it was its deficiency that first led to its discovery, it now appears that its clinical significance also lies when it is in excess. Emerging evidence from animal studies is implicating leptin in infertility and in a number of obesity-related diseases. Chronic leptin administration to non-obese rats decreases sperm count, increases the fraction of sperm with abnormal morphology, increases sperm DNA fragmentation and alters its histone:protamine ratio. These changes are accompanied by the up-regulation of respiratory chain enzyme genes and down-regulation of the antioxidant enzyme genes in the testes of leptin treated rats. Expressions of TNF-α, p53, p21 and AIF are also up-regulated while the expression of Bcl2-like-1 is down-regulated following leptin treatment. Most of these changes are however reversible upon cessation of leptin treatment and recovery is near complete in 7-8 weeks. These changes are also prevented by concurrent administration of melatonin. It appears that the adverse effects of leptin involves increases in free radical activity that then induce DNA fragmentation and a necrotic-like cell death of sperm and seminiferous tubular cells through activation of TNF and JNK pathway. No COI.
**Symposium 21: Advances in reproductive physiology for wildlife conservation**

**S 21**

**Biodiversity and Reproductive Endocrinology in Mammals**

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Pregnancy with placentation and lactation are the peculiarity of mammals. A remarkably growth of fetus occurs in uterus during the late stages of pregnancy in any mammals. The most important factor is rapid elongation and cylindrical changes of uterus to maintain fetal survival in the stage of rapid fetal growth during the late stages of pregnancy. Progesterone, estrogen, prolactin and relaxin play key roles in the implantation of embryos, maintenance of pregnancy and the induction of parturition. Although the synergistic action of progesterone and estrogen is generally essential for most mammals in maintaining a successful pregnancy, estrogen plays a dominant role in the maintenance of uterine elongation when the fetus grow rapidly in the late pregnancy. However, the origin and mechanism responsible for secretion of a large amount of estrogen in the late pregnancy are different among species. There are two types of groups. The first group is the ovary and placenta unit type which can be seen in rat, mouse, golden hamster, rabbit, dog, goat, cow and elephant. For the first group, the mother’s ovary is an essential endocrine organ throughout pregnancy. The second group is the fetus and placenta unit type which can be seen in guinea pig, cat, sheep, horse, monkey and human. In this latter type, mother’s ovary is not essential in the late stages of pregnancy. The currently known aspects of the unique biodiversity of endocrine mechanism in pregnancy and the comparison between those two groups of animals will be reviewed in this paper. No COI.

**S 21**

**Shp2 is essential for normal embryo implantation in mice**

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Ovarian steroid hormones and their nuclear receptors PR and ER coupled with locally produced signaling molecules have been well demonstrated essential for embryo implantation, however, the hierarchical landscape of the molecular pathways that govern this process remains largely unexplored. The crosstalk and interaction between the RTK and steroid hormone receptor has been well studied in the cancer related research, but whether this happens in the physiological implantation process remains unclear. Shp2 is a positive signal transducer of RTK signaling and its systemic knockout would cause the embryo lethal. We show herein that uterine conditional deletion of Shp2 by utilizing PR-Cre mouse model derails normal uterine receptivity leading to a complete implantation failure. Unexpectedly, this compromised uterine receptivity exhibiting progesterone resistance upon Shp2 depletion is seeded by limited ERα activation and thus reduced PR expression in uterine stroma independent of ERK pathway activation. Further analysis reveals that nuclear Shp2, rather than cytosolic Shp2 physically interacts with ERα, facilitating ERα binding to DNA sequence of targeting PR promoter and subsequent cofactor recruitment for ERα transcriptional activation in peri-implantation uteri. Besides uncovering a novel regulatory mechanism, this study could be clinically relevant to dysfunctional ERα-caused endometrial disorders in women. No COI.
Using science to understand factors related to zoo elephant reproduction and welfare

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Questions have been raised about whether environmental and social needs are being met for elephants in zoos. Lack of reproductive function has long been considered an indicator of reduced welfare and poor sustainability of zoo elephants. A large, epidemiological study was conducted in the U.S. that involved 250 elephants at 64 zoological institutions to identify how social, facility, management, keeper factors affect ovarian function in elephants. Rates of normal cycling, non-cycling and irregular cycling were 73.2, 22.5 and 4.2% for Asian, and 48.4, 37.9 and 13.7% for African elephants, which differed between species. Several management factors were related to ovarian cycle status. Enrichment diversity was found to increase the chance that a female African elephant will cycle normally, possibly due to elephants making a positive appraisal of the quality of the zoo environment. Social experience was also associated with ovarian cycling for female African elephants. Females that are socially separated, even if they have access to one or more other elephants through a barrier, have a decreased chance of cycling. Although a larger percentage of female Asian elephants had normal ovarian cycles, univariate analyses indicated that more time spent in spaces with free choice of being indoors or outdoors was associated with an increased likelihood of normal cycles. Thus, there are many factors in the captive environment that can be managed to improve reproduction and improve welfare of elephants under human care. No COI.
Role of Thai Traditional Medicine in the Health Care System of Thailand
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Humanity has long been faced with a variety of illnesses, and are determined to find a way to relieve suffering caused by them. Knowledge has continuously evolved and has been passed down from generation to generation. Medical knowledge from the western world has been accepted for its advanced methods and technology and has become mainstream medicine in most parts of the world. But the advancement of western medicine has also created problems, such as the burden of the increasing cost of the health care system, and many patients still suffer from diseases for which western medicine has yet to develop successful treatments. Society is now beginning to realize that the advances of western medicine may not be the answer for all problems, and wants to learn more about whether traditional medicine can be integrated with modern medicine to help solve or at least reduce these problems. In Thailand, attempts have been made to conserve and make use of Thai traditional medicine. Work related to Thai traditional medicine, such as education, health services and herbal medicine production, has been improved. At present, some university hospitals and most hospitals under the Ministry of Public Health provide services in Thai traditional medicine; these services cover diagnosis and treatment with herbal medicine, traditional maneuvers including massage, hot herbal compresses, herbal steam baths and postpartum treatment. Some development of the works from the Faculty of Medicine Siriraj Hospital, Mahidol University, the oldest hospital and medical school in Thailand, established 127 years ago will be presented. No COI.

Thai Traditional Medicine: Moving Towards Precision Medicine
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In order to accelerate the integration of Thai traditional medicine into the health care system, Thai government has established the national essential herbal drug list comprising traditional drugs and herbal items for many health conditions since 1999. Although the prescribed items can be reimbursed from the government under the universal health coverage scheme, only a small fraction of modern physicians agreed to prescribed some products on the list, but most of the physicians asked for scientific evidences on safety and efficacy. Therefore, the recent trend in researches on Thai traditional medicine has shifted to clinical trials assessing the efficacy and safety of herbal medicines rather than in vitro screening of interested pharmacological properties which was performed extensively in the past. Thai traditional practitioners have been asked to perform documentation on the actual uses of Thai traditional herbal remedies, so called Actual Use Research with the hope that the information will be evidence to support the usage and ensure the safety of the remedies. It can also be used as basic information to investigate pharmacological mechanisms and help in the explanation of the effects of such remedies to human body. A new approach of Multi-omics analysis (genomics, proteomics, metabolomics, phenomics and etc.) are used to create databases with the ultimate aim to generate massive data network of Thai traditional medicine in patients and reaching out towards disease mechanisms, precision diagnosis and treatment for individual patient, calling precision medicine. This approach will help not only creating evidence-based Thai traditional medical practices, with rational use of traditional drugs as personalised medicine, but also help to conserve Thai traditional medical knowledge, one of the wisdoms of mankind. No COI.
Thai Traditional Massage: Explaining the Art of Healing with Modern Scientific Knowledge

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Court-type Thai traditional massage (CTTM), an art of healing, is a popular alternative treatment for musculoskeletal disorders nowadays. Researches have been done in this field to reveal the effects of CTTM in terms of evidence-based medicine. The main characteristic of CTTM is the use of thumbs, fingers or palm pressing with gentle force on points along basic massage lines in the initial phase and later on pressing some specific body points, called major pressure point or major signal point (MaSP). There are 50 MaSPs all over the body. Thai traditional practitioners believe that by pressing these MaSPs, the blood and heat would be regulated to specific body parts to cure the symptoms or ailments. Anatomical positions of MaSPs have been studied by various groups, the result of 15 MaSPs on neck and upper extremities will be presented. Surface landmarks can be used to distinguish location of each point in cadaver as well as in practice. Muscles were the main anatomical structure underneath MaSPs together with their nerves and arterial supplies. Physiological effects of the massage on these 15 MaSPs were studied in healthy volunteers to determine blood flow and thermal skin using Duplex ultrasound and thermographic camera, respectively. After pressing each MaSP for 30 seconds, blood flow increased immediately and lasted for 60 seconds. Thermal skin was also conformed to blood flow, skin temperature increased immediately but lasted longer for 3 minutes. In clinical practice, practitioners who perform CTTM usually massage along the basic massage lines and on specific MaSPs related to the symptom for a period of 45 minutes. This study demonstrated that massage of the MaSPs may increase local blood circulation and improve muscle relaxation via the increase of blood flow and thermal skin. No COI.
Symposium 23  **Systems biology investigations of renal epithelia**

**S 23**

**Systems Biology of Vasopressin Signaling in Kidney**

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Vasopressin regulates water excretion by controlling the water channel aquaporin-2 (AQP2) in two ways: 1) regulation of aquaporin-2 (AQP2) trafficking to and from the apical plasma membrane; and 2) regulation of transcription of the Aqp2 gene. We are using large-scale proteomics (LC-MS/MS) and deep sequencing of DNA to identify the signaling mechanisms involved. Phosphoproteomics studies in collecting duct cells identified several hundred phosphorylation sites that increase with vasopressin (including Ser-256, 264 and 269 of AQP2) and a similar number of sites that decrease with vasopressin (including Ser-261 of AQP2). The former have sequences predicted to be targets of basophilic kinases (AGC and CAMK families), while the latter are predicted to be phosphorylated by proline-directed kinases (CMGC family). Of the 521 protein kinases present in the rat genome, we have identified 217 that are expressed in rat collecting duct (RNA-seq analysis). These protein kinases were ranked in terms of likelihood of phosphorylating each of the four phosphorylation sites in AQP2 by employing Bayes’ rule to integrate data from multiple sources (transcriptomics data, proteomics data, data from reductionist studies of vasopressin signaling, kinase inhibitor data and kinase target specificities). We are systematically deleting these kinases using CRISPR-Cas9 followed by phosphoproteomics to identify which of these candidate kinases play critical roles in vasopressin signaling. To identify upstream kinases in the relevant signaling cascades, we have determined the effects of 7 broad spectrum protein kinase inhibitors on the phosphoproteome of mpkCCD cells. The result is a vasopressin-activated signaling network responsible for regulation of AQP2 phosphorylation. No COI.

**S 23**

**Systems Biology of Renal Epithelium**

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The success of the “human genome project” has marked the beginning of 21st century inter-disciplinary field of study so-called “Systems Biology” that focuses on complex interactions within biological systems, using a more holistic perspective approach to biological and biomedical research. Currently, all of the elite research institutes around the globe have set their missions toward this new approach in science. It is now clear that systems biology has brought us a magnitude of information that never before attained in the history of biological science. Systems biology studies require an integration of basic and clinical science standpoints, large-scale “omics” technologies, and high-level computational methods for data analysis and interpretation. Many laboratories have successfully applied the state-of-the-art methods in systems biology viz. protein mass spectrometry, microarrays, next-generation sequencing, and bioinformatics to investigate the fundamental mechanisms involved in the regulation of water and solute transport by the kidney. In this symposium, we will discuss the development of systems biology approaches in the investigation of physiology and pathophysiology of renal epithelium. Numerous new findings from these studies that help us understand more about the underlying molecular mechanisms of vasopressin-related disorders such as the syndrome of inappropriate secretion of antiuretic hormone (SIADH), diabetes insipidus, and polycystic kidney disease will be highlighted. Finally, we will explore approaches to investigate the heterogeneity of phenotypic responses down to the single-cell resolution. No COI.
Investigation of heterogeneity in cellular phenotypic responses at the single-cell resolution

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How cells convert the extracellular cues to intracellular signals and commit on a specific phenotypic outcome has still been an active area of research. Using time-lapse microscopy and the construction of live reporter proteins, we can now begin to investigate how cells respond heterogeneously at the single-cell level. Using live reporters of ERK activity and the translocation reporter of FoxO3a protein, we investigated how the activity of these two proteins temporally vary upon stimulation with six different growth factors at varying doses. In addition to confirming the pulsing characteristics of ERK activity, we demonstrated that FoxO3a translocations are also truly dynamic, involving both early synchronous phase and the late asynchronous pulsatile response. Co-expression of ERK and FoxO3a reporters showed for the first time how ERK activation contributes towards the pulsing of FoxO3a translocation. We then explored the AKT/ERK/FoxO3a connectivity in different breast epithelial and breast cancer cell lines and showed that FoxO3a can be distinctly regulated by AKT and ERK pathways. Our discovery of FoxO3a pulsatile translocation is consistent with the already reported pulsing dynamics of other transcription factors including p53, NFκB and NFAT4. Such temporally dynamic cellular responses cannot be identified using conventional bulk assays, emphasizing the necessity to reinvestigate other common physiological processes using these novel technologies. No COI.
S 24

Thermoregulation in the tropics

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It has been reported recently that humans have developed such a remarkable system for endurance exercise in the heat, that we could outrun almost all other mammals, including horses. The key for maintaining exercise in the heat may reside in controlling both core temperature and systemic blood pressure simultaneously. Thus, thermoregulatory researches, specially, during exercise in the heat, are critical to understanding human adaptation to the tropic conditions. During exercise, heat loss (via sweating and skin blood flow) is regulated by two main factors; thermal (core and skin temperature) and work factors (non-thermal; central command, muscle mechanoreceptors etc). Although it is well known that increases in thermal factors have a positive effect on sweating and cutaneous blood flow response, the effects of non-thermal factors on the heat loss responses are not well understood. Previous research indicates that afferent signals from working muscles (muscle metaboreflex) are one of the non-thermal factors, which increases sympathetic nerve activity and are important in controlling cardiovascular responses during exercise. Therefore, it has been suggested that this muscle metaboreflex also modulates heat loss responses during exercise. Thus, we will discuss an integrative control of heat loss responses during exercise based on muscle metaboreflex. No COI.

S 24

Exertional heat stroke in the tropics

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With the influence of global warming and increasing surface temperature about 1.8- 4oC by 2100 (IPCC 2007), heat related illness (HRI) in the tropical countries is likely to be more prevalent. Exertional heat stroke (EHS) is a key concern for athletes, military recruits, workers under outdoor and indoor without air conditioning even following heat acclimatization. The risk of HRI is dependent on work intensity and heat exposure time. The incidence of EHS in each tropical country may be under-diagnosed as symptoms are non-specific. The incidence of EHS can be minimized with education to increase awareness. Furthermore, the strategies for heat mitigation at work/drill settings including exercise and work/rest guidelines, identifying individuals with high heat strain, fluid and electrolyte replacement and effective cooling should be made known to the supervisors. If EHS is detected, rapid ice immersion with immediate hospital evacuation is the most effective way to reduce poor prognosis. In the Royal Thai Army, heat related injuries in new recruits during military training are still a health problem. The prevention program provided includes health education, display color flags in accordance with environmental temperature and humidity, water replacement with exercise/rest guidelines, measurement of tympanic temperature and urine color daily. Research is being undertaken to develop more effective preventive method such as real time monitoring. As the trend of EHS is increasing for high risk populations in the tropics, in addition to awareness through education, heat preventive policies and appropriate effective preventive methods need to be implemented in work places. No COI.
Endurance performance in the tropics
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Excessive heat stress compromises endurance performance and increases the risk of heat stroke. The rapid rise of body core temperature (Tc) when exercising in the heat often results in an impairment of exercise capacity and performance. As such, coaches and employers apply various heat mitigation strategies to counteract the debilitating effects of heat strain in their athletes and workers, respectively. These strategies include behavioural alteration, aerobic fitness, heat acclimation/acclimatisation, pre-exercise cooling and fluid ingestion. An ideal heat mitigation strategy would: (i) lower Tc before exercise; (ii) attenuate the rise of Tc during exercise; and (iii) extend Tc at the end of exercise within safe limits. Through these alterations of Tc, an athlete would increase his capacity for heat storage during exercise, and therefore optimise performance in the heat. Current understanding in this field of research is largely based on data collected from non-heat acclimatised cohorts and therefore its validity remains unanswered for tropical natives where the exposure to heat is higher. For example, a conventional heat acclimatisation programme that is usually effective when employed on Caucasians did not induce any physiological adaptation in tropical natives. This lecture will have practical implications for individuals seeking to optimise performance and health in the heat by making informed decisions when choosing the appropriate heat mitigation to employ. No COI.
Oral Presentations

Young Scientist Awards (Oral)
Neural activity sets endocytic and motor proteins for synaptic vesicle recycle
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Presynaptic nerve terminals must maintain stable neurotransmission via synaptic vesicle (SV) resupply despite encountering wide fluctuations in the number and frequency of incoming action potentials (AP). However, the molecular mechanism linking variation in neural activity to SV resupply is unknown. Three isoforms of dynamin are essential endocytic proteins and myosins II and VI are actin-based cytoskeletal motors that drive dendritic actin dynamics and membrane transport, respectively, at brain synapses. We combined genetic knockdown or molecular dysfunction with the specific antibodies by microinjection into a cultured rat superior cervical ganglion neuron and direct physiological measurement of synaptic transmission from paired neurons to show that dynamin isoforms or myosins IIB and VI work individually in SV reuse pathways, having distinct dependency and time constants with physiological AP frequency. Dynamin-3 or myosin VI resupplied the readily releasable pool (RRP) with slow kinetics independently of firing rates but acted quickly within 50 ms after AP. Under high frequency AP firing, dynamin-1 or myosin IIB resupplied the RRP with fast kinetics in a slower time window. Knockdown of both myosin and dynamin isoforms by mixed siRNAs microinjection revealed that myosin IIB-mediated SV resupply follows amphiphysin/dynamin-1-mediated endocytosis, while myosin VI-mediated SV resupply follows dynamin-3-mediated endocytosis. Collectively, our findings show how dynamin isoforms select appropriate vesicle reuse pathways and how distinct myosin isoforms work as vesicle motors associated with specific firing patterns. No COI.

Morphometric plasticity of nitric oxide containing neurons in the barrel cortex of de-whiskered rats
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The rodent somatosensory barrel cortex is an ideal model to examine the effect of experience-dependent plasticity on developing brain circuitry. Sensory deprivation such as whisker deprivation may affect neuroanatomical aspects of the brain during developmental processes. The present study designed to investigate the possible effects of whisker deprivation on the morphometric characteristics of NADPH-d positive neurons in the barrel field cortex of adolescent rats. Pups were divided into the intact (n=4) and whisker-deprived groups (n=4). In whisker-deprived group, the total whiskers of subjects were trimmed every other day from postnatal day (PND) 0 to PND 60. NADPH-d histochemistry reaction was processed to quantitatively analyze the feature of NADPH-d containing neurons of barrel cortex. Our results showed that the number of NADPH-d positive neurons remained unchanged in whisker-deprived group. However, the mean soma diameter, dendritic length and the number of 3rd order processes were significantly decreased in the whisker-deprived rats (P<0.05). Our results indicate that postnatal whisker deprivation possibly alter NADPH-d/NOS neuronal features in the barrel cortex. The functional implications of these data may relate the plasticity of synaptic receptive field and developmental brain circuits. No COI.
Predicting the future position of moving objects is an essential cognitive function used for many daily activities. In spite of a wealth of studies on visual motion prediction by psychophysicists, it has not been investigated with reference to circadian modulation so far. This study examined the diurnal time-of-day modulation of visual motion prediction in a task to predict the position of moving objects at 9:00, 12:00 and 18:00. In the experiments, the inflating and the deflating computer images were displayed as moving objects to prevent the contaminating effects of eye pursuit. The results demonstrated that participants showed a marked diurnal time-of-day modulation in predicting times related to the inflating images in a light-adapted environment (p<0.004). This motion prediction was more accurate in the afternoon than in the morning. Such diurnal time-of-day modulation was, however, not found in predicting times related to the inflating images. Our experiments were done in light-adapted environments. Thus, the results can reflect the functions of cone photoreceptors. Cone photoreceptors also have circadian dependency. These cells were shown to contain the clock genes in mouse retina. Moreover, those gene expressions fluctuate throughout the day. In physiological experiments, the light adapted electroretinogram shows a marked circadian rhythm which peaks at 20:00 in human subjects (Danilenko et al., 2011). Therefore, it is plausible that visual motion prediction is circadian-dependent in light-adapted environments. The discrepant results may be explained by the gradient distribution of cone photoreceptors in the human retina. This study is scheduled to be published in Chronobiology International. No COI.
ABS0205

Antiallodynic and antihyperalgesic effects of zerumbone on a mouse model of chronic constriction injury-induced neuropathic pain

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Neuropathic pain is a chronic condition that is difficult to be treated. Noting the seriousness of the problem, the International Association for the Study of Pain (IASP) announced that 2014-2015 to be the Global Year Against Neuropathic Pain. Among many, some of the major challenges in managing neuropathic pain is the ineffectiveness and non-specificity of the currently available drugs, thus requiring the discovery of newer therapies. In this study, we investigated the antiallodynic and antihyperalgesic effects of zerumbone, a bioactive sesquiterpene from Zingiber zerumbet in chronic constriction injury (CCI)-induced neuropathic pain animal model. Our findings showed that single and repeated dose of intra-peritoneal administration of zerumbone (5, 10, 50, 100 mg/kg) significantly attenuated the CCI-induced neuropathic pain when evaluated using the electronic von Frey anesthesiometer, cold plate, Randall Selitto analgesiometer and the Hargreaves plantar test. Zerumbone significantly alleviated tactile and cold allodynia as well as mechanical and thermal hyperalgesia. Our findings are in comparison to the positive control drugs used gabapentin (20 mg/kg i.p.) and morphine (1 mg/kg i.p.). Together, these results showed that the systemic administration of zerumbone produced marked antiallodynic and antihyperalgesic effects in the CCI-induced neuropathic pain in mice and may serve as a potential lead compound for further analysis and development. No COI.

ABS0277

Arginine vasopressin V1b receptor regulates cell growth and promotes neurite outgrowth in PC12 cells

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The neurohypophysial hormone arginine vasopressin (AVP) is essential for a wide range of physiological functions, including water reabsorption, cardiovascular homeostasis, hormone secretion, and social behavior. These actions of AVP are mediated by at least three distinct receptor subtypes: V1aR, V1bR, and V2R. AVP action through V1bR is known to regulate social memory and social aggression in rodents. However, it is largely unknown how V1bR contributes to the regulation of these behaviors. Since alterations in connectivity in neuronal circuits has been postulated to be a critical step in social behavioral deficits, we here examined the role of V1bR in the formation of neurite outgrowth, which is indispensable for shaping neuronal circuit, by using rat PC12 pheochromocytoma cells. PC12 cells can be differentiated into neuron-like cells with elongated neurites by exposing to neurotrophic factors. Interestingly, we found that Nerve growth factor (NGF) treatment, which is well known to induce neurite outgrowth in PC12 cells, decreased the gene expression level of V1bR in a dose-dependent manner, implying the involvement of V1bR in the formation of neurite outgrowth. In facts, we found that blocking or knock down of V1bR promotes neurite outgrowth in PC12 cells. Moreover, knock down of V1bR promotes the formation of neurite outgrowth induced by NGF. On the other hand, blocking or knock down of V1bR inhibits cell proliferation, and overexpression of V1bR promotes cell proliferation in PC12 cells. These data suggest that V1bR plays an important role in neurite outgrowth formation through regulation of cell proliferation, and downregulation of V1bR might alter the cell status from a proliferative phase into a differentiation phase. No COI.
ABS0294

Novel hybrid kernel function exploring hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) in lacunar stroke during cerebrovascular reactivity (CVR)

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Lacunar stroke is deep small artery disease caused by overwhelmed oxidative stress and vascular inflammation leading to neuronal necrosis and glia activation. H\textsubscript{2}O\textsubscript{2} – derived glia activation is one of free radicals that plays a role in neurovascular glia coupling process. Releasing H\textsubscript{2}O\textsubscript{2} is a key biomarker indicates degree of brain damage. We studied in Lacunar stroke because of its etiology in vascular origin and using CVR for cerebral reserve function test. In this study, we examine the role of H\textsubscript{2}O\textsubscript{2} by using kernel function of support vector machine (SVM). Kernel function is a key mapping of SVM supervised by learning algorithms. Plasma H\textsubscript{2}O\textsubscript{2} was assessed by real time electrochemistry method. The training data sets were prepared from H\textsubscript{2}O\textsubscript{2} concentration in basal, experimental and recovery phases during CRV of both healthy subjects (n= 16, aged 27.33±3.85) and acute lacunar infarcts (n=15, aged 65±4.50). The general binary classification can be stated as follows: given a data set of N samples; each sample consists of a training example of length M with elements and a target value. The classifier performance by each single kernel function presents only radial basis function (RBF) has highest performance (90 % classified accuracy) than linear, polynomial, and sigmoid functions in experiment phase. Combined with highest performance, a hybrid model was developed and given 92 % accuracy. This novel hybrid model is the best classifier for H\textsubscript{2}O\textsubscript{2} biomarker in lacunar stroke. No COI.

ABS0296

The role of dorsal hippocampal orexin-2 receptors in the acquisition and expression of morphine-induced place preference in rats

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Orexinergic system is involved in reward processing and drug addiction. In the present study, we investigated the effect of intrahippocampal CA1 injection of orexin-2 receptor (OX2r) antagonists on the acquisition and expression of morphine-induced place preference in male Wistar rats. Animals weighing 230-280 g were bilaterally implanted with two separate cannulae into the CA1 region. Different doses of TCS OX2 29 (1, 3, 10 and 30 nM/0.5µl DMSO) as a selective antagonist of OX2rs were microinjected into the CA1 prior to subcutaneous injection of morphine during a 3-day conditioning phase in four treatment groups while some groups just received the antagonist in the expression phase following a single morphine injection during the conditioning phase. Conditioning scores and locomotor activities were recorded by Ethovision software during the test. The results demonstrate that subcutaneous administration of 5 mg/kg morphine sulphate produces conditioned place preference (CPP), while intrahippocampal administration of the OX2rs antagonist attenuates the induction of CPP during the acquisition and expression phases. Furthermore, the effect of TCS OX2 29 on the reduction of morphine CPP in the acquisition phase was dose-dependent and also was more pronounced in the acquisition than the expression. Nevertheless, TCS OX2 29 at the dose of 30 nM alone had no effect on conditioning score. Also, the administration of different doses of TCS OX2 29 did not have any influence on locomotor activity of all phases. Our findings suggest that OX2rs in the CA1 region of the hippocampus are involved in the development of the acquisition and expression of morphine CPP. No COI.
ABS0320

Electrical stimulation of the Ventral Tegmental Area effects the acquisition and expression of morphine–induced conditional placed preference

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Ventral tegmental area (VTA) is an important section of reward pathway involved in opiate reinforcement. Herein, we studied the effect of electrical stimulation with varying levels of current intensities from 50 µA to 10µA at constant frequency of 100 Hz, on VTA with effective and ineffective doses of morphine (5 mg/kg and 0.5 mg/kg, respectively), during conditioning and post-conditioning phases of Conditioned Placed Preference. Our results demonstrate that subcutaneous administration of 5 mg/kg of morphine produced significant CPP in comparison with that of the saline group. Electrical stimulation of VTA blocked the effect of both acquisition and expression of morphine-induced CPP and stimulation of VTA at the highest current intensity (50 µA) at the ineffective low dose of morphine significantly enhanced the acquisition phase of CPP. Our findings suggest that the electrical stimulation of VTA has a notable effect on memory and learning formation during the conditioning induced process by morphine. No COI.

ABS0352

Optogenetic induction of contractile ability in C2C12 myotubes

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Myoblasts can be differentiated into multinucleated myotubes, which provide a well-established and reproducible muscle cell model for skeletal myogenesis in vitro. However, under conventional differentiation conditions, each myotube rarely exhibits robust contraction as well as sarcomere arrangement. Previously, it was reported that muscle fiber stimulation with electrical, mechanical or pharmacological methods, which mimic motor neuron inputs, facilitate the maturation of developing muscles as well as the maintenance of contractility. Here, we applied trains of optical stimulation (OS) to C2C12 myotubes, which were genetically engineered to express a channelrhodopsin variant, channelrhodopsin-green receiver (ChRGR), to investigate whether membrane depolarization facilitates the maturation of myotubes. We found that light pulses induced membrane depolarization and evoked action potentials in ChRGR-expressing myotubes. Regular alignments of sarcomeric proteins were patterned periodically after OS training. In contrast, untrained control myotubes rarely exhibited the striated patterns. OS-trained and untrained myotubes also differed in terms of their resting potential. OS training significantly increased the number of contractile myotubes. Treatment with nifedipine during OS training significantly decreased the fraction of contractible myotubes, whereas tetrodotoxin was less effective. These results suggest that oscillations of membrane potential and intracellular Ca²⁺ accompanied by OS promoted sarcomere assembly and the development of contractility during the myogenic process. The optogenetic techniques could be used to manipulate the activity-dependent process during myogenic development. No COI.
**ABS0421**

**Relationship between serum tri-iodothyronine(T3), thyroxin (T4) and thyroid-stimulating hormone(TSH) levels with major depressive disorder(MDD)**

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Depression is one of the most common psychiatric disorders. Over the last few years, the relationship between hypothalamic-pituitary-thyroid axis and depression has been the focus of attention. In this study, the serum levels of T3, T4 and TSH in patients with MDD have been compared to the healthy adults in Hamedan (Iran). In this study, serum levels of T3, T4 and TSH were measured in 32 patients with major depression (diagnosed according to Beck Depression Inventory) referred to psychiatric hospital and in 32 age- and sex-matched normal adults. Sampling was exerted through Convenience sampling by a completely randomized design. Data were analyzed using independent t-test and One-Way ANOVA. Logistic regression was used for depression occurrence probability prediction. Serum T4 and TSH levels were significantly higher in depressive than control group (P=0.01), whereas there was no significant difference in T3 serum levels between two groups (P=0.08). Serum TSH level was significantly higher in depressive compared to non-depressive groups (P=0.001). According to logistic regression analysis, a one unit increase in serum T4 or TSH level may enhance non-clinical depression probability by 1.3 or 1.7 times and clinical depression probability by 1.2 or 2.9 times, respectively. Serum T4 and TSH levels in depressive were significantly higher than control group, indicating the association between serum T4 and TSH level and depression. No COI.

**ABS0435**

**Visualization of neurotransmitter release in the rat-derived neurosphere cells using enzyme-linked photo-assay**

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The detection of neurotransmitter release gives us much knowledge of neuronal conditions. In order to observe the spatio-temporal transmitter release in cerebellar cortex, we have developed the enzyme-linked photo-assay system for glutamate, γ-amino butyric acid and ATP, with an immobilized specific enzyme on a quart surface and the CMOS camera. Using this device, we have succeeded in visualizing the transmitter releases in both developing and juvenile cerebellar slices. However, it has been unclear whether this system could apply to the detection of the transmitter releases from cultured cells. In this study, we proposed a new simple device for cell-level detection with both the two UV-LED lights at different angles, and a signal processing system for the reduction of autofluorescence. Using this improved system, we investigated the glutamate release in the differentiated rat-derived neurosphere. Cultured neural stem/progenitor cells derived from rat embryo were plated on uncoated dishes in a medium containing fibroblast growth factor (bFGF) and epidermal growth factor (EGF). After a week, the aggregated cells were transferred onto the coated glasses and induced their differentiation by removing bFGF and EGF. After 2-3 weeks of differentiation, both of spontaneous and stimulation-induced glutamate releases were observed using the new photo-assay system. In addition, we confirmed the expressions of beta3-tubulin, and a glutamate receptor 2/3 in the differentiated neurosphere. We suggest that the new photo-assay system would become useful to detect neurotransmitter releases in cultured cells. No COI.
EXERCISE PHYSIOLOGY / CARDIOVASCULAR PHYSIOLOGY

ABS0048

Creatine phosphokinase (CPK) and myoglobin as potential indicators for skeletal muscle training adaptation
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Altered exercise intensity may change pattern of many genes expression and metabolic fuel utilization of muscle tissues differently. Therefore determining optimal training intensity will also give us best outcome and also importantly, to avoid muscle injury. There is no really good marker for alarming athletes about their muscle condition during training. We proposed that creatine phosphokinase (CPK) and myoglobin which may release during muscle injury might be a potential indicator for early injury in skeletal muscle. We used male Wistar rat and trained them with different exercise intensity (15 and 25 m/min) for 30 minutes per day for 14 days. Rats were sacrificed under ether anesthesia after last exercise. Soleus muscle was dissected out for gene expression study and hematoxylin eosin (HE) staining. Then jugular vein was carefully exposed via small skin incision. Blood sample was collected and proposed for measuring CPK and myoglobin levels. We observed that at day fourteen, CPK level was increased double until 1587+15 U/L only in anaerobic compare to aerobic and control group. There is no significant difference between low and high intensity groups but it showed trend that CPK levels was higher compared to control and aerobic groups. In addition, we observed consistent increase of myoglobin mRNA expression until 1.5 +/- 0.1 arbitrary units in both groups and myoglobin levels in plasma were until 1.2 +/- 0.5 ng/ml in anaerobic group at day fourteen. Histological results showed that there was more muscle dystrophic area found only in group with high intensity exercise. Taken together, change in myoglobin and CPK may be able to reflect the physiological changes in muscle, and it may be a good marker for muscle injury. No COI.

ABS0182

The effects of hurdle aerobic exercise on neuroglobin, VEGF, and drebrin-A levels in the brain, and cognitive ability of middle aged mice
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Aerobic exercise has been proven to improve cognitive structure and function. Study about the type of aerobic exercise effects on angiogenesis, neuroplasticity and oxidative homeostasis in brain specific regions is still limited. The present study was conducted to investigate the effects of hurdle aerobic exercise on brain neuroglobin level, angiogenesis and neuroplasticity proteins in hippocampus and prefrontal cortex, and relational memory among middle aged CBS-Swiss strain mice. Mice, age 10 months were subjected to hurdle running wheel for 8 weeks. They ran at speed of 10 m/min, 30 min/day, and 5 days/week with hurdles for every 78 cm. Three types of hurdles were changed for every 3 days. Another group of same age mice ran at same speed, time, and period, without hurdle as comparison, while other control group never exercises. The hurdle group has significant higher level of developmentally regulated brain protein-A (drebrin-A) in hippocampus compared to non-hurdle group. Both of exercise groups have significant higher ability on paired associative cognitive test, and they have significant higher expression of vascular endothelial growth factor (VEGF) and higher level of drebrin-A compared to control. Neuroglobin level was not significantly different among all groups. More complex aerobic exercise has better effect on hippocampus neuroplasticity. Both types of aerobic exercise have better effect on angiogenesis and neuroplasticity in the brain, and also on cognitive function. Aerobic exercise does not resulting high hypoxic stress and could be tolerated by brain. No COI.
ABS0306

Prolonged QTc interval in rat after long-term exercise

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Long-term exercise causes cardiac remodeling or exercise-induced ventricle hypertrophy. Changes in cardiomyocyte structure due to intensive exercise training are accompanied by electrical remodeling, reflected on 12 ECG lead recording, and are associated with an increase of developing arrhythmia, which may lead to sudden death. This study aimed at recognizing electrocardiographic changes, specifically QTc interval changes, in rats undergoing long-term intensive exercise. Four groups of young adult male Wistar rats were randomly selected. Groups 1 and 2 were assigned as control group for ECG recording on week (4 and 8) and (12 and 16) respectively. Group 3 was given 4 weeks of intensive training, followed by 4 weeks of detraining, while group 4 was given 12 weeks of intensive training followed by 4 weeks of detraining. ECG examination was performed at the end of each period of training or detraining and compared to control group of the same period of age. P wave in exercising group was significantly increased. PR interval was significantly decreased in group 4 after detraining. QRS amplitude was increased although not significantly different compared to control group. However, a significant persistent prolonged QTc interval was observed in the exercising group (62.76±4.03ms and 64.24±3.78ms) compared to control group (48.88±2.15ms and 47.33±3.43ms). Detraining did not restore QTc interval (57.81±1.96ms and 61.16±5.02ms) vs (48.93±2.40ms and 48.13±1.66ms). The results showed cardiac remodeling after long-term intensive exercise caused ventricular hypertrophy with persistent repolarization disturbances after a period of detraining, indicated by an increase in QRS amplitude and a significant prolonged QTc interval. No COI.

ABS0082

Snapin Involved in the Atrial Fibrillation-Related Ca1.3 Calcium Channel Dysregulation

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Systemic deletion of Ca1,3 Ca2+ channels rendered mice susceptible to atrial fibrillation (AF). This study was designed to explore the mechanisms underlying the regulation Ca1.3 involved in atrial arrhythmogenesis. A novel Ca1,3 associated protein, snapin was identified using YHT system. By co-immunoprecipitation and immunostaining assays, a physical interaction and co-localization between snapin and Ca1.3 were confirmed in both heterologous expression system and mouse atrial myocytes. These were additionally addressed in GST pull down assay. Furthermore, both total and membrane expressions of Ca1.3 were significantly impaired by snapin overexpression, causing Ca1.3 ubiquitination-proteasome degradation. Accordingly, the densities of whole-cell ICa-L were significantly abated. Interestingly, in a tachypaced HL-1 cell model, enhanced expression of snapin while decreased that of Ca1.3 were documented by western blot analysis. Of note, the significant changes in the status of phosphorylation of snapin was revealed using proteomic analysis of the right appendages from AF patients, implying that Snapin involved in AF-related Ca1.3 Ca2+ channel dysregulation. The precise molecular mechanisms contributing to snapin related regulation of Ca1,3, particularly in AF condition, should be addressed in future detailed studies. No COI.
Nox2 contributes to the arterial endothelial specification of mouse induced pluripotent stem cells

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Reactive oxygen species (ROS) have a crucial role in stem-cell differentiation; however, the specific mechanisms by which ROS regulate the differentiation of induced pluripotent stem cells (iPSCs) into endothelial cells (ECs) have yet to be deciphered. We aim to determine whether ROS production by NADPH oxidase 2 (Nox2) promotes endothelial-lineage specification in mouse iPSCs (miPSCs). miPSCs were generated from wild-type (WT miPSCs) and Nox2-knockout (Nox2check for correct symbol miPSCs) mouse embryonic fibroblasts and then differentiated into ECs (miPSC-ECs). Measurements of ROS production and the expression of endothelial markers, arterial endothelial markers, pro-angiogenic cytokines, and Notch pathway components were all lower for Nox2check for correct symbol miPSC-ECs than for WT miPSC-ECs, and the declines of these genes expression were rescued by Nox2 or Notch1 overexpression, while higher levels of Nox2 expression or exogenous H2O2 increased Notch signaling and arterial EC differentiation, and this increase was abolished by inhibition of ROS generation or by silencing of Notch1 expression during the early stages of differentiation. The Nox2 deficiency in miPSC-ECs was associated with declines in the cell migration, proliferation, tube formation, and cell survival, as well as the vascularization of Matrigel plugs, while measurements of perfusion, capillary and arterial density, and Notch target gene expression were lower in the ischemic limbs of mice after treatment with Nox2check for correct symbol miPSC-ECs than after WT miPSC-ECs treatment. Nox2-mediated ROS production promotes arterial EC specification in differentiating iPSCs by activating the Notch signaling pathway and contributes to the potency of transplanted iPSC-derived ECs. No COI.
Effect of automated emails linked to Moodle quizzes on self learning
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'Step-by-step study of human life sciences' is an innovative digital educational material with straight-forward illustration/animation and simple multi-choice questions presented in very small steps for beginners. The materials were installed on Moodle, which was customized to automatically send a celebratory email immediately after submission of a quiz which was successfully passed, and an encouragement email immediately after submission of a quiz which was a not passed, and also a reminder email on 6, 5, 4, 3, 2, 1 day(s), and at 12 hr and 1 hr before the deadline of non-submitted quizzes. The present investigation studied whether or not such emails enhance self learning. An introductory ‘step-by-step’ material including 144 steps, 200 illustrations, 119 animations and 394 questions comprised the Moodle course with 27 quizzes. This was assigned to perspective students before entrance to a health care school to be completed by self learning in 15 weeks. Compared to the 2014 year students (n = 124), when the email function was not used, the 2015 students (n = 132) showed significantly higher results: quiz-submission rate: 87.6 ± 7.9% vs 92.5 ± 2.5% (avg±SD n = 27 p < 0.01), percentage of students passing each quiz: 86.0 ± 8.1% vs 91.2 ± 3.1% (p < 0.01), average score of each quiz: 92.6 ± 2.7 vs 93.8 ± 2.4 (points p < 0.01), and score of the evaluation paper test given in class upon completion of the introductory course: 85.9 ± 13.1 vs 89.1 ± 10.4 (points p < 0.05 t-test). The scores of a test given before the introductory course, however, were the same in both 2014 and 2015 students, 67.1 ± 12.9 vs 67.8 ± 15.4 points, respectively. In conclusion, automated emails linked to Moodle quizzes may enhance not only the amount of self online learning, but also the level achieved. No COI.

Early Clinical Exposure in Medical Students during Pre-Clinical Phase
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The objective of the present study is to introduce Basic Life Support (BLS) in 1st yr MBBS students and to evaluate the enhancement of their knowledge and develop clinical skills and attitudes right from 1st yr MBBS. In our traditional curriculum in 1st year MBBS, the students are mainly exposed to didactic lectures for development of cognitive domain, followed by practical and other teaching learning methods. However, very little attention is being paid to development of their clinical skills and attitude. With advancing times and technology, the Early Clinical Exposure (ECE) of MBBS 1st year students is very important so that they start developing competency based learning right from 1st yr MBBS. One of the teaching learning methods which can help in achieving this can be, by training the students about Basic Life Support. BLS hands on training of 1st year MBBS students will help in making students confident in dealing medical emergencies in future. The Study group consisted of 133 MBBS 1st yr Students of J.N Medical College, AMU. Lecture, Demonstration and Hands-on practice on Mannequin were used as Teaching and learning method. Evaluation was done by Pre & Post test by standardized validated Questionnaire. The Workshop evaluation was performed by Feedback questionnaire on Likert scale. There was statistically significant improvement in knowledge of students as shown from results of Pre Test when compared to Post test Questionnaire. 90.22% students found the Demonstration extremely useful and many were confident to do Basic Life Support (BLS) in future. 93.22% students were of the opinion that this sort of workshop should be included in 1st year MBBS curriculum. The results of this study suggest that the workshop provided the students with sound basic knowledge and adequate practical skills in BLS and many students were of the view, that BLS workshop should be carried out every year among Pre-clinical Undergraduate students which can form base for better competency based learning. Hence “Introduction of BLS in 1st year MBBS students will be a good early clinical exposure”. No COI.
A rarely existing natural sugar, D-allulose, prevents obesity and progression of diabetes in Type 2 diabetic OLETF rats

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Prevalence of global obesity has emerged as the single most lifestyle-related health problem. The inextricably interlinked pathological progression from excessive weight gain, obesity, and hyperglycemia to T2DM, typically originates from the overconsumption of sugar and high-fat diets. This situation warrants attentive consideration of alternative medicines that provide better protection with lesser side effects. Recently, we have discovered the advantages of a rare sugar D-allulose, a zero-calorie sweetener which has been identified as a non-toxic compound having strong anti-dyslipidemic and anti-hyperglycemic effects and thus represents to maintain blood glucose levels. 5% D-allulose fed for 60 weeks significantly maintained body weight (p<0.01), blood glucose (p<0.05) and insulin (p<0.05) levels than control rats. Oral glucose tolerance tests also showed significant reduction (p<0.01) of glucose rise by D-allulose at 30 and 60 weeks. D-allulose significantly reduced both body fat levels (p<0.5) and abdominal fat accumulation (p<0.01), and also markedly attenuated progressive beta-islet fibrosis evaluated by HE, Masson’s trichrome staining and immuno-staining for insulin, glucagon and alpha-smooth muscle actin. Serum pro-and anti-inflammatory adipocytokines were also controlled well. It is concluded that rare sugar D-allulose might be a promising strategy for the prevention of lifestyle-related diseases through controlling obesity, maintaining blood sugar, and preserving pancreatic beta-cells. No COI.

A hybrid signal processing of RR interval from QTc variation proving arrhythmia and improving heart rate variability assessment in acute stroke

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Abnormal QTc and arrhythmia (AF) with high risks for sudden death have been reported in stroke. Brain-heart axis by heart rate variability (HRV) is shifted affecting autonomic modulation with arrhythmic event during acute stroke. Missing beats of R-R interval have been shown contributing abnormal QTc and AF during acute stroke. In this study, we develop a hybrid signal processing by Pan Tompkins QRS detection and Kalman filter estimator for missing beat correction in order to examine proving AF with abnormal QTc and improving HRV. We investigate missing beat behavior in long QTc with AF and normal QTc with non-AF during 24 hours acute stroke and then assessed both groups by HRV analysis. Methods: fifteen acute stroke patients with LQTc-AF Kalman and NQTc-nonAF Kalman (seven men, eight women, age 65±4 years old) were studied. All subjects gave informed consent NO. MTU-EC-IM-4-018/54. QTc is determined by Bazett’s method. R-R intervals of Lead II ECG recordings were performed by Labchart. RR intervals were examined by hybrid signal processing and then by HRV. Comparison between both groups, mean heart rate, mean R-R interval and SDNN are significant difference. Mean R-R in LQTc-AF-Kalman is lesser than in NQTc-non AF- Kalman. Predominant parasympathetic activity indicating power drive for sympathetic vagal balance is evident in LQTc-AF-Kalman as shown by HF, SD2 and SD2/SD1. Obviously, greater SamEn is evident in LQTc-AF-Kalman group which it is correspond with an irregularity of signals in geometry of Poincaré plot. Compared with conventional Labchart, fractal scaling exponent of α1 (DFA) is greater in AF-stroke patient. This finding indicates remarkable complexity in physiological response associated with predominant parasympathetic drive. No COI.
This study was aimed to determine the effect of Citrus sinensis waste in ration on cholesterol and fat meat level of Padjadjaran sheep. Twenty (20) tail sheep male were randomly allocated to four (4) treatment groups as T1, T2, T3 and T4 with 5 sheep per treatment group replicated four times with one (1) sheep per replicate in a Complete Randomized Design (CRD). The sheeps in the control group (T1) were given normal basal diet without the addition of Citrus sinensis waste, while as other groups (T2, T3, T4) were supplemented with 7.0%, 12.0 % and 19.0 % waste respectively. The meat samples were randomly collected from two (2) sheep per replicate at the end experimental period (6th week) and analyzed for the estimation of meat cholesterol and fat. The results revealed that meat cholesterol and fat decrease in the groups fed citrus sinensis waste at various level when compare to the control Further, the highest cholesterol meat reduction of 54.71 mg/100gr in the group supplemented with 7% Citrus sinensis waste (T2) compared to 66.14 mg/100 mg in the control group, and the highest fat meat reduction of 10.48 mg/100gr in the group supplemented 19% Citrus sinensis waste (T4) compare to 24.15 mg/100 gr in the control group. In conclusion, dietary inclusion of Citrus sinensis waste had beneficial effect with regard to its ability in reducing the cholesterol and fat meat of Padjadjaran Sheep. No COI.

The beating cilia play a key role in the mucociliary transport system. The rate of mucociliary transport is controlled by two parameters regulating ciliary beating, ciliary beat angle (CBA) and ciliary beat frequency (CBF). Ambroxol (ABX), a mucolytic agent, is known to be a drug activating mucociliary transport. However, ABX actions on the ciliary beating remain uncertain. In this study, we examined the effects of ABX on CBA and CBF using bronchiolar ciliary cells of mice. Ciliary cells isolated from mice lungs by an elastase treatment were observed with a high speed camera (500 Hz) at 37 °C ABX gradually increased CBA and CBF via pH increase and [Cl–] decrease. 1) pHi pathway: ABX increased pHi by activating NBC, leading to increases in CBA and CBF. 2) [Cl–]i pathway: ABX elevated [Ca2+]i by activating nifedipine-sensitive calcium channels, resulting in cell shrinkage via an increase in Ca2+-dependent KCl efflux associated with a decrease in [Cl–], of ciliary cells. This decrease in [Cl–] increased CBA. In conclusion, ABX increased CBF and CBA via a pH increase by activating NBC and increased CBA via a [Cl–] decrease by causing cell shrinkage. Thus, ABX stimulated the ciliary beating coupled with transepithelial HCO3-/Cl– secretion by modulating activity of ion channels/transporters. No COI.
Differentiation forced by reactive oxygen species. The case of the NADPH oxidases Nox4
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Reactive oxygen species (ROS) play an essential role in cellular homeostasis and differentiation. One of the most important sources of ROS is the family of NADPH oxidases, which compromises 7 members (Nox1-5, Duox1 & Duox2). Out of those, Nox4 is special as it is ubiquitously expressed, is constitutively active and directly produces hydrogen peroxide. The expression of Nox4 is higher in differentiated than in undifferentiated cells and induction of Nox4 expression is needed for the differentiation of mesenchymal cells. In the cardiovascular system, the absence of Nox4 in endothelial cells induces activation of a pro-inflammatory phenotype as well as attenuation of angiogenesis. In a model of in vitro adipocyte differentiation, we identified Nox4 as a switch from insulin-induced proliferation towards differentiation. Even in the bone, Nox4 is involved in differentiation. In a study where we analyzed the role of Nox4 in osteoporosis, it was observed that Nox4 maintains intracellular calcium, via the µ-calpain/calcineurin/NFATc1 system promoting osteoclastogenesis. The ability of Nox4 to maintain cellular quiescence and differentiation together with its anti-inflammatory activity in vivo let us to analyze the role of Nox4 in cancer, where we found it to be protective. No COI.

Physiological regulation of cell surface expression of membrane transport proteins by an actin-binding protein, ezrin
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Ezrin is an actin-binding protein, which cross-links membrane proteins and actin cytoskeleton directly or indirectly through scaffold proteins such as Na+, H+-exchanger regulatory factors, NHERFs. It is concentrated on apical surface of many epithelial cells especially in small intestine, stomach, and kidneys. Here, we introduce several phenotypes of transgenic ezrin knockdown (Vil2kd/kd) mice, in which expression level of ezrin was decreased to less than 5% compared with the wild-type mice. In the kidney, ezrin is located at the brush border membrane of proximal tubules where it interacts with a Na+/phosphate cotransporter, Npt2a, through a scaffolding protein, NHERF1. The Npt2a and NHERF1 expressions at the brush border membrane were reduced in the Vil2kd/kd mice. As a consequence, the Vil2kd/kd mice exhibited hypophosphatemia, osteomalacia, and urinary loss of phosphate. These results suggest that ezrin is involved in cell surface expression of Npt2a and required for the regulation of systemic Pi homeostasis. In the liver, ezrin is specifically expressed at the brush border membrane of cholangiocytes, which are involved in modulating the fluidity and alkalinity of canaliculi bile. The CFTR, anion exchanger 2 (AE2), and aquaporin 1 (AQP1) expressions at the brush border membrane were impaired in the bile ducts of Vil2kd/kd mice. As a consequence, the Vil2kd/kd mice developed intrahepatic cholestasis characterized by extensive bile duct proliferation, periductular fibrosis, and intrahepatic bile duct accumulation. These results suggest that ezrin is involved in cell surface expression of CFTR, AE2, and AQP1, and required for modulating the canaliculi bile. No COI.
**ABS0512**

**Antiflammin-1 inhibits the TGF-β1 induced epithelial-mesenchymal transition in A549 cells through ERK pathway**

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Epithelial to mesenchymal transition (EMT) is a process by which an epithelial cell alters its phenotype to that of a mesenchymal cell and may play a critical role in the lung fibrosis. Antiflammin-1 (AF-1, MQMKKVLDS) is a synthetic nonapeptide with a similar sequence to the conserved sequence of uteroglobin (UG) secreted by lung Clara cells. Studies suggest that it has many biological functions. Our previous studies indicated that AF-1 could suppress the TGF-β1-induced EMT in A549 cells. This report is the first to demonstrate the cell signal pathway of AF-1 in inhibiting TGF-β1 induced EMT in A549 cells. A549 cells were seeded in culture dish and grown for 24 h. Before the experiments, the medium was changed to the incubation medium containing TGF-β1 (5 ng/mL) in the absence or presence of AF-1 (100 μM) and with or without ERK inhibitor (10 μM). Then, cells were cultured for an additional 48 h. After that, cells were lysed in RIPA buffer and the expressions of E-cadherin and α-smooth muscle actin (α-SMA) were analyzed by western blot. The results showed that ERK inhibitor had no effect on TGF-β1 induced EMT in A549 cells. However, the effect of AF-1 was reversed by pretreatment with ERK inhibitor. In conclusion, AF-1 can inhibit TGF-β1 induced EMT in A549 cells through ERK pathway. No COI.

**ABS0390**

**TLR4 triggered inflammation signaling pathway was inhibited by integrin β4 on airway epithelial cells**

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Excessive or prolonged activation of the TLRs triggered inflammation immune response can lead to a cytokine storm, which results in pathological tissue damage and even lethal septic shock. To prevent that, TLR signaling is subject to negative regulation and feedback inhibition that tightly control the magnitude of the innate immune response. Although many factors negatively regulate TLR signaling to avoid excessive production of proinflammatory cytokines, the detailed mechanisms is remain unclear. On the airway epithelial cells of asthma patients, excessive inflammation reaction existed along with decreased expression of integrin β4. Given that integrin β4 engaged in multiple signaling pathways, we studied whether disruption of integrin β4 may regulate the TLR4 triggered inflammation pathway. Here, we silenced integrin β4 expression with an effective siRNA vector and studied the effects of integrin β4 silencing on the TLR4 inflammation pathway by ELISA and immunoblot analysis respectively. We found that integrin β4-deficient mice have more production of proinflammatory cytokines when challenged with endotoxic shock. Integrin β4 inhibited TLR4 signaling by activating the tyrosine kinases Src which could induced phosphorylation degradation of downstream signaling molecules MyD88 and TRIF. Thus, our results provide evidence that integrin β4 negative regulate TLR4 signaling by phosphorylation degradation of MyD88 and TRIF through Src. It would contribute to better understand of the mechanisms of the regulation of TLR4 triggered innate inflammatory responses on airway epithelial cells. (This work was supported by grants #81270065, #81370116 from NSFC and grant#2013JJ4030, #2015JJ2147 from Hunan Natural Science Foundation). No COI.
ABS0514

NMDA receptors activation promotes epithelial-mesenchymal transition in MLE-12 cells

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Background: Pulmonary fibrosis is a sever disease which threaten human health, with progressing myofibroblast proliferation, extracellular matrix accumulation. Epithelial-mesenchymal transition (EMT) is an important resource of myofibroblast in pulmonary fibrosis. We had found that NMDA receptors participated in bleomycin induced acute lung injury. Whether NMDA receptors activation contributes to EMT remains unclear. Method: Mice alveolar epithelial cell line MLE-12 cells are treated with NMDA or TGFβ1 for 48h. Mesenchymal cell marker α-SMA and alveolar epithelial cell markers E-cadherin expression and phosphorylation of MAPK signal pathway were detected by western blot. Results: NMDA receptors expressed on MLE-12 detected by RT-PCR and western blot. 10 mM NMDA can increase α-SMA mRNA expression (P<0.01) and protein expression, and decrease E-Cadherin protein expression (P<0.05) in MLE-12. The phosphorylation levels of MAPKs were increased, including ERK, JNK, p38 protein. Conclusion: 10 mM NMDA can induce EMT and MAPKs phosphorylation in MLE-12. NMDA receptors activation promote EMT may be involved in MAPKs phosphorylation. No COI.

ABS0515

Mobilization of bone marrow cells by G-CSF inhibits the bleomycin induced pulmonary fibrosis

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Background: Pulmonary fibrosis is a progressive disorder characterized by the excessive proliferation of fibroblasts and deposition of extracellular matrix, which destroy normal tissue architecture and function. The mechanisms of pulmonary fibrosis are not completely understood, and the effects of drugs on idiopathic pulmonary fibrosis (IPF), a fatal respiratory disease in humans, are not satisfactory. The granulocyte colony stimulating factor (G-CSF) can mobilize bone marrow stem cells from bone marrow into peripheral blood and make them migrate to the damaged site, which may play an important role in tissue injury and repair process. Method: To investigate the antifibrotic effect of bone marrow stem cells which are mobilized by G-CSF, mice were randomly divided into four groups: control group, G-CSF(40 μg/kg/d) group, BLM group and BLM+G-CSF(40 μg/kg/d) group. The collagen content was examined by hydroxyproline (HYP) assay and the expression of procollagen I and procollagen III were quantified by real time PCR. The histopathological observation was also used to evaluate the degree of pulmonary fibrosis. Results: Bleomycin induced an increase in the HYP content and G-CSF significantly reduced the HYP content. We also found that G-CSF decreased the expression of procollagen I and procollagen III in bleomycin-treated mice. The severe fibrosis was found using light microscopy in all bleomycin-treated mice. In contrast, the pulmonary fibrosis was markedly alleviated in the G-CSF treated mice. Conclusion: The bone marrow stem cells, which are mobilized by G-CSF, have a protective effect against pulmonary fibrosis. No COI.
Lesion of medulla’s catecholaminergic neurons is associated with cardiovascular dysfunction in rotenone-induced Parkinson's disease rats

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Non-motor symptoms are of vital importance in Parkinson’s disease (PD) in recent years, among which cardiovascular dysfunctions are commonly seen in PD patients before their motor signs. It is largely unknown the role of cardiovascular dysfunction in the progression of PD pathology and its underlying mechanisms. In the present study, in rotenone-induced PD rats, there was a gradual reduction in the number of nigral tyrosine hydroxylase-immunoreactive (TH-ir) neurons after 7, 14 and 21 days treatment. With the 56% reduction of striatal dopamine content and 52% loss of TH-ir neurons on 14th day, the rats showed motor dysfunctions. However, normalized LF power (LFnu), low-frequency power (LF)/high-frequency power (HF) ratio and mean blood pressure (MBP) reduction was observed as early as the 3rd day. Plasma norepinephrine (NE) and epinephrine (E) levels were decreased by 39% and 26% at the same time, respectively. Pearson’s correlation analysis showed that either plasma NE or E levels positively correlated with MBP. Our results also showed that only the loss of catecholaminergic neurons in the rostral ventrolateral medulla (RVLM) emerged earlier than the nigral dopaminergic neurons, neither that of the caudal ventrolateral medulla (CVLM) nor the nucleus tractus solitarii (NTS). These suggest that dysfunction of catecholaminergic neurons in the RVLM might account for the reduced sympathetic activity, MBP and plasma catecholamine levels in the early stage of PD. No COI.

Long term follow up study of at-risk children for developing sensorineural hearing defects using brain stem auditory evoked potentials (BAEP)

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Long-term follow up of hearing impaired infants is important to decide on therapeutic interventions. Our objective was to evaluate the long-term hearing outcome of at-risk infants. Initially we evaluated 19 infants who had ≥1 risk factors for hearing impairment (viz. prematurity <37weeks, birth weight <1.5 kg, meningitis, septicemia, exposure to ototoxic drugs, neonatal intensive care unit stay >5 days and mechanical ventilation) using brain stem auditory evoked potentials (BAEPs). The degree of impairment was stratified according to BAEP thresholds (normal: 30dB, mild: 31-40dB, moderate: 41-55dB, moderately severe: 56-70dB, severe: 71-90dB). We followed them up after 6 months and 4-5 years clinically and with repeat BAEP testing. All had undergone conservative management. Eight of 19 infants had elevated hearing thresholds initially. Of them, five had moderately severe impairment while one each had mild, moderate and severe impairment. At 6 months, five had normal thresholds, while three (one severe, one moderately severe and one moderate) did not show any improvement in BAEPs. At 4-5 years, all had improved their BAEP thresholds to normal, except the infant with severe impairment who remained severely impaired with a speech delay. The infants with normal hearing thresholds remained normal in both follow up assessments. Our data suggest that there is improvement in hearing status in most infants with mild to moderate impairment while severely impaired may need therapeutic interventions. Large scale studies are necessary to examine how the pattern of recovery depends on different risk factors. No COI.
ABS0139  Young Scientist Award

Experimental study: Neural stem cell density in hippocampal versus lateral ventricle regions of the mouse brain and use of gelatin and collagen as alternative neural differentiation matrices
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In-vitro grown neural stem cells (NSCs) are vital for research into neurological diseases and injury. Readily available sources of neural cells are rare, therefore it is important to identify suitable neurogenic sites for NSC harvesting and develop low cost protocols for NSC growth. Variations in NSC density in hippocampus and lateral ventricle regions of the mouse brain, and parallels between mouse and human brains have been reported. Protocols for NSC differentiation use poly-D-lysine, laminin and Matrigel as neurosphere attachment matrices. We aimed to confirm regional variations of NSC density in mouse brains and to establish a protocol for in-vitro growth of NSCs using gelatin and collagen as differentiation matrices. Tissue sections from the hippocampus and lateral ventricle regions of two, 8-week-old ICR strain mice were grown in NSC culture and differentiation media (Stemcell Technologies) according to standard protocol. The cultures were observed for neurosphere formation, serially passaged to validate their true stemness, and population counts of NSCs from both sites were taken. At passage 2, the neurospheres were allowed to differentiate into neurons. Neuronal differentiation was confirmed using cytoplasmic Nestin IgG1 antibody and nuclear DAPI counterstaining. Our observations were that NSC density in the lateral ventricle region was significantly higher (p=0.038) and passage 0 lateral ventricle derived neurosphere clusters were more prominent than hippocampal derived NSCs. These results are comparable with previously reported data on NSCs isolated from the mouse brain. Gelatin and collagen could be used as alternative matrices for NSC differentiation; this needs to be validated. No COI.

ABS0152  Young Scientist Award

Perfusion of adrenergic agonists into the preoptic area and anterior hypothalamus do not affect thermoregulatory responses in the rat.
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It has been reported that catecholamines (noradrenaline: NA and dopamine: DA) in the brain plays an important role in thermoregulatory mechanism. However, it is not clear relationship between thermoregulatory mechanism and individual NA subtypes, despite the different pharmacological manipulations. Therefore, the purpose of this study is to clarify the effect of individual NA subtypes on thermoregulatory systems using freely moving techniques. Wistar rats were used for this study and we selected two adrenergic agonists, Cirazoline (α1-adrenocepter agonist) and Clonidine (α2-adrenocepter agonist), in order to verify the effect of individual NA subtype in the preoptic area and anterior hypothalamus (PO/AH). We perfused these drugs to the hypothalamus using a microdialysis in order to prevent some damages to the brain per se. We measured core body temperature (Tcore), tail skin temperature (Tskin), oxygen consumption (VO2), concentrations of DA, NA and serotonin (5-HT) in the PO/AH, which is the center of thermoregulation. These drugs are perfused over a 1 h, and all experiments were carried out same ambient temperature (23 °C). Perfusing with Cirazoline (100 μM) and Clonidine (100 μM) to the PO/AH, NA levels were significantly increased (at 30 min, 201 ± 31%; 212 ± 47%, respectively) compared with control condition in the PO/AH (p<0.05), while DA and 5-HT levels in the PO/AH did not change. Although NA level in the PO/AH significantly changed, Tcore, Tskin and VO2 was not altered by perfusing with Cirazoline and Clonidine. These results suggest that the increase of α1 and α2-adrenocepters in the PO/AH do not affect the thermoregulatory responses. No COI.
Correlated pallidal activity during voluntary reaching movements in a macaque monkey

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The basal ganglia (BG) play a crucial role in control of voluntary movements. There are many reports on movement-related activity in the internal (GPi) and external (GPe) segments of the globus pallidus: the former sends BG outputs to the thalamo-cortical and brainstem motor systems, and the latter projects to many areas of the BG and may control whole BG activity. On the other hand, task-related correlated activity, which is suggested to represent neuronal information in other brain areas, has yet to be examined. In the present study, we recorded neuronal activity in the GPi/GPe of a behaving monkey and analysed their correlations. A female Japanese monkey (Macaca fuscata) was trained to perform a voluntary reaching task with her hand to the left or right target, which was instructed by LED. Activity of GPi/GPe neurons was recorded using a multichannel electrode with 16 equally spaced contacts by 150 µm. Following results were obtained. (1) GPi/GPe neurons responded to cortical stimulation through chronically implanted electrodes in the hand regions of the motor cortices, and their response was mainly composed of early excitation, inhibition and late excitation. (2) GPi/GPe neurons changed their activity in relation to reaching movements. (3) GPi/GPe neurons increased their correlated activity during movement period, while no correlations were observed in other periods, such as around target presentation and reward release. These results suggest that GPi/GPe neurons change firing correlations as well as firing rates and transfer movement-related information to target structures. No COI.

Therapeutic comparisons of three iron chelators in the brain of iron-overload rats

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We previously showed that iron overload caused blood brain barrier (BBB) break down, brain mitochondrial dysfunction and cognitive decline. Iron chelators, including desferoxamine (DFO), deferiprone (DFP) and desferasirox (DFX), are widely used to treat iron overload to protect cardiac and liver functions. However, their neuroprotective effects under the iron overload have not been investigated. The present study was 1) to investigate whether iron overload constructed the BBB breakdown, induced brain mitochondrial dysfunction and decreased dendritic spine density, and 2) the administration of iron chelators can reverse these impairments. Male wistar rats were divided into two groups to receive either normal diet (ND, n=6) or high-iron diet (HFe: 0.2% Fe/kg diet, n=24) for total 4 months. At 2nd-month, HFe-fed rats were subdivided into four subgroups to receive vehicle (0.9% NSS), DFO (25 mg/kg), DFP (75 mg/kg) or DFX (20 mg/kg), while ND group was orally received vehicle. At the end of experiment, animals were sacrificed and brains were rapidly removed to determine brain iron level, brain mitochondrial function, the expression of BBB protein (occludin) and dendritic spine density. We found that HFe-fed rats treated with vehicle demonstrated increased occludin expression, indicating BBB breakdown, increased brain iron level, induced brain mitochondrial dysfunction and reduced dendritic spine density, compared with ND-fed rats. The administration of DFO or DFP, but not DFX, in HFe-fed rats significantly reduced these impairments. Our findings suggest that iron overload can induce brain iron toxicity and iron chelators, particularly DFO and DFP, have the beneficial effects on the brain. No COI.
The probiotic therapy with Lactobacillus paracasei increased cognitive function in obese-insulin resistant rats

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Chronic high-fat diet (HFD) consumption causes not only peripheral insulin resistance, but also impaired cognition. Recent studies demonstrated that probiotic therapy attenuated gut inflammation and improved glycemic control. However, the effects of probiotics on insulin sensitivity and cognition in obese-insulin resistant model have never been investigated. The present study hypothesized that the administration of probiotics increases insulin sensitivity and improves cognitive function of obese-insulin resistant rats. Sixteen male wistar rats (200-250 g) were divided into 2 groups to receive either normal diet (ND) or high-fat diet (HF) for 12 weeks. At week 13, each group was subdivided into 2 subgroups to receive either vehicle or probiotics (10^7 colony forming unit (cfu)/day of Lactobacillus paracasei HP4) for 12 weeks. At the end of the experimental protocol, rats were determined cognitive function by Morris water maze test, before determining insulin sensitivity by oral glucose tolerance test (OGTT).

We found that HF-fed rats with probiotics improved cognition by decreased time to reach platform and increased time spent in target quadrant, when compared those rats with vehicle (p<0.05). In addition, probiotics significantly improved insulin sensitivity in HF-fed rats by decreased AUg of OGTT (p<0.05; 2.78±1.0 AUg in probiotic vs. 3.5±0.5 AUg in vehicle). These findings suggest that probiotics improves insulin sensitivity and attenuated cognitive decline in obese-insulin resistant subjects. No COI.

Rice bran protein hydrolysates alleviate metabolic syndrome and vascular remodeling in high carbohydrate, high fat-fed rats

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Protein hydrolysates-derived from rice bran (RBPH) possess strong antioxidant and angiotensin converting enzyme (ACE) inhibition activity. Diet-induced metabolic syndrome (MS) is an experimental model that shares ethologic and pathophysiology with MS in humans. This study aimed to establish whether RBPH could alleviate MS and vascular remodeling in a rat model of MS. Male Sprague-Dawley rats were fed with high carbohydrate, high fat (HCHF) diet and 15% fructose in drinking water for 16 weeks. RBPH (250 or 500 mg/kg/day) was orally administered to HCHF diet-fed rats for the last 6 weeks of the experiment. After 16 weeks, rats fed with HCHF diet had developed hypertension, dyslipidemia, hyperglycemia, impaired vascular function, and elevated oxidative stress. RBPH dose-dependently normalized blood pressure, reduced blood glucose, improved glucose tolerance, and lowered serum triglyceride concentration of HCHF-fed rats. RBPH also restored vascular function and vascular remodeling by increasing vascular responsiveness and reducing mesenteric arterial wall thickness, medial wall thickness to lumen diameter ratio, and MMP-2 and MMP-9 levels. Improvement of MS was associated with a reduction of plasma ACE, plasma measures of oxidative stress and inflammation, and up-regulation of arterial eNOS protein expression. These findings clearly suggest that RBPH reduces MS in HCHF-fed rats by enhancing NO bioavailability and reducing oxidative stress and inflammation. NO COI.
Vascular hyperpermeability remains the main cause of underlying disorders in myocardial ischemia and atherosclerosis. Tualang Honey (TH) has been used in traditional medicine for decades and proven to possess multiple pharmacological actions. However, to date, little is known about the use of TH in anti-inflammatory activity specifically in endothelial barrier protection. Thus, this study aimed to investigate the effects of TH on H2O2-induced endothelial hyperpermeability. In order to determine the effect of TH on endothelial hyperpermeability, HUVEC was pre-treated with pre-defined non-cytotoxic concentration (via MTT assay) of TH for 4 h and then exposed to 0.5 mM H2O2. FITC-dextran was used as permeability indicator. To examine the morphological alterations, adherence junction proteins in HUVEC were identified using Fluorescein Phalloidin and β-catenin immunofluorescence labeling. Intracellular calcium and cAMP signaling were also investigated. All data was analyzed using SPSS. LD50 of TH was found to be 3.7% and concentrations ranging from 0.01% - 1% showed no cytotoxic effect to HUVEC. Induction with H2O2 was found to increase HUVEC permeability but the effect was significantly reversed by TH (p<0.05), of which the permeability inhibition peaked at 0.1% (83.10%), while capable to maintain the level of cAMP when induced with H2O2. In conclusion, TH ameliorates H2O2-induced endothelial hyperpermeability via suppression of adherence junction protein re-distribution and calcium level. No COI.
Young Scientist Award

Role of TRPC3 in a Slow Force Response to Stretch on Mice Cardiomyocytes
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When cardiac muscle is held in a stretched position, its intracellular Ca2+ transient and twitch force slowly increase over several minutes. This response is called a slow force response to stretch (SFR). The stretch-induced release of angiotensin II has been implicated in the SFR, to raise intracellular Na+, followed by an increase in intracellular Ca2+ via Na+/Ca2+ exchanger. However, the cation (Na+) influx pathway remains unclear. TRPC3 is known as receptor-operated cation channel. We focused on the functional linkages between angiotensin II type 1 (AT1) receptor and TRPC3 via diacylglycerol (DAG) on SFR. Mouse ventricular myocytes were enzymatically isolated. A pair of carbon fibers was attached to each cell end to apply stretch. The myocytes were electrically stimulated (1 Hz) in normal Tyrode solution at room temperature. The Ca2+ transient was measured with Fura-4F. The myocytes were stretched for 300 seconds. The stretch slowly increased the Ca2+ transient. AT1 receptor blocker (Olmesartan), DAG inhibitor (U-73122) and TRPC3 inhibitor (Pyrazole-3) significantly suppressed the SFR. The SFR tended to be depressed in TRPC3 knockout mice. Then, we used Angiotensin II, instead of stretch, to record the slow increase in Ca2+ transient. U-73122 and Pyrazole-3 significantly suppressed this increase. To speculate on the potential location of TRPC channels, we used the mathematical cardiomyocyte model with cation channels on either sarcolemma or sarcoplasmic reticulum. The model with cation channels on sarcolemma successfully reproduced SFR, while the other did not. These results suggest that TRPC3, activated by AT1 receptor on sarcolemma, is involved in SFR. This study is supported by Daiichi Sankyo Co., Ltd., which provided Olmesartan.

Reducing reperfusion injury in diabetic myocardium through combined postconditioning with ischemia and cyclosporine-A: oxidative stress and histo-pathological changes
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Protecting the diabetic heart would have important clinical outcomes. We evaluated whether it is possible to protect the diabetic heart against reperfusion injury through concomitant application of ischemic-postconditioning (IPostC) and inhibition of mitochondrial permeability transitions pores by cyclosporine-A (CsA). Streptozocin-induced diabetic rat's hearts and non-diabetic controls in eight subgroups (6 rats/each; with or without receiving IPostC, CsA or both of them) were received 30-min regional ischemia followed by 45-min reperfusion. The levels of lactate dehydrogenase (LDH), and oxidative stress markers including 8-isoprostane, superoxide dismutase, glutathione peroxidase and total antioxidant capacity in myocardial supernatant of ischemic zone and histopathological studies (using hematoxylin-eosin staining) were assayed. Administration of IPostC and CsA (alone or together) in non-diabetic hearts significantly reduced the severity of histological changes and level of LDH release and oxidative stress as compared with untreated-controls (P<0.05). Alone administration of procedures in diabetic hearts did not show significant cardioprotection (P>0.1). However, the combined postconditioning with ischemia and CsA exerted significant protection in diabetic hearts and this was associated with decreased 8-isoprostane level and increased antioxidant capacity in both diabetic and non-diabetic hearts (P<0.05). Therefore, with enforcing the protective effects of IPostC and CsA through their combined application at the onset of reperfusion, the cardioprotection in diabetic heart is achieved. No COI.
Elucidation of endocrinological basis on sexual behavior in postmenopausal female Japanese macaques

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Generally in mammals, sexual behavior is for reproduction. Sexual behavior in female mainly depends on estradiol, which increases both the sexual motivation of the female and her attractiveness to males. In primates, however, sexual behaviors occur in non-reproductive context. In Japanese macaques (Macaca fuscata), it has been reported that postmenopausal females copulate as frequently as young females. We conducted study on sexual behavior of females after menopause to understand the endocrinological basis of sexual behavior and its purposes. Study site was Arashiyama Monkey Park in Kyoto, Japan. Fourteen aged females which were born before 1986 were selected as subjects. Behavioral observation and fecal sampling were conducted by a single observer during 2013-2014 mating season. We collected 746 fecal samples from 14 females, and analyzed contents of estrone conjugates and pregnanediol-3-glucronide by enzyme immunoassays. Sexual behaviors were observed in 8 of 14 females which were estimated to be in postmenopausal phase, but there was no correlation between sexual activity and hormonal levels. On the other hand, females which had grooming relationship with males appeared to copulate more frequently. Although we need more studies, the present observation suggests that aged females may have used grooming and copulation for similar purposes, to have opportunities for social communication and/or social reward. No COI.

Mitochondrial dysfunction with increased inflammatory levels in salivary glands of obese-insulin resistant rats without hypo-salivation

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Obesity leads to the development of insulin resistance and hypo-salivation. Our previous studies demonstrated that 12-week high-fat diet (HFD) consumption caused insulin resistance and mitochondrial dysfunction in heart and brain, leading to cardiac and brain dysfunction. However, the effects of obese-insulin resistance on salivary gland function as well as salivary mitochondria have not been investigated. The present study hypothesized that obese-insulin resistance caused salivary mitochondrial dysfunction, leading to damaged salivary gland. Twelve male Wistar rats were divided into two groups to receive either normal diet (ND) or high-fat diet (HFD) for 12 weeks. At the end of week 12, blood sample from each rat was collected to determine the metabolic parameters. Salivary flow rate was measured in each rat, before being sacrificed. Then, submandibular salivary gland was removed to 1) measure the inflammatory levels and 2) to determine salivary mitochondrial function. The results showed that HFD-fed rats developed peripheral insulin resistance, characterized by hyperinsulinemia with euglycemia. The salivary flow rate was not significantly different between the two groups. However, an increase in inflammatory cytokines (TNF-α and TGF-β) was observed in submandibular gland of HFD-fed rats, when compared with ND-fed rats (p<0.05). In addition, the isolated submandibular mitochondrial dysfunction, as indicated by increased ROS production, membrane potential depolarization and mitochondrial swelling was found in HFD-fed rats. These findings suggest that obese-insulin resistant condition leads to defective salivary gland by inducing mitochondrial dysfunction and initiating inflammation, even though change in the salivary flow rate could not be observed. No COI.
ABS0315
Pancreatic 5-HT stimulates insulin secretion through 5-HT4 receptors sited on the islet β-cells
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Backgrounds and aims: 5-HT involves the regulation of blood glucose. It is reported that activating 5-HT1A receptor (5-HT1AR) and 5-HT2B/2CR could induce a hyperglycemia by facilitating adrenaline release, while application of 5-HT4R agonist produced a decrease in the level of blood glucose. The underlying mechanism is unknown. Methods: In the present study, immunofluorescence, western blot, HPLC, radioimmunoassay, pancreatic tissue incubation, INS-1 cell culture and SD rat were employed. Results: The enzymes involving 5-HT synthesis, tryptophan hydroxylase (TPH) and L-aromatic amino acid decarboxylase (L-AAAD) were examined by means of double-label immunofluorescence. The TPH and L-AAAD immunoreactivities (IR) were respectively distributed in the exocrine acinar cells and endocrine islets. L-AAAD-IR was co-localized with insulin-IR, glucagon-IR, somatostatin-IR, and polypeptide-IR. Incubation of INS-1 cells with 10μM 5-HT precursor, 5-hydroxytryptophan (5-HTP) increased 5-HT content in supernatant from 0 to 9.53±1.69 ng/ml. 5-HTP and 5-HT were detected in pancreatic juice respectively 87.22±16.81, 453.0±18.32 ng/ml. 5-HT4R was expressed in rat islets. 5-HT4R-IR was only co-localized with insulin-IR. Under a high glucose stimulation, 5-HT4R agonist, mosapride 2.5 mg/kg significantly decreased blood glucose at 15 minutes (from 15.08±2.411 to 11.59±2.047 uIU/ml, P<0.05) and 30 minutes (from 14.69±2.27 to 12.03±0.78 uIU/ml, P<0.05). Similar result was also observed with prucalopride, another 5-HT4R agonist. Treatment pancreatic tissue with mosapride and prucalopride significantly increased the insulin secretion by 50%. Conclusion: Pancreatic 5-HT is able to elicit islet insulin secretion through 5-HT4R. No COI.

ABS0441
Estrogen secretion and estrogen-producing enzymes expressions in the male goat gastrointestinal tract (GI)
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Estrogen is mainly produced in gonads, but also in extragonadal tissues, such as adrenal cortex, skin and brain. Among numerous physiological functions, estrogen is known to stimulate cell proliferation and differentiation. Rat gastric parietal cells express steroidogenic enzymes and secrete 17β-estradiol (E2) into the portal vein. Since digestive system of rodents differ from ruminants, and estrogen production in ruminant’s GI tract could have unique functions, this study was performed to study estrogen production in adult male goat GI tract. To detect the difference of steroid hormones concentrations between portal vein and mesenteric artery, blood from portal vein and mesenteric artery was collected and measured by radioimmunoassay. To find out which parts of GI tract can express estrogen-producing enzyme mRNA and aromatase protein, samples were harvested from stomach, small intestine and large intestine. Progesterone concentrations were higher in mesenteric artery, but testosterone and estrogen concentrations were slightly higher in portal vein. Expressions of P450scc, 3β-HSD, 17β-HSDtype2 and P450arom were analyzed. There was a weak expression of P450scc in GI tract. 3β-HSD was expressed strongly in jejunum, duodenum and weakly in other parts of GI tract. 17β-HSD type2 was expressed in rumen, abomasum, reticulum and duodenum. P450arom was expressed strongly in the body and pylorus of abomasum. Immunohistochemistry results demonstrated that the mucosa cells of abomasum are the major source of aromatase enzymes. In conclusion, abomasum is suggested to be responsible for estrogen production in the GI tract. No COI.
Phytosterol (PS) which has been widely used as cholesterol lowering agent since 1950 was found to decrease LDL-C and prevent atherosclerosis. In the body, adrenal gland has the highest rate of uptake of plant sterols on a weight basis when compared to the gonads and other tissues. On the other hand, PS disrupts the reproductive function in the zebrafish and causes infertility in male and female sterolin-deficient mice. Also PS overloading disrupts the adrenal function in male Japanese quail. Thus, we hypothesize that feeding PS in a higher dose will disrupt the adrenal function in quail. Two experiments were conducted: In the 1st study, adult males and females were subcutaneously (SC) injected with PS (8, 80 and 800 mg/kg BW) and exogenous E2 (10 mg/kg) in one shot. After injection, blood samples were collected at 3, 6 and 24 h to see the acute direct effects of PS. In the 2nd study, PS was gavaged (same doses as in the 1st exp.) into the crop sac of treated animals. After 44 days, 6-day ACTH challenge was performed to artificially stimulate the adrenals and to study long term effects of PS. Results: PS significantly increased the corticosterone (CORT) levels in male and female after SC injection. In long term, PS decreased body and adrenal weight in both sexes. Moreover, CORT level was increased in the ACTH challenged animals. In conclusion, PS food-additives in higher doses enhanced adrenal function and increased the CORT level as a consequence. No COI.
ABS0211  Young Scientist Award
Combined preconditioning with cinnamon extract and aerobic training reduces oxidative stress following myocardial reperfusion injury in rat model
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Coronary artery disease is one of the main causes of death worldwide. Nowadays, using natural strategies for prevention of ischemic heart disease is very important. The aim of this study was to evaluate the combined effects of cinnamon extract and aerobic exercise on the oxidative stress following myocardial ischemia-reperfusion (IR) injury in a rat model. Wistar male rats were divided in 4 groups (6 rats/each), including control, cinnamon, exercise, and combination of cinnamon and aerobic. The aerobic exercise was performed on a treadmill and cinnamon extract (200 mg/kg) was administered by gavage for a month. The isolated hearts of rats were received regional ischemia for 30 minutes and reperfusion for 60 minutes. The indicator of tissue damage (lactate dehydrogenase), the marker of lipid peroxidation (malondealdehyde) and myocardial antioxidant enzymes (superoxide dismutase and glutathione peroxidase) were measured with specific kits and ELISA on samples obtained from ischemic tissue. The lactate dehydrogenase level was significantly decreased in group receiving combination of cinnamon and aerobic exercise in comparison with control group (p<0.05). In addition, each of aerobic exercise and cinnamon extract significantly increased the values of antioxidant enzymes, and this effect was greater in combined group than those of individual treatments. The amount of malondealdehyde in the combined treatment was significantly reduced as compared with controls (p<0.05). Therefore, combination of aerobic training with cinnamon supplementation has better cardioprotective influences, and cinnamon may increase the aerobic exercise potency in enhancing the heart antioxidant capacity against oxidative insult in reperfusion injury. No COI.

ABS0225  Young Scientist Award
Fibroblast growth factor 21 improved cardiac function and cardiac autonomic regulation by attenuates metabolic disturbance, inflammation, and oxidative stress in obese insulin resistance rats
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Long-term high-fat diet (HFD) consumption leads to metabolic disturbance, inflammation, oxidative stress and insulin resistance which causes cardiac autonomic dysregulation and impaired cardiac function. Fibroblast growth factor 21 (FGF21) is the novel peptide which plays a role in metabolic regulation and cardioprotection in myocardial injury. However, the effects of long term FGF21 administration in the heart in HFD-induced obese-insulin resistance have not been investigated. We tested the hypothesis that long term FGF21 administration attenuates metabolic disturbance, inflammation, oxidative stress and increased insulin sensitivity leads to increased cardiac autonomic regulation and cardiac function in obese-insulin resistant rats. Rats were fed either normal diet (ND) or HFD for 12 weeks. Then, rats in the HFD group were divided into 2 subgroups to receive either vehicle or rhFGF21 (0.1 mg/kg/day) injected intraperitonealy for 28 days. Then, metabolic parameters, serum TNF-α, serum and cardiac tissue malondialdehyde (MDA), heart rate variability (HRV), and left ventricular (LV) function were determined. The results showed that FGF21 improved metabolic parameters, insulin sensitivity, attenuate serum TNF-α, and serum and cardiac tissue MDA. Moreover, FGF21 decreased LF/HF ratio and increased LV function by increased %fractional shortening. Our data indicate that FGF21 improved metabolic parameters, insulin sensitivity, oxidative stress, and HRV, leading to improved cardiac function in obese-insulin resistant rats. No COI.
Recanalization of occluded coronary arteries is a gold standard of treatment for acute myocardial infarction (AMI). However, reperfusion itself can cause myocardial damage, which is known as ischemia/reperfusion (I/R) injury. Several pharmacological interventions have been extensively studied to reduce myocardial damage from reperfusion injury, but conclusive evidence in the clinical setting has been lacking. Recently, a 24-amino acid peptide Humanin has been demonstrated to exert an anti-oxidative effect. However, its beneficial effects against reperfusion injury in AMI have not been investigated. We tested the hypothesis that Humanin exerts its cardioprotection against I/R injury through anti-oxidative effect and cardiac mitochondrial protection. Twenty male rats were divided into 4 groups. Rats were subjected to 30-min of left anterior coronary artery (LAD) occlusion followed by a 120-min reperfusion. In groups 1 and 2, saline or Humanin analog (HNG, 84 µg/kg) was injected (IV) at 15 min before LAD occlusion. In groups 3 and 4, saline or HNG was injected at 15 min after LAD occlusion. The arrhythmia incidence, infarct size, and mitochondrial function were determined. We found that HNG administered before LAD occlusion exerted cardioprotection against I/R injury as demonstrated by decreased arrhythmia incidence, decreased infarct size, and restored cardiac mitochondrial function. However, HNG applied during ischemic period could only decrease the reactive oxygen species (ROS) production. These findings suggest that HNG provides cardioprotection against myocardial I/R injury by preserving mitochondrial function. No COI.

Iron overload cardiomyopathy is a major cause of death in transfusion dependent thalassemia (TDT) patients due to the occurrence of left ventricular dysfunction, arrhythmia, and heart failure. Deferiprone (DFP) is an iron chelator that has been reported to improve cardiac function under iron overload conditions. Our previous studies demonstrated that T-type calcium channel (TTCC) played an important role in Fe²⁺ entry into thalassemic cardiomyocytes under iron overload conditions. However, the comparisons of therapeutic effects between TTCC blocker (efonidipine) and DFP on heart rate variability (HRV), left ventricular (LV) function, and cardiac mitochondrial function, under iron overload conditions have not been investigated. A wild-type (WT) and heterozygous bK⁰ type (HT) mice were fed with iron diet (0.2% ferrocene w/w) to induce iron overload condition for 90 days. Then, mice were treated with DFP and efonidipine for 30 days with continuous iron diet feeding. HRV, echocardiography, and cardiac mitochondrial function were determined. Chronic iron overload caused depressed HRV and decreased %LV fractional shortening, and cardiac mitochondrial dysfunction, which are indicated by increased ROS production, mitochondrial membrane depolarization, and mitochondrial swelling, both in WT and HT mice. Treatment with efonidipine and DFP showed similar improvement in HRV, %LV fractional shortening, and cardiac mitochondrial function in iron-overload WT and HT mice. These findings suggested that TTCC blocker could improve cardiac function and cardiac mitochondrial function in iron-overloaded mice similar to iron chelator. Therefore, the inhibition of TTCC may be an alternative target for treating iron-overload cardiomyopathy in TDT patients. No COI.
Iron overload cardiomyopathy is the major cause of death in transfusion dependent thalassemia (TDT) and hereditary hemochromatosis patients. Currently, deferiprone (DFP) is a common iron chelator for treating iron overload cardiomyopathy. Moreover, an antioxidant N-acetyl cysteine (NAC) has been shown to reduce oxidative stress and DNA damage in thalassemia patients. However, the protective effects of NAC alone or the combination of DFP and NAC on left ventricular (LV) function impaired by iron overload condition have not been investigated.

In this study, we determined the effects of DFP, NAC or combined DFP plus NAC on LV function in iron-overloaded rats. Male Wistar rats were fed with either normal diet (control group; n=6) or high iron (HFe) diet for 4 months. At 2 months, iron-overloaded rats were divided into 4 groups (n=6/group) to receive treatment with DFP, NAC, DFP plus NAC or vehicle and continued feeding with HFe diet for 2 months. Heart rate variability, echocardiography, and cardiac iron concentration were determined. The results showed that DFP or NAC alone had similar efficacy in improving LV function and reducing cardiac iron concentration. Combined DFP plus NAC could restore cardiac autonomic imbalance and LV fraction shortening, as well as decrease cardiac iron concentration to normal level. Although either iron chelator or antioxidant attenuated LV dysfunction and cardiac iron concentration, combined therapy provided more robust results. Therefore, combined DFP plus NAC may provide better efficacy in treating patients with iron overload cardiomyopathy. No COI.

Odontoblasts play an important role in the sensory signal transduction sequence in generating dentinal pain. However, the functional expression and their biophysical properties of voltage-dependent ionic currents in odontoblasts has remained unclear. We characterized plasma membrane voltage-dependent ionic currents in odontoblasts by whole-cell patch-clamp recording in a voltage-clamp configuration. The mean resting membrane potential of rat odontoblasts was –48 mV (n = 17). Depolarizing voltage steps to +80 mV from a holding potential of –70 mV with 10mV increments evoked outwardly rectifying currents with extracellular K+ concentration ([K+]o) of 5 mM. When we replaced Cl– to gluconate equimolarly in both the intracellular (150 mM) and extracellular solution (141 mM), the reversal potential (Erev) of the current was shifted 10–20 mV to hyperpolarizing potential (n = 6). Selected changes in [K+]o in the gluconate-based intracellular and extracellular solution without Cl– showed a shift in the Erev of tail currents as expected for a K+ equilibrium potential (n = 22). The relatively slow activation and inactivation kinetics exhibited dependence on the membrane potential. The steady-state inactivation was well described by a Boltzmann function with a half-maximal inactivation potential of ~29 mV (n = 6), showing that K+ currents in odontoblasts exhibit voltage-dependency. These results indicate that the odontoblasts express voltage-dependent K+ current showing slow activating/inactivating properties with residual Cl– conductance. No COI.
Computational studies on urinary bladder biophysics with special reference to nerve evoked signaling in bladder overactivity
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The overactive bladder (OAB) is an urological problem with serious social consequences. OAB is often associated with detrusor smooth muscle (DSM) instability. Spontaneous contractile activity is recorded in DSM strips from all species due to neurogenic, myogenic, and autonomous hypothesis. Micturition depends on a forceful and coordinated contraction of DSM that is brought by activation of muscarinic and purinergic receptors in response to nerve-released Acetylcholine (ACh) and ATP, respectively. Although both purinergic and muscarinic pathways are important to contraction, their relative contributions and signaling mechanisms are not well understood. A biophysically detailed computational model of the DSM cell in the bladder, and of its neural signaling (both purinergic and muscarinic) pathways towards calcium signaling and underlying membrane biophysics, can help provide new insights into mechanisms of over activity. Here, we aimed to model single cell of bladder along these lines and explore the factors that determine initialization of membrane excitation in this tissue. Our model shows that ACh increases membrane excitability, which depends on voltage gated ion channels. Purinergic membrane excitation is about 2.5 mV from the resting membrane potential of −55mV. The purinergic receptor inhibits the muscarinic response because activation of muscarinic receptor is slower than the activation of P2X receptor. This depolarization is sufficient to open the some voltage-gated channel to generate spike in DSM. In summary, this mathematical model provides an elemental tool to investigate the physiological both purinergic and muscarinic mechanisms underlying the spikes in DSM cell, which in turn can shed light in genesis of OAB. No COI.

Expression of Homer 2 proteins as novelty skeletal muscle regeneration factor
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The calcineurin-NFAT pathway is an important pathway that regulates skeletal muscle regeneration. Homer2 that modulates signal transduction in the central nervous system directly binds to NFATc1. However, its role is not descriptively understood in the skeletal muscle. We aimed to investigate the change of Homer 2 protein levels and expression patterns during muscle regeneration. Male ICR mice (12 weeks) were used in the experiment (n=6/group). Their left tibialis anterior (TA) muscle was damaged via intramuscular injection of 0.5% bupivacaine hydrochloride. The TA muscles of both legs were dissected at 2, 4, 6 days post-injection and performed immunofluorescence staining with Homer 2, NFATc1 and muscle regeneration markers [Pax7, myogenin, Neonatal MHC]. We calculated their expression frequency by counting the number of immunoreactivity per 500 nuclears. We observed Homer 2 immunoreactivity in TA muscles at 2, 4 and 6 days post-injection (p<0.001 vs. control). Homer 2 and Pax7, the satellite cells marker, were co-localized mononuclear cells also in regenerating TA muscles. Many Homer 2-positive mononuclear cells possessed expression of myogenin and Neonatal MHC respectively. The frequency of Homer 2 and NFATc1 positive cells significantly increases in 4 and 6 days rather than 2 days post injection (p<0.05). In conclusion, we demonstrated that expression of Homer 2 protein increases in TA muscle regeneration. Homer 2 can expect to work in the muscle regeneration process via the calcineurin-NFAT pathway. No COI.
Expression of Large Conductance Calcium-activated Potassium Channels (BKCa) and the Role in Myogenic Tension Regulation of Colonic Smooth Muscle

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The increase of gastrointestinal contents, especially in the stomach and colon, will not cause the cavity pressure sustained rise. The myogenic regulation mechanism is unclear. Large conductance calcium-activated potassium channels (BKCa) are widely expressed in gastrointestinal smooth muscles and are mechanosensitive. The present study is to investigate the role of BKCa in myogenic regulation of relaxation process of colonic smooth muscle. RT-PCR, Western Blot and patch clamp techniques were used to detect BKCa. Passive tension and active contraction of smooth muscle strips was recorded using stress transducer. Both α and β1 subunits of BKCa were detected in gastrointestinal smooth muscle layer. BKCa protein levels in stomach and colon are higher than other gastrointestinal segments. Both STREX and ZERO type splice variants of α subunit were detected. The density of BKCa is high according to patch clamp data and the tetraethylammonium (TEA) sensitive potassium channels are mostly BKCa currents. BKCa in rat colonic smooth muscle cells could be activated by stretch. The relaxation of colonic smooth muscle strips induced by stretch was decreased by charybdotoxin (ChTX), a specific BKCa blocker. While after using tetrodotoxin (TTX) to block intrinsic nervous activities, the relaxation of colonic strips induced by stretch did not change. Still ChTX increased the passive tension under stretch. Conclusions BKCa may play a role in myogenic tension regulation of colonic smooth muscle enduring stretch. Expression and function of BKCa may be of great significance in gastrointestinal motility regulation under physiological and pathological conditions. No COI.

5-fluorouracil-induced necroptotic death in colorectal cancer cells was prevented by glycolytic pyruvate

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Chemoresistance to 5-fluorouracil (5-FU), the first line anticancer agent, is commonly reported in colorectal cancer (CRC). 5-FU is a pyrimidine analog which suppresses cell proliferation and induces cell death. Upregulated expression of glucose transporters and glycolytic enzymes in CRC has been incriminated in chemoresistance, and high glucose modulates the effect of 5-FU in CRC cell lines. Our aim is to study the mechanism of glucose metabolism modulating cytotoxicity of 5-FU in CRC cells. Human CRC cell lines HT29, Caco-2, HCT116, and SW480 were exposed to 5-FU for 48 hrs in presence of 1, 5, and 25 mM glucose or a cell-permeable pyruvate derivative, ethyl pyruvate. Levels of cell apoptosis and necrosis were examined. Mitochondrial-derived reactive oxygen species (ROS) was measured by fluorometric analysis of MitoSOX. Staining of Ki67 and propidium iodide (PI) was used for cell cycle analysis. Under normal glucose (5mM), 5-FU caused necrotic death in CRC cell lines. Cell necrosis was mediated by RIP-1/3 complex formation and mitochondrial ROS production, but independent of caspase activation. High concentration (25 mM) of glucose and pyruvate attenuated receptor interacting protein kinase 1/3 complex formation and suppressed mitochondrial superoxide in cells exposed to 5-FU, resulting in a lower level of necroptosis. Glucose-mediated cytoprotection was reversed by iodoacetate (a glycolytic enzyme inhibitor) but not UK5099 (a mitochondrial pyruvate carrier). Furthermore, high concentration of glucose or pyruvate did not recover intracellular ATP drop or S-phase arrest caused by 5-FU. We concluded that glycolytic pyruvate confers resistance to 5-FU, through reduction of necroptotic death but not by promoting cell proliferation in CRC. NO COI.
Eritoran acts as a CD14 agonist and TLR4 antagonist to suppress colon cancer growth by dual mechanisms of apoptosis-inducing and anti-proliferative effects
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Colorectal carcinoma (CRC) is characterized by unlimited proliferation and death resistance. Overexpression of bacterial lipopolysaccharide (LPS) receptor CD14/TLR4 is involved in intestinal carcinogenesis. Eritoran is an investigational drug for treatment of severe sepsis as a TLR4 antagonist based on its structural similarity to the LPS lipid A moiety. We explored potential therapeutic use of eritoran in cancer reduction and examined underlying molecular mechanisms for its anticancer effect. Our study showed that eritoran administration via intracolonic, intragastric, or intravenous routes caused significant reduction of tumor multiplicity and sizes in mice by using a chemical-induced CRC model. Decreased tumor proliferation and increased cell apoptosis were seen after eritoran treatment in mouse CRC models. LPS/TLR4-dependent hyperproliferation in primary mouse cancer spheroids and human adenocarcinoma cell lines was inhibited by eritoran. Moreover, eritoran-induced cell apoptosis was ablated by gene silencing of CD14 and PKCζ, but not TLR4, in mouse spheroids and human adenocarcinoma cells. Finally, LPS caused hyperphosphorylation of PKCζ at Thr410, Thr560 and tyrosine sites in cancer cells. Blockade of PKCζ activation by inhibitors to Src kinase and serine/threonine phosphatase, or by PKCζ pseudosubstrate prevented cell apoptosis. In conclusion, eritoran treatment suppressed colon cancer growth by induction of CD14/Src/PKCζ-mediated apoptosis and blockade of TLR4-dependent proliferation. Our findings provide novel strategies for intervention against colorectal cancer. No COI.
Characterization of cold-induced remodeling reveals depot-specific differences across and within brown and white adipose tissues in mice
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Brown and beige adipose tissue dissipate energy in the form of heat via mitochondrial uncoupling protein 1, defending against hypothermia and potential obesity. The latter has prompted renewed interest in understanding the processes involved in browning to realize potential medical benefits. Aim: To characterize the temporal profile of cold-induced changes and browning of brown and white adipose tissue in mice. Methods: Male C57BL/6J mice were singly housed in conventional cages under cold exposure (4 °C) for 1, 2, 3, 4 and 5 days. Food intake and body weight were measured daily. Interscapular brown adipose tissue (iBAT), inguinal subcutaneous (sWAT) and epididymal white adipose tissues (eWAT) were harvested for histological, immunohistochemical, gene and protein expression analysis. Results: Upon cold exposure food intake increased but body weight and adipocyte size were transiently reduced. iBAT mass was increased whilst sWAT and eWAT were transiently decreased. A combination of morphology, genetic (Ucp-1, Pgc-1α and Elov13) and biochemical (UCP-1, PPARγ and aP2) analyses demonstrated depot-specific remodeling across the three depots in response to cold exposure. Conclusion: Our results demonstrate differential responses to cold-induced changes across discrete BAT and WAT depots and support the notion that the effects of prolonged cold exposure can be divided into two major phases, ‘cold remodelling’, for the first three days, and ‘cold adapted’, from four days onwards. No COI.

CRISPR-Cas9-sgRNA targeted NMDAR1 knockout attenuated high glucose-induced β-cells dysfunction
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The excessive activation of N-methyl-D-aspartate (NMDA) receptor by glutamate, an important neurotransmitter in CNS, evoked toxic effect on neural tissues. NMDA receptors are found in neural tissues and many peripheral non-neural tissues including islet β-cells. NMDAR, an important ionotropic glutamate receptor, is a heterotetramer composed of NMDAR1 and NMDAR2. NMDAR1 is the indispensable component for the activation of this receptor. We have found that NMDAR antagonist (MK-801) could enhance the insulin secretion and decrease the apoptosis induced by high glucose in β-cells. To investigate the exact role of NMDAR in high glucose-induced β-cell dysfunction, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9) - single-guide RNA (sgRNA) was used to knockout the NMDAR1 expression. CRISPR-Cas9 can be programmed with a sgRNA to generate site-specific DNA breaks. The NMDAR1 gene was silenced by CRISPR-Cas9-sgRNA in RINm5f cells. RINm5f cells infected with NMDAR1 sgRNA expressed around 80% less NMDAR1 protein compared to cells treated with control sgRNA. The cells were divided into control sgRNA group, control sgRNA-treated with high glucose (33.3 mM) group, NMDAR1 sgRNA group and NMDAR1 sgRNA-treated with high glucose group. RINm5f cells treated with NMDAR1 sgRNA partly inhibited the downregulation of GSIS induced by high glucose. Moreover, NMDAR1 sgRNA partially blocked high glucose-induced suppression of insulin gene expression. NMDAR1 sgRNA also attenuated the increased expression of caspase-3. These observations suggest that high glucose-induced β-cell dysfunction is mediated, at least in part, by NMDARs. No COI.
The effects of meniran extracts (Phyllantus niruri Linn) to COX2 mRNA expression in colitis associated model mouse

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Background: the aging of cells have an influence on the duration of prolonged inflammation and free radicals that can cause cancer, such as colorectal cancer. Meniran (Phyllantus niruri Linn) is known to contain various types of substances active compounds such as flavonoids and lignans useful as anti-inflammatory, antioxidant, anti-proliferative, immunomodulatory and antineoplastic, have considerable potential to be developed as a complementary medicine. Objective: to analyze the effect ethanol extract of meniran (Phyllantus niruri Linn) on gene expression of cyclooxygenase-2 (COX-2) and histopathological assessment (the type of cell founded, the degree of inflammation and degree of dysplasia) in mouse model colitis associated cancer. This study was an experimental laboratory Completely Randomized Design (CRD) using 15 mice were divided into 3 groups: group 1 (positive control) were administration by azoxymethane (AOM) and Dextran Sulfate Sodium (DSS), group 2 (treatment) were administration by AOM, DSS and ethanol extract of meniran 6 mg/mouse/day and group 3 (negative control) were mice as normal without administration both AOM, DDS and meniran. The study was conducted during the months of March through December 2014. The treatment, induction of experimental animals, mRNA extraction and RT-PCR was performed in the Laboratory of Medical Science Research Center (PPIK), Faculty of Medicine, University of Maranatha Bandung. Histopathologic examination carried out in the Laboratory of Pathology Anatomy Hasan Sadikin Hospital. Measurement parameters were the expression of COX-2 gene by electrophoresis of RT-PCR method using an internal control HPRT and histopathological assessment based on the type of cells, the degree of dysplasia, and the degree of inflammation. Mann-Whitney test was used for statistical analysis (p < 0.05). Results: The expression of COX-2 gene in group 1 was administrated with AOM and DSS had the greatest density up to 8× greater than group 2 with AOM, DDS and meniran administration. Group 3 as a negative control showed no gene expression of COX-2 in each animal. Statistical assessment showed significant p value between group 1 (positive control) with group 2 (treatment) for all parameters with p = 0.009 (gene expression), p=0.042 (type of cell founded), p=0.005 (degree of inflammation) and p = 0.011 (degree of dysplasia). Conclusion: the ethanol extract meniran decrease gene expression of COX-2 and meniran improved histopathology (types of cells were found, the degree of dysplasia, the degree of inflammation) on mice colon cancer model colitis associated cancer. No COI.
Roscovitine, a Cdk inhibitor, suppresses androgen receptor activation and prostate cancer cell proliferation

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It has been reported that cyclin-dependent kinases play important roles in modulating androgen receptor (AR) function and proliferation of prostate cancer. Roscovitine is a specific Cdk inhibitor and had been applied in clinical trials and combination therapy in many types of cancer. The aim of this study is to investigate whether AR is a target of Roscovitine and therefore affects prostate cancer cell growth. Roscovitine was treated in culture medium of prostate cancer cell lines (LNCaP, LNCaPdc, 22Rv1) as well as normal prostatic epithelial cells. Cell growth (in vitro and in vivo) and AR activation (including localization, reporter assay, PSA (prostate-specific antigen) expression) were evaluated. Roscovitine treatment resulted in significant growth inhibitions in both prostate cancer cell lines and normal prostatic cells. The results of xenografted tumor growth also support the finding. The indices of AR activation, including subcellular localization, promoter activity, and PSA production/secretion, were all inhibited after Roscovitine treatment. In conclusion, AR might be one of the pharmaceutical targets of Roscovitine in prostate cancer cells and, therefore, Roscovitine might be a potential drug candidate in prostate cancer therapy. No COI.
Processed food that contained saturated fat and beverages that use fructose as a sweetener can cause several metabolic abnormalities such as insulin resistance and non-alcoholic fatty liver disease (NAFLD). Although the protective role of female sex hormones against the development of metabolic defects has been reported, the degree by which processed food influences hepatic fat accumulation in estrogen-deprived state is unknown. Thus, this study investigated how high-fat high-fructose (HFF) diet modulates hepatic fat metabolism in ovariectomized rats. Adult female Sprague-Dawley rats were sham-operated and fed with control diet (SHAM) or ovariectomized and fed with either control diet (OVX) or HFF (OHF) for 12 weeks. NAFLD activity score and some hepatic lipogenic proteins including acetyl-coA carboxylase (ACC), fatty acid synthase (FAS) and sterol regulatory element binding protein-1c (SREBP-1c) in OVX rats were significantly increased when compared to SHAM. Comparison of OHF rats with OVX rats showed an increase in hepatic triglyceride content. Hematoxylin-eosin staining and NAFLD activity score also revealed a greater extent of fat infiltration with macro- and micro-vesicular steatosis in OHF rats, which was associated with higher expression of all lipogenic proteins as well as hepatic oxidative marker PPAR-α above the values of OVX rats. These results indicated that estrogen deprivation alone enhances only some proteins in lipogenic pathway, whereas HFF diet augments both lipogenic and oxidative proteins. Importantly, HFF diet progressively aggravates hepatic steatosis in estrogen-deprived state by upregulating lipogenic proteins. No COI.

A growing body of evidence indicates epigenetic regulation plays pivotal roles in the development of endometriosis, a common gynecological disease defined as the presence of endometria outside of uterus. We hypothesized that hypoxia, an immediate stress encountered by cast-off endometrial tissues, may regulate DNA methylation. Indeed, ectopic endometriotic stromal cells from patient with endometriosis had lower level of DNA methylation compared to their eutopic counterparts. Subsequent analyses using quantitative PCR and Western blot showed that DNA methyltransferase 1 (DNMT1), but not DNMT3a or 3b, was downregulated in the ectopic stromal cells. Treatment of eutopic endometrial stromal cells with hypoxia reduced levels of DNMT1 and DNA methylation. They revealed that hypoxia-suppressed DNMT1 expression was mediated through shortening DNMT1 mRNA half-life. RNA immunoprecipitation showed that hypoxia enhanced the binding of AU-rich element binding factor 1 (AUF1) to ARE by inhibiting HuR expression. Binding of AUF1 to ARE subsequently facilitated miR-148a-loaded AGO2 targeting to DNMT1 3'-UTR. Mutating miR-148a binding site or ARE abolished hypoxia-induced AUFI-mediated DNMT1 downregulation. Hypoxia-mediated DNMT1 downregulation and global DNA hypomethylation derepressed several genes involving in endometriosis pathogenesis such as GATA6, HOXA3, and SLC16A5. A murine model of endometriosis further demonstrated the decrease of Dnmt1 expression and DNA methylation during the development of endometriosis. Taken together, our data demonstrate that RNA binding proteins and microRNA coordinate to regulate DNMT1 under hypoxia, which may be an important regulatory mechanism for epigenetic alteration during the development of endometriosis. No COI.
Young Scientist Award
Effect of heat stress on serum glucose, insulin, luteinizing hormone and testosterone in Bama miniature pig
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Many stressors could impact endocrinology system, promote catabolism, inhibit reproductive activities and reduce growth performance in heat-stressed animals. The present study aimed to determine whether high ambient temperature-induced stress change the energy metabolism and reproduction in a Chinese local breed, Bama miniature pig. Twelve male 7-mo-old Bama miniature pigs were randomly allotted to 2 groups: 1) the thermal neutral (TN) group remained at 25 °C, 2) the heat treatment (HT) group exposed to ambient temperature at 40 °C for 5 h daily for 8 consecutive days. Pigs were sacrificed on day 8 and the blood samples were collected immediately after HT. The results showed that serum concentrations of cortisol were increased by 14.6% but not significant in HT, compared with that in TN group. Serum glucose level was increased by 5.9% but not significant, while serum insulin level was decreased by 8.6% but not significant. Interestingly, pigs in HT showed a significant decrease in luteinizing hormone (LH) as compare to TN (P<0.01), while serum testosterone level was increased by 10.5% but not significant. It is concluded that high ambient temperature at 40 °C induced heat stress in Bama miniature pig characterized by the obvious increased cortisol, as an indicator of stress. The decreased insulin and increased glucose well documented that cortisol could promote the cerebral use of glucose and enhance the ability to prevent stress-induced damage. The decreased LH implied the inhibited hypothalamus-pituitary-gonadal (HPG) axis, but the increased testosterone might be attributed to the interaction between the HPG axis and the hypothalamus-pituitary adrenal axis in Bama miniature pigs under heat stress. No COI.

Young Scientist Award
Short Palate Lung and Nasal Epithelial Clone 1 (SPLUNC1) dissociates and internalizes the Epithelial Sodium Channel (ENaC)
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αβγENaC regulates sodium and water absorption across airway epithelia. In cystic fibrosis airways, hyperactive ENaC dehydrates airway surfaces which results in mucus thickening and increased probability of infection. SPLUNC1 is a negative regulator of ENaC but its underlying mechanism of action is unknown. Here, we tested the hypothesis that SPLUNC1 works by internalizing ENaC. Surface biotinylation was performed in HEK293 and Human bronchial epithelial cells (HBECs) to investigate ENaC surface level. Immunoprecipitation, immunostaining and acceptor-photobleaching fluorescent resonance energy transfer (FRET) were performed in HEK293 to investigate ubiquitination, co-localization, and conformational change of ENaC respectively. A Nedd4-2 dominant-negative construct was a gift from Dr. Peter Snyder (UI). SPLUNC1 reduced the %FRET efficiency between βENaC-GFP and γENaC-mCherry from 9.8±1.4 to 5.0±1.1%. SPLUNC1 decreased plasma membrane αENaC by 6.7-fold in HEK293 cells and 2.6-fold in HBECs without affecting the plasma membrane βENaC. When αγENaC was co-expressed, SPLUNC1 did not affect plasma membrane αENaC. SPLUNC1 ubiquitinated αENaC by 4.5 fold, which was abolished when Nedd4-2 ubiquitin ligase function was blocked by αENaC PY-motif truncation or Nedd4-2 dominant negative transfection. Pre-treatment with chloroquine, a lysosome inhibitor, but not MG-115, a proteasome inhibitor, abolished intracellular αENaC degradation without affecting ENaC internalization. Internalized αγENaC by SPLUNC1 co-localized. In conclusion, upon the binding of SPLUNC1 to βENaC, SPLUNC1 allosterically triggers Nedd4-2 mediated αENaC ubiquitination that results in the dissociation of ENaC subunits, internalization and degradation of αγENaC but not βENaC via the lysosomal pathway. No COI.
Activation of volume-regulated anion channel by nanomolar concentrations of ouabain in human cancer cells
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Cardiac glycosides at nanomolar concentrations are known to block cancer cell growth without affecting Na,K-ATPase activity. However, these mechanisms have not been fully understood. In the present patch-clamp study, we found that nanomolar concentrations of ouabain increased outwardly rectifying Cl- currents in human cancer cells but not in non-cancer cells. The effect was concentration-dependent and EC50 value was 24 nM. This value was close to IC50 value for the ouabain-induced inhibition of cancer cell proliferation (34 nM). The ouabain-induced Cl-currents were dramatically inhibited by DCPIB, a specific inhibitor of volume-regulated anion channel (VRAC) and by knockdown of LRRC8A, a component of VRAC, suggesting that the molecular nature of ouabain-induced currents is identical to the hypotonicity-induced VRAC. LRRC8A and Na,K-ATPase were distributed in both the cholesterol-enriched membrane microdomains and non-microdomains in human cancer cells. The disruption of the microdomains by methyl-β-cyclodextrin significantly suppressed the ouabain-induced VRAC currents but not the hypotonicity-induced VRAC currents in cancer cells. In addition, inhibitors of NADPH oxidase (NOX) such as apocynin and VAS2870 significantly attenuated the ouabain-induced VRAC currents. Interestingly, the ouabain-induced inhibition of cancer cell proliferation was weakened by DCPIB, NOX inhibitors, methyl-β-cyclodextrin, and knockdown of LRRC8A. These results suggest that Na,K-ATPase, NOX and VRAC form a signalosome in the membrane microdomains of human cancer cells, and that the cardiac glycoside exerts anti-cancer activity. No COI.

Hypoxia induces angiogenesis via depressing COUP-TFII-suppressed angiogenin expression in endometriosis
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Hypoxia plays an important role in promoting angiogenesis during the development of endometriosis; however, the underlying mechanism remains largely unknown. Herein, we identified angiogenin (ANG), a novel angiogenic factor, is increased under hypoxia. Since there is no hypoxia responsive element in the ANG promoter region, we analyzed the potential transcription factor binding site in the promoter region of ANG and identified a potential binding site for chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). Consistent with this notion, knockdown of COUP-TFII in eutopic endometrial stromal cells increased ANG expression. Treatment of endometrial stromal cells with hypoxia decreased COUP-TFII expression and concomitantly induced ANG expression. In contrast, overexpression of COUP-TFII under hypoxia significantly reduced hypoxia-induced ANG expression. Chromatin immunoprecipitation-PCR assay revealed that binding of COUP-TFII to ANG promoter region was significantly reduced under hypoxia. Treatment with hypoxia or knockdown of COUP-TFII increased ANG promoter activity. Treatment of human umbilical vein endothelial cells with conditioned media collected from stromal cells with COUP-TFII knockdown significantly promoted tube formation. In contrast, the increased angiogenic capacity induced by COUP-TFII knockdown was abolished by simultaneously knocking down of ANG. Taken together, our results demonstrated that ANG involves in the angiogenesis of endometriosis through hypoxia-mediated loss-of-COUP-TFII expression and suggested ANG may be a novel therapeutic target for treatment of endometriosis. No COI.
Interferon-gamma disrupts barrier integrity of human umbilical vein endothelial cells

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Endothelial barrier function is regulated by multiple regulatory molecules including cytokine, actin cytoskeleton, β-catenin and p38 mitogen-activated protein (MAP) kinase. Dysregulation of any of these molecules will lead to endothelial hyperpermeability. Interferon-gamma (IFN-γ) has been reported to disrupt barrier integrity of various cells, and potentiated the progression of inflammatory disorders. However, the mechanisms of IFN-γ on increasing human umbilical vein endothelial cells (HUVECs) permeability remain unknown. This study aimed to investigate the effect of IFN-γ on HUVECs permeability; and how IFN-γ affects cell morphology, actin cytoskeleton and β-catenin protein expression. The involvement of p38 MAP kinase in all these events was assessed using a specific inhibitor, SB203580. The increased permeability to FITC-dextran was evaluated using permeability assay kit. The altered cell morphology and actin cytoskeleton were studied using rhodamine-phalloidin staining, and viewed under confocal microscope. Total expression of β-catenin was studied using immunoblotting. For p38 MAP kinase study, HUVECs were pretreated with SB203580 followed by IFN-γ. Permeability data showed that IFN-γ increased HUVECs permeability in a biphasic manner. Imaging studies showed that IFN-γ caused cell rounding and condensed actin ring followed by cell elongation and stress fiber formation. IFN-γ also reduced β-catenin expression. p38 MAP kinase partially inhibited IFN-γ-induced HUVECs hyperpermeability, actin rearrangement, but did not affect reduced β-catenin expression. In conclusion, IFN-γ increased permeability is associated with actin rearrangement and downregulation of β-catenin expression; and these are partially regulated by p38 MAP kinase. NO COI.

Effects of dietary Kaempferia parviflora on contractile force in rat skeletal muscle

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The aim of this study was to investigate the effects of Kaempferia parviflora (KP) on force production in skeletal muscle. Male Wistar rats ingested KP extract (0.15 mg/ g body weight) for 4 weeks. After the supplementation, intact gastrocnemius muscles were electrically stimulated in vivo. KP ingestion resulted in an increase in maximal force. Repetitive contractions (fatiguing stimulation) were applied to the muscles for 2 min. KP ingestion brought about an increase in the initial force of fatiguing stimulation, whereas there was no difference in the force at the end of fatiguing stimulation between control and ingested group, indicating that the potentiating effect of KP were progressively diminished during fatiguing stimulation. When skinned fibers from gastrocnemius muscles were exposed to the solution containing KP extract (0.15 mg/ml), Ca2+-induced maximal force was increased. These results suggest that i) KP ingestion brings about an increase in maximal force, possibly caused by an improvement of cross-bridge cycle and ii) fatigue resistance is not affected by the KP ingestion-induced force potentiation. No COI.
Effects of asiatic acid on TNF-α-induced vascular inflammatory events in human aortic endothelial cells
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Asiatic acid (AA) is a major triterpenes isolated from Centella asiatica and its biological activities such as anti-inflammatory and anti-hyperlipidemic effects have been reported previously. In this study, we aimed to evaluate protective effects of AA against TNF-α-induced vascular inflammatory events in human aortic endothelial cells (HAECs). We examined the effects of AA on TNF-α-induced hyperpermeability, actin cytoskeleton alterations, increased protein expression of cellular adhesion molecules (ICAM-1 and VCAM-1) and monocyte adhesion. Cytochalasin D, an actin depolymerizing agent, was used to correlate the anti-hyperpermeability effect of AA with actin cytoskeleton. In vitro vascular permeability assay kit was used to measure permeability of HAECs. F-actin was stained with rhodamine phalloidin and viewed under confocal microscope. Protein expressions of ICAM-1, VCAM-1 and F/G-actin ratio were determined by western blot. For monocyte adhesion assay, fluorescently labeled-U937 monocytes were added to HAECs and fluorescent intensities of adhered monocytes were measured. AA (20-40 µM) significantly suppressed TNF-α-induced hyperpermeability, and prevented TNF-α- as well as cytochalasin D-induced redistribution of F-actin. However, AA failed to suppress TNF-α-increased F/G-actin ratio and cytochalasin D-induced permeability. In addition, AA inhibited TNF-α-induced VCAM-1 expression but did not suppress up regulated ICAM-1 and monocyte adhesion. In conclusion, the barrier protective effects of AA were demonstrated through inhibition of hyperpermeability and VCAM-1 expression. AA also stabilizes F-actin without altering total actin pool of HAECs. However, stabilization of F-actin by AA does not lead to its anti-hyperpermeability effect. No COI.

Gac fruit (Momordica cochinchinensis) extract enhances proliferation and differentiation in rat osteoblast-like UMR-106
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Osteoporosis is one of the major public health problems in elderly and most of anti-osteoporotic drugs are expensive and inaccessible by Thai people. Gac fruit (Momordica cochinchinensis), a local Thai plant, which is cheap and contains high amount of antioxidant lycopene becomes a focal point of our interest. Although it was reported that lycopene could suppress oxidative stress and prevent osteoporosis in elderly, no report of Gac fruit on bone was found. Here, we investigated the effects of gac extract (GAC) on bone formation. Rat osteoblast-like UMR106 cells were cultured and incubated with 0.3% DMSO, 1, 10, 100 µM of lycopene, and 0.1, 10, 1000 µg/ml of GAC, then cell viability, cell proliferation and expression of osteoblast differentiation (alkaline phosphatase; ALP) and antioxidant (glutathione (GSH), superoxide dismutase (SOD), heme oxygenase 1 (HO-1) and catalase) marker genes were determined. The results indicated that 1000 µg/ml of GAC and 10 µM of lycopene could increase osteoblast proliferation with no effect on cell viability. Moreover, quantitative real-time PCR revealed that GAC and lycopene at these doses could up-regulate the expression of ALP gene. Although no significant changes of expression of GSH and SOD genes were detected, the HO-1 and catalase mRNA levels were decreased as observed in the BG-12, positive control group of antioxidant. Our results indicate that GAC could enhance bone formation by stimulating osteoblast proliferation and differentiation via the anti-oxidation pathway. Since currently Gac fruit has been popularly used by Thai people as drinks, foods, and cosmetics, our finding should be value-added to this plant. No COI.
The evaluation of acute and repeated dose 28 days oral toxicity testing of Jerusalem Artichoke (Helianthus tuberosus) product in Wistar rats

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Jerusalem Artichoke tubers are an important source of inulin used as a dietary fiber in food manufacturing. However, toxicological study of Jerusalem Artichoke product are still lacking. This study was aimed at evaluating of acute and repeated dose 28 days oral toxicity of Jerusalem Artichoke product in Wistar rats. The study was in compliance with OECD/OCDE 423 and 407. For acute toxicity study Jerusalem Artichoke product at the dose of 300 and 2,000 mg/kg body weight was given orally to Wistar rats. No sign of toxicity or deaths were observed for 14 days. The results showed that Jerusalem Artichoke product was classified in GHS category 5 or Unclassifed, the LD50 cut off at 5,000 - ∞ mg/kg body weight. Repeated dose 28 days oral toxicity was studied by daily oral dose of 1,000, 2,000 and 4,000 mg/kg body weight for 28 days. The study revealed that all treated rats survived through the whole experimental periods without adverse effects observed in either sex of rats after repeated dose. Growth pattern (body weights, food consumption, and relative organ weights), hematology analysis, and clinical biochemistry analysis in all treated rats were in normal physiological ranges. The no-observed-adverse-effect-level (NOAEL) was considered to be 4,000 mg/kg body weight per day for rats. The results from the study suggest that the Jerusalem Artichoke product had no toxicologically effects on acute and repeated dose 28 days oral administration in rats. NO COI.
ABS0109  Young Scientist Award
Feeding of Pueraria mirifica phytoestrogen containing herb increases bone mass in post-menopausal monkeys
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Since estrogen-replacement therapy poses more risk than benefit for osteoporotic treatment, it has no longer recommended and the natural based chemicals such as phytoestrogens are attracted attention. Pueraria mirifica, a phytoestrogen-rich herb, was reported its positive effects on bone in rodent models. Regarding the regulatory guideline of US-FDA, to develop a new therapeutic agent for human osteoporosis, testing in monkey which has similar intracortical bone remodeling to human is suggested. Here, the anti-osteoporotic effect of P. mirifica was investigated in post-menopausal cynomolgus monkeys. Monkeys were divided into two groups (5 monkeys each), and fed daily with standard monkey diet only (PM0) or mixed with 1,000 mg/kg BW of P. mirifica (PM1000) for 16 months. Bone mass at the distal radius and proximal tibia and serum bone markers were measured in every two months. In each bone type, two bone sites of metaphysis consisting of trabecular and cortical bone and diaphysis consisting only cortical bone were analyzed. After 16 months of PM1000 treatment, total BMDs and BMCs in both radius and tibia were increased, especially at the cortical diaphysis. Comparing the increase in diaphysis cortical BMCs, treatment of PM1000 at the early (<5 years) and mid (5-10 years) period of menopause seems to be more effective than the late (>10 years) period. Increased bone mass in PM1000 group was caused by a decrease in bone turnover rate indicating by low bone formation (serum BAP and osteoclacin) and bone resorption (urinary NTX) markers. This corroborates the high potential of P. mirifica for human osteoporotic treatment. No COI.

ABS0217  Young Scientist Award
Steviol stabilizes polycystin 1 expression and promotes lysosomal degradation of CFTR and β-catenin proteins in polycystic kidney disease
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The mutation of PKD1 gene encoding polycystin 1 (PC1) causes autosomal dominant polycystic kidney disease (ADPKD), which is characterized by abnormally high epithelial cell proliferation and fluid secretion leading to end-stage renal failure. Currently, there is no effective treatment for this disease. PC1 is a complex protein functioning as a calcium channel involved in several signaling cascades including renal tubulogenesis. PC1 malfunction contributes to the cyst formation in human ADPKD. The recent studies reported that PC1 regulates CFTR chloride channel and β-catenin levels in normal renal epithelial cells. Concurrently, our previous study found that steviol retards cyst enlargement in both renal epithelial cyst model and PKD mice by reducing both expression and activity of CFTR. Therefore, it is interesting to explore whether steviol has an effect on PC1 function. The current study was aimed to determine the effect and mechanism of steviol action on PC1, CFTR, and β-catenin levels in renal epithelial cell that has defect of PC1 biogenesis and expression (PRKCSH - deficient cell). Interestingly, it was found that treating these cells with steviol at a dose of 100 µM for 24-48 hours enhanced and stabilized PC1 C-terminal expression. Steviol also inhibited CFTR and β-catenin protein expression. In addition, steviol promoted LAMP2, a marker of lysosomal enzyme. These findings indicate that steviol slows cyst progression in cell and animal models of PKD, in part, by enhancing PC1 protein expression as well as promoting lysosomal degradation of CFTR and β-catenin. Therefore, steviol may represent a promising compound for the treatment of polycystic kidney disease. No COI.
Procaterol stimulated ciliary beat frequency via PDE1 in mouse bronchiolar cilia.  
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Procaterol (an β2-agonist) stimulates an immediate increase in ciliary bend angle (CBA) followed by a gradual increase in ciliary beat frequency (CBF) via cAMP accumulation. The time course of procaterol-stimulated CBF increase is faster at a low [Ca^{2+}] than that at a high [Ca^{2+}]. Moreover, in unstimulated cells, an extremely low [Ca^{2+}] increases CBF, which is inhibited by PKI-amide (a PKA inhibitor). On the other hand, 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase (PDE) inhibitor) increases both CBA and CBF in a similar time course. These observations suggest that cAMP accumulation in the microdomain regulating CBF is controlled by the Ca^{2+}-dependent PDE (PDE1). Inhibition of PDE1 by 8-methoxymethyl-IBMX (8MmIBMX) increased CBF, and a further procaterol stimulation increases both CBF and CBA in a similar time course. Moreover, in immunohistochemical examination, PDE1A was detected in the microdomain between the nine doublet tubules and the cell membrane, where the outer dynein arms (ODAs, molecular motors of CBF regulator) function, and an extremely [Ca^{2+}] and 8 mM IBMX accumulates cAMP in isolated lung cells. Thus, PDE1 regulates CBF via modulation of cAMP accumulation. In bronchiolar cilia, PDE1A, which delays cAMP accumulation in the ODA-functioning microdomain (between the nine doublet tubules and the cell membrane), causes an increase in CBF to be slower than that in CBA during procaterol stimulation. No COI.

Discrimination of optogenetic whisker-barrel inputs using channelrhodopsin-2 transgenic rat

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The rodent whisker-barrel system has been a model to study somatosensory representation in the cortex. Optogenetics would facilitate this with high spatio-temporal resolutions. Recently, we have identified the expression of channelrhodopsin-2 (ChR2) in the mechanoreceptive neurons in the trigeminal ganglion in one of Thy1.2-ChR2-Venus transgenic rat lines, W-TChR2V4. Each whisker follicles were thus innervated by ChR2-positive nerve endings. Here, we evaluated the ability of this rat to discriminate the irradiation patterns on their whiskers. A W-TChR2V4 rat was irradiated blue light on each whisker with a certain pattern conditioned with a reward. The Go task was designed so as the rat is allowed to get a reward, when it licked the nozzle within 5 s after irradiation of one of whiskers. The No-go task was designed so as the rat have to withhold licking least at 5 s to get a reward after irradiation of another whisker. The rat learned to discriminate these optogenetic whisker patterns successively with sessions and even with days (success rate 80%). In another series experiment, the rat was trained to learn Go task to the whisker pad irradiation. When the blue LED light was irradiated on the barrel cortex where many neurons are also expressing ChR2, it induced to lick the nozzle within 5 s (success rate, about 80%). It is suggested that the W-TChR2V4 rat can discriminate the spatiotemporal pattern on whiskers and that the signal pattern on whiskers can be reproduced by the direct photostimulation to barrel cortex. Our optogenetic approach would facilitate to study how the spatio-temporal pattern of the mechanoreception would be interpreted in the cortex. No COI.
**ABS0129 Young Scientist Award**

**PPARα modulation mediated via PI3K/Akt pathway of Ca\(^{2+}\)-regulated exocytosis in antral mucous cells**

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In antral mucous cells, Ca\(^{2+}\)-regulated exocytosis activated by acetylcholine (ACh) is the main mechanism for mucin release. We have demonstrated that arachidonic acid (AA)/PPARα autocrine mechanism modulates Ca\(^{2+}\)-regulated exocytosis mediated via NOS1/NO/cGMP. However, we do not know how PPARα activates NOS1, such as NOS1 phosphorylation. We studied the signal followed by the PPARα activation in ACh-stimulated antral mucous cells. Male guinea pigs were anaesthetized by pentobarbital-Na (70 mg/kg, ip). Antral mucous cells were isolated by a collagenase digestion. The exocytotic events were measured by video-microscopy. The actions of GW7647 (a PPARα agonist) on ACh-stimulated exocytotic events, the enhancement of initial transient increase, were abolished by GW6471 (PPARα antagonist). However, GW6471 produced a delayed, but transient increase (delayed increase) in the exocytotic events via cGMP-activated phosphodiesterase 2 inhibition. The effects of GW6471 on ACh-stimulated exocytotic events with GW7647 were mimicked by wortmannin (a PI3K inhibitor) and Akt 2/2 kinase inhibitor (an Akt inhibitor). Moreover, the western blotting revealed that GW7647 evoked phosphorylation of PI3K, Akt, or NOS1 in antral mucosa. NO production stimulated by GW7647 was inhibited by wortmannin and Akt2/2 kinase inhibitor. Thus, PPARα phosphorylates NOS1 mediated via PI3K/Akt signal, leading to NO and cGMP accumulation, which enhances the Ca\(^{2+}\)-regulated exocytosis in antral mucous cells. No COI.

**ABS0212 Young Scientist Award**

**The role of primary somatosensory cortex in chronic pain**

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There is an increasing evidence that the plasticity of neuronal circuits is important in the pathophysiology of chronic pain. We previously reported that chronic pain in the hindpaw of mice increased the neuronal activity and synaptic remodeling in contralateral primary somatosensory cortex (cont-S1). In addition to activation of cont-S1, an MRI study demonstrated that the ipsilateral primary somatosensory cortex (ipsi-S1) was also activated in chronic pain patients. However, despite increased activity in ipsi-S1, these patients did not display any changes in pain sensitivity with regard to their contralateral extremities. To understand this discrepancy, we investigated the activity in ipsi-S1 using in vivo 2-photon Ca\(^{2+}\) imaging under chronic pain conditions. Following peripheral nerve ligation (PSL), we observed increased Ca\(^{2+}\) transients in layer 1 inhibitory neurons and astrocytes, but the spine turnover rate of pyramidal neurons remained unchanged. To examine the role of enhanced inhibitory neuronal activation, we observed the dendrites of layer 5 pyramidal neurons in ipsi-S1 of PSL mice and examined the peripheral sensitivity of intact hindpaw before and after application of a GABAA receptor antagonist. Chronic inhibition of the GABAA receptor to ipsi-S1 with PSL increased the spine turnover rate, and decreased the threshold of mechanical stimuli in the intact hindpaw contralateral to the PSL site. Thus, synaptic remodeling in S1 is a potentially important underlying mechanism for the change in peripheral sensitivity. An impairment of GABAergic function of ipsi-S1 with PSL might provide an underlying for mirror image pain, which persists at uninjured sites contralateral to the peripheral nerve injury. No COI.
Poster Presentations

Young Scientist Awards (Poster)
Protective effect of vitamin E against ethanol-induced structural alteration, oxidative stress and inflammatory reaction in small intestine of rat
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The role of oxidative stress and inflammatory reaction has been reported in various ethanol-induced complications. The purpose of this study was to evaluate the effect of ethanol consumption-induced structural alteration, oxidative stress and inflammatory reaction in small intestine of rat, and plausible protective effect of vitamin E to determine if it inhibits the abnormality induced by ethanol in small intestine. Twenty-four male wistar rats were divided into three groups, namely: Control ©, ethanol and vitamin E treated ethanol groups. After 6 weeks treatment, the length of small intestine, villus height, crypt depth and muscular layer thickness, as well as oxidative stress and inflammatory parameters showed a significant changes in ethanol treated group compared to the control group. Vitamin E consumption along ethanol ameliorate structural alteration of small intestine and reduced, elevated amount of oxidative stress and inflammatory markers such as protein carbonyl, OX-LDL, IL-6, Hey, TNF-α, as well as increased total antioxidant capacity significantly compared to the ethanol group. These findings indicate that ethanol induce small intestine abnormality by oxidative and inflammatory stress and that these effects can be alleviated by using vitamin E as an antioxidant and anti-inflammatory molecule. No COI.

Cholesterol-lowering activity of Spirogyra neglecta extract
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High plasma cholesterol levels are a risk factor for development of cardiovascular diseases (CVD). Dietary natural plants are an option and receiving much attention to reduce cholesterol levels. Spirogyra neglecta (SN) contains both macronutrients and micronutrients and shows anti-gastric ulcer, anti-inflammatory, anti-hyperglycemic anti-hyperlipidemic actions and antioxidant properties. However, the impact of SN on cholesterol-lowering properties is still little evidence. The cholesterol-lowering activity of SN was examined. The results showed that SN significantly increased cholesterol micelles size in a dose-dependent manner. At the concentration of 0.1 to 10 mg/mL of SN inhibited the solubility of cholesterol micelle 25.85% to 83.68%. Moreover, SN bound to taurocholic acid, taurodeoxycholic acid, and glycodeoxycholic acid at levels ranging from 24.01% to 49.58%. These findings demonstrate that SN extract have cholesterol-lowering activity by increasing cholesterol micelles size, reducing solubility of cholesterol in micelles and inhibiting binding of bile acids which may result in delayed cholesterol absorption and reduce the chance of developing CVD. No COI.
**ABS0259**

Effect of essential oil extracted from Ocimum sanctum L. leaf on serum lipid and blood glucose in rat fed with high calorie diet

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Ocimum sanctum Linn. leaves (OS) have been reported to reduce blood glucose and serum lipids in DM rats. OS is enriched of essential oil. The objective of the present study was to evaluate the effect of essential oil extracted from OS (EOOS) on blood glucose and serum lipid in rats fed with high calorie diet (HCD). The rats were divided in to three groups including normal control group and HCD group treated with or without EOOS for 4 months. HCD increased blood glucose, serum insulin, serum lipid, HOMA IR and atherogenic index (AI). Area under the curve of glucose (AUG) and area under the curve of insulin (AUI) were also increased. Liver lipid content and fecal lipid excretion were raised. EOOS had no effect on the high levels of blood glucose, serum insulin, HOMA IR, AUG and AUI whereas it decreased serum lipid without effect on liver lipid content and fecal lipid excretion. It can be concluded that HCD increased insulin resistance, resulting increased blood glucose and serum lipid. EOOS could not decrease the high level of blood glucose whereas it decreased serum lipid. Its lipid-lowering effect is not due to either decreased liver lipid content or increased fecal lipid excretion. No COI.

**ABS0264**

Organ protective effect of essential oil extracted from Ocimum sanctum L. in rats fed with high calories diet.

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The present study was conducted to investigate the effect of essential oil extracts from Ocimum sanctum L. leaves (EOOS) on blood glucose, serum lipid and organ protection in rats fed with high calorie diet (HCD). The rats were divided in to three groups including normal control group, HCD group and HCD group treated with EOOS for 4 months. The results show that HCD increased blood glucose, serum insulin and HOMA IR. It also raised serum lipid and atherogenic index (AI). Serum levels of AST, ALT and LDH were significantly increased in HCD rats. The liver and myocardial tissues were damaged as shown by histopathological examination. EOOS decreased serum lipid and AI without effect on the high levels of blood glucose, serum insulin and HOMA IR. It also decreased the high serum levels of ALT and LDH with slightly decreased serum levels of AST and CK-MB. The liver and myocardial tissues were preserved in rats treated with EOOS. It can be concluded that HCD increases insulin resistance, resulting increased both blood glucose and serum lipid. EOOS decreased serum lipid without effect on blood glucose and insulin levels. It also protects the liver and myocardial tissues against HCD. No COI.
Rice bran oil (RBO), is recently gaining commercial interest due to its health benefit and biological effects. Gamma-oryzanol has been suggested to be the important ingredient for health promotion, especially improving the plasma lipid profile. The present study was carried out to compare the cytotoxicity of rice bran oil containing different concentration of gamma-oryzanol. Cytotoxic activity of RBO on human prostate cancer cells, DU145, was performed by proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent. Cells were treated with RBO in a humidified atmosphere of 5% CO\textsubscript{2} at 37 °C for 7 days. Culture medium containing 1 mg/ml of MTT dye was added into each well, incubated for 4 h and then replaced with DMSO. The blue color of the oxidized MTT was determined by measuring the absorbance at 570 nm. Three concentrations of RBO, 5,000, 8,000 and 15,000 ppm, significantly inhibited prostate cancer cell growth in a dose- and time-dependent fashion. Half of the cells were dead at RBO concentration (IC\textsubscript{50}) of 1.06 ± 0.06, 0.36 ± 0.06 and 0.27 ± 0.04 mg/ml, respectively. These results showed the potential of RBO for anticancer activity. Its mechanism might be both direct, such as cytotoxicity, and indirect effect to the cancer cells, such as antioxidant activity as claimed about the plasma cholesterol and triglycerides in previous papers. Further study of the antioxidant gene expression is worthwhile. No COI.

Artificially made CO\textsubscript{2}-hot spring water (CO\textsubscript{2}-water: contains CO\textsubscript{2} > 1000ppm), as well as natural hot spring water, dilates skin blood vessels in the body part immersed in it, hence increases skin blood flow. Previous observations indicated that a similar vasodilation could occur in the skeletal muscle under the skin of immersed part. If muscle blood flow is improved by CO\textsubscript{2}-water immersion, recovery of muscle fatigue also might be promoted. In the present study we investigated this hypothesis by means of water immersions of the forearms between three series of a continual measuring of grip strength. Subjects (Ss) were 11 healthy student volunteers. Ss were measured the grip strength in both hand simultaneously with one's utmost effort for 10 s and then rested for 5 s, which was repeated 25 times continuously as a set of trials. Each Ss performed 3 sets in one experiment. During 10 min of resting between two sets, Ss put both forearms in right and left separate bathtubs of two arm-bath apparatus filled with a tap water or CO\textsubscript{2}-water in a same temperature (32–35 °C) or with a room air (24 °C). Grip strength decreased 20 to 30 % of initial level by 25 times repetition in each set. Initial levels of grip strength of each set decreased gradually as a set advances, but forearm treatment for 10 min between the sets did not affect the decreases. In the third set, attenuation of the grip strength to the end of a set was significantly smaller in the arm with water immersion than in non-immersion arm. Though a significant difference between treatments with CO\textsubscript{2}-water and tap-water was not detected under the present conditions, CO\textsubscript{2}-water immersion might be effective in suppressing progression of muscle fatigue. No COI.
ABS0371

Active fraction from Bixa orellana leaves ameliorates bradykinin-induced hyperpermeability via NO-cGMP-PKC pathway

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Alteration in endothelial permeability is a hallmark of inflammatory processes. It causes severe disruption in endothelial barrier function which eventually leads to vascular pathologies, including atherosclerosis. Previous studies showed aqueous extract of Bixa orellana leave (AEBO) exhibited anti-hyperpermeability against bradykinin. Thus, an active fraction (FAEBO) that separated from AEBO through nitric oxide (NO)-guided fractionation was tested for its anti-hyperpermeability activity. Human umbilical vein endothelial cells cultured at 1 μM bradykinin showed increases in endothelial barrier permeability to FITC-dextran compared to cells cultured at media only. Pre-loading of the cells for 60 min with FAEBO before the permeability assay prevented the bradykinin induced increase in permeability. In addition, FAEBO was found to inhibit NO production with maximal inhibition, 82% at concentration 0.2 mg/ml. Furthermore, it was also found that FAEBO attenuated the production of cyclic guanosine monophosphate (cGMP) induced by bradykinin with inhibition 79%. Bradykinin induced activation of protein kinase C (PKC) in endothelial cell leading to reorganization of intercellular junction, however, this abolished by pre-incubation with FAEBO. FAEBO suppressed almost 53% of the PKC activity. Collectively, FAEBO exhibited anti-hyperpermeability properties via suppression of NO-cGMP signaling and PKC activity induced by bradykinin. This activity may partly contributed by the dominant compound of the FAEBO, 2-propanamine,2-methyl which was identified through GC-MS. However, further study is needed to determine the activity of the compound. No COI.

ABS0428

Analysis of blood pressure and heart rate variability in hypertensive patients before and after treatment with Dulyapabbumbud acupuncture.

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The autonomic nervous system (ANS) plays a fundamental role in the control of arterial blood pressure and heart rate, and, therefore, is considered an important pathophysiologic factor in the development of arterial hypertension. Previous studies indicate enhancing of sympathetic activity and reducing of parasympathetic in hypertensive subjects. Dissimilar to traditional Chinese acupuncture, Dulyapabbumbud acupuncture (DA) offers integration and correlation between alteration of superficial anatomy and physiology as a holistic approach. In the present study, we aimed to illustrate the influence of DA on blood pressure and behavior of the ANS in hypertensive individuals. The study comprised 11 patients diagnosed with arterial hypertension for the first time. Blood pressure and heart rate variability were recorded before and after receiving the DA treatment. Hypertensive patients, after treatment with DA, showed recovery in blood pressure (P<0.001). In addition, LF/HF ratio, LF, and SDNN were significantly reduced after the DA treatment (P<0.05). Moreover, HF was distinctively increased (P<0.05) comparing to prior DA treatment. These data suggested that a functional autonomic adjustment after DA treatment was observed, indicating recovery of sympathetic and parasympathetic tonus. No COI.
P1 ALTERNATIVE AND COMPLEMENTARY MEDICINE

ABS0462
Vasoactive effects of semen from Psoralea Corylifolia on rat artery
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PCE (PS) is an herbal compound extracted from Psoralea corylifolia seed and has been used in traditional medicines for many years. We studied vasoactive mechanisms of PCE in isolated rat aortic rings using organ bath technique. Phenylephrine (Phe) induced pre-contracted aortic rings were relaxed with endothelial- and dose-dependent. The L-NAME and ODQ significantly attenuated PCE-induced relaxation, respectively. However, extracellular potassium induced pre-contraction was minimally regulated upon treatment of PCE and pretreatment of potassium channel inhibitors, TEA, Glibenclamide, and 4-AP did not result in significant changes in PCE-induced relaxation on Phe-induced precontraction. Blockage of neither L-type calcium channel nor TRPC were not significantly alter PCE-induced vasorelaxation. While Indomethacin pretreatment significantly inhibited the vasodilatory effect of PCE. Paradoxically, PCE pretreatment inhibited Carbachol-induced vasorelaxation and Atropine and Hexamethonium reduced vasodilatory action of PCE. PCE-dependent inhibition were measured from muscarinergic receptor-dependent and -independent TRPC3 channels current in heterologous overexpressed HEK293 cells using patch clamp techniques. Based on the results from present study, the vasodilatory action of PCE was dependent on endothelial NO synthesis and cyclic guanylate, addition to prostaglandin. Although the compounds in PCE had antimuscarinic action may attenuate the vasodilative action, majority of compounds in PCE attributed vasodilation on aortic ring of rat and it may useful to cardiovascular therapeutic agent in the case of hypertension and related diseases. No COI.

ABS0466
The preventive effect of Thunbergia laurifolia, Linn. on alcohol detoxification in rats
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Thunbergia laurifolia Linn (TL) or Rangchert is a thai herbal medicine which has been used as crude extracts for various detoxification including alcohol intoxication. This study aims to evaluate the preventive effect of TL on alcohol intoxication using various behavioral models. Balance and motor coordination were determined using a narrow balance beam elevated 50 cm from the ground. The rats were placed at 30, 50, 80 and 100 cm away from the goal box and allowed to traverse the beam to the goal box and the mean number of hindlimb footslips during three trials was recorded. The effect of alcohol and TL on anxiety or sedation and exploratory behavior were also tested using elevated plus-maze and hole-board, respectively. A methanol extract of TL (200 mg/kg, orally) was administrated to Wistar rats (300–350 g) 60 minutes before 20% ethanol (2 g/kg, i.p.) administration. The behavioral tests were performed 30 minutes later started with the elevated plus maze (5 min.), hole-board (10 min) and balance beam. The results demonstrated that ethanol administration significantly increased (P < 0.05) the number of hindslips by 100, 250, 50 and 145% at 30, 50, 80 and 100 cm, respectively, indicating the poor motor performance when compared to control group. When TL was pretreated to the rats prior to ethanol exposure, the number of hindslips were significantly reduced (P < 0.05) by 20, 80, 58 and 40% at 30, 50, 80 and 100 cm distance, respectively. The results indicated that TL can reverse the effect of alcohol intoxication represented by the improvement of balance and motor behavior. However, there are no significant effect of ethanol and TL on anxiety, sedation and exploratory behavior. No COI.
Lumbrokinase attenuates myocardial ischemia-reperfusion injury by inhibiting TLR4 signaling
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Lumbrokinase, a novel antithrombotic agent purified from the earthworm Lumbricus rubellus, has been clinically used to treat stroke and cardiovascular diseases. However, inflammatory responses in the cardioprotective effect of lumbrokinase remain unknown. In this study, the signaling pathways involved in lumbrokinase-inhibited expression of inflammation mediators were investigated in rats subjected to myocardial ischemia-reperfusion (I-R) injury. The left main coronary artery of anesthetized rats was subjected to 60 min occlusion and 3 h reperfusion. The animals were administrated with and without lumbrokinase, and the severity of I-R-induced arrhythmias and infarction were compared. Lumbrokinase inhibited I-R-induced arrhythmias and mortality. Lumbrokinase decreased the lactate dehydrogenase levels in carotid blood during the same period. Lumbrokinase also inhibited the enhancement of the I-R induced expression of cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), and matrix metalloproteinase (MMP)-9 through toll-like receptor 4 (TLR4) signaling pathway. Our results also demonstrated that stimulation with lumbrokinase decreased the phosphorylation of JNK, IκB, and NF-κB. These findings suggest that lumbrokinase is a potent antiarrhythmic agent with cardioprotective properties in rats with I-R injury. The cardioprotective effects of lumbrokinase may be correlated with its inhibitory effect on the I-R-induced expression of COX-2, iNOS and MMP-9 and mediated by the TLR4 signaling through the JNK and NF-κB pathways. No COI.

Alterations in vascular functions in experimental androgen deprivation.
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Aim: To investigate the mechanisms underlying vascular dysfunction and to shed light on the cardiovascular risk factors in androgen-deprived state. Materials and Methods: Adult male Wistar rats were exposed to androgen-deprived state whether through surgical orchidectomy (ORX) or through finasteride treatment. Both groups were subjected to body weight (BW) measurement, recording of ECG, measurement of ABP, plasma androgens, plasma catalase activity and plasma malondialdehyde (MDA) levels. For the surgically (ORX) rats, baroreflex functions, aortic vascular reactivity, and aortic nitrate level were determined. Estimation of plasma lipid profile as well as cardiac weights were done in the finasteride-treated rats. Results: Significant reduction in the plasma androgens was present in both groups with a significant increase in their final BW. In the ORX rats, significant increases in the heart rate, QRS complex duration, aortic ring ACh/PE ratio, plasma MDA level existed. But significant decreases in the aortic ring responses to phenylephrine, plasma catalase activity and aortic tissue nitrate were evident. In the finasteride-treated rats, the QTo and QTc and high density lipoprotein-cholesterol were significantly increased. Moreover, the plasma levels of triglycerides, total cholesterol, and low density lipoprotein-cholesterol were significantly decreased. Conclusion: The hypo-gonadal state has a limited safety margin, and deterioration in cardiac and vascular functions in the absence of male sex hormones may occur, thereby, suggesting protective effects of androgens on the cardiovascular system. No COI.
ABS0081

Cardiac-specific ablation of Ppp2ca causes electrical remodeling in mouse heart

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We have previously observed that the mice with cardiac-targeted deletion of Ppp2ca gene (KO) developed hypertrophic cardiomyopathy at the postnatal day 11 and died around the day 13. The aim of present study was to analyze the electrical remodeling features in KO mice. As compared to control, cardiac hypertrophy was evidenced by the increases in left ventricle weight to body weight ratio and left ventricle mass, by the significant elevation of hypertrophy markers expression, by the increase in both LVED and LVSD as well as by the decrease in the FS and EF in KO mice (P<0.05). In addition to morphological changes observed in HE staining, notable ultrastructural alterations in KO myocytes were addressed in TEM analysis. Moreover, electrophysiological recordings indicated that the duration of APs in KO myocytes were markedly prolonged as compared with those in control. In contrast, no changes were observed in RPs and other parameters of APs. Consequently, a significant reduction in whole-cell Ito, and intermediate increase of whole-cell ICa-L were documented in KO myocytes. In consistence with changes in currents, a down-regulations of Kv4.2, Kv4.3, Kv1.4 and KChIP2 proteins co-assembling α and β subunits of Ito channel were detected by western blot analysis. Whereas, notably, not an increased expression of Cav1.2 but the increased phosphorylation of CaV1.2 Ca2+ channels in KO mice was demonstrated by immunohistochemistry and western blot analysis in comparison with those of control. Taken together, these findings are indicative of the fundamental role of PP2A in mouse heart and imply that deficiency of PP2A may perturb phosphorylation status of CaV1.2 and intracellular Ca2+ homeostasis, leading to hypertrophic cardiomyopathy along with electrical remodeling. No COI.

ABS0128

Endogenous Angiotensin-(1-7)/MAS receptor/NO is a novel pathway involved in the cardioprotective effects of pacing postconditioning

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Background: Pacing-induced postconditioning (PPC) has been shown to protect the heart against ischemia reperfusion injury. The aim of the present study was to investigate the role of Angiotensin-(1-7) [Ang-(1-7)] receptor (Mas) and nitric oxide (NO) in PPC-mediated cardioprotection against ischemia reperfusion (I/R) injury. Methods: Cardiac contractility and hemodynamics were assessed using a modified Langendorff system, infarct size was evaluated using 2,3,5-Triphenyl-2H-tetrazolium chloride (TTC) staining and levels of phosphorylated and total endothelial NO synthase (eNOS) were determined by Western Blotting. Isolated hearts were subjected to 30 min of regional ischemia, produced by ligation of left anterior descending (LAD) coronary artery followed by 30 min of reperfusion (n=6). Hearts were also subjected to pacing postconditioning (3 cycles of 30 seconds left ventricle (LV) pacing alternated with 30 seconds right atrium (RA) pacing) and/or treated during reperfusion with Ang-(1-7), L-NAME or Ang-(1-7) receptor (Mas) antagonist ((D-Ala7)-Angiotensin I/II (1-7). Results: PPC-mediated a significant (P<0.01) improvement in cardiac contractility and hemodynamics, infarct size and eNOS phosphorylation. These improvements were significantly attenuated upon treatment with (D-Ala7)-Angiotensin I/II (1-7) or L-NAME. Treatment with Ang-(1-7) significantly (P<0.01) improved cardiac function and reduced infarct size, however, the effects of Ang-(1-7) were not additive with PPC. Conclusions: These data provide novel insights into the mechanisms of PPC in that they involve MAS receptor and eNOS in PPC-mediated cardioprotection. This study is supported by grant #YM09/11 from Kuwait University. No COI.
Combined NSAID to antidepressant treatment induces cardiovascular alterations in an animal model of depression

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Depression is associated with dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic hyperactivity, and inflammation. Treatment of depression may be achieved by adding NSAID to a typical antidepressant, but combination therapy may cause a predisposition to cardiovascular disease. We investigated the cardiovascular effects of combined fluoxetine with celecoxib in a chronic mild stress (CMS) rat model of depression. The rats were divided into 4 groups including control, CMS, CMS with fluoxetine treatment (5 mg/kg/d), and CMS with fluoxetine and celecoxib treatment (5mg/kg/d each). At the end of 5-week treatment, the following parameters were examined: body weight, plasma cortisol using ELISA kit, heart rate variability (HRV) determined from tail pulse, blood pressure (BP) using a tail-cuff method, and in vitro aortic responses to acetylcholine. CMS induced increases in plasma cortisol and BP, but decreases in body weight and HRV. Both fluoxetine and drug combination partially lowered the plasma cortisol, indicating attenuated HPA axis activity. Celecoxib co-treatment caused significantly higher BP than fluoxetine alone. Fluoxetine did not alter HRV, whereas combination treatment significantly enhanced HRV, reflecting an improvement of cardiac ANS regulatory function. Drug combination produced significantly higher body weight gain than fluoxetine monotherapy, suggesting a possibility of body fluid retention. CMS induced a decrease in vasorelaxation to ACh which was not reversed by fluoxetine. The adjunct celecoxib partially enhanced ACh-induced vasorelaxation, indicating alleviated endothelial dysfunction. This study suggests that combined NSAID to antidepressant may cause risk of hypertension associated with body fluid retention. No COI.

The calmodulin inhibitor CGS 9343B inhibits voltage-dependent K⁺ channels in rabbit coronary arterial smooth muscle cells

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We investigated the effects of the calmodulin inhibitor CGS 9343B on voltage-dependent K⁺ (Kv) channels using whole-cell patch clamp technique in freshly isolated rabbit coronary arterial smooth muscle cells. CGS 9343B inhibited Kv currents in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC50) value of 0.81 µM. The decay rate of Kv channel inactivation was accelerated by CGS 9343B. The rate constants of association and dissociation for CGS 9343B were 2.77 ± 0.04 μM⁻¹s⁻¹ and 2.55 ± 1.50 s⁻¹, respectively. CGS 9343B did not affect the steady-state activation curve, but shifted the inactivation curve toward to a more negative potential. Train pulses (1 or 2 Hz) application progressively increased the CGS 9343B-induced Kv channel inhibition. In addition, the inactivation recovery time constant was increased in the presence of CGS 9343B, suggesting that CGS 9343B-induced inhibition of Kv channel was use-dependent. Another calmodulin inhibitor, W-13, did not affect Kv currents, and did not change the inhibitory effect of CGS 9343B on Kv current. Our results demonstrated that CGS 9343B inhibited Kv currents in a state-, time-, and use-dependent manner, independent of calmodulin inhibition. No COI.
ABS0250
The signaling mechanisms of cilostazol-induced vasodilation by activation of big conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in aortic smooth muscle
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We investigated the effect of cilostazol in phenylephrine (Phe)-induced pre-contracted aortic rings. Cilostazol induced vasorelaxation in a concentration-dependent manner. Application of the voltage-dependent K\textsuperscript{+} (Kv) channel inhibitor 4-AP, the ATP-sensitive K\textsuperscript{+} (KATP) channel inhibitor glibenclamide, and the inwardly rectifying K\textsuperscript{+} (Kir) channel inhibitor Ba\textsuperscript{2+} did not alter the vasorelaxant effect of cilostazol; however, treatment with the big-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (BKCa) channel inhibitor paxilline inhibited the vasorelaxant effect of cilostazol. This vasorelaxant effect of cilostazol was reduced in the presence of an adenylyl cyclase or a protein kinase A (PKA) inhibitor. Inside-out single channel recordings revealed that cilostazol induced the activation of BKCa channel activity. The vasorelaxant effect of cilostazol was not affected by removal of the endothelium. In addition, application of a nitric oxide synthase and a small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (SKCa) channel inhibitor did not affect cilostazol-induced vasorelaxation. We conclude that cilostazol induced vasorelaxation of the aorta through activation of BKCa channel via a PKA-dependent signaling mechanism. No COI.

ABS0251
The inhibitory effect of efonidipine, a T-type Ca\textsuperscript{2+} channel inhibitor, on voltage-dependent K\textsuperscript{+} current in rabbit coronary arterial smooth muscle cells
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The effect of efonidipine, a commercially available antihypertensive drug and Ca\textsuperscript{2+} channel inhibitor, on voltage-dependent K\textsuperscript{+} (Kv) channels was studied in freshly isolated rabbit coronary arterial smooth muscle cells using the whole-cell patch clamp technique. The amplitude of Kv current was decreased by application of efonidipine in a dose-dependent manner, with IC\textsubscript{50} of 0.26 \mu M and a Hill coefficient of 0.91, which suggests 1:1 binding stoichiometry. Efonidipine did not affect voltage-dependent activation of the Kv channel, but shifted the inactivation curve by \textbf{\textendash}8.87 mV. The inhibitory effect of efonidipine was not significantly changed by depletion of extracellular Ca\textsuperscript{2+} or intracellular ATP, which indicated no involvement of the Ca\textsuperscript{2+} channel or intracellular protein kinase-dependent cascades. We conclude that efonidipine dose-dependently inhibits Kv current in a phosphorylation- and Ca\textsuperscript{2+} channel-independent manner. No COI.
ABS0252
The direct inhibition of trifluoperazine, an antipsychotic drug and calmodulin inhibitor, on voltage-dependent K⁺ channels in coronary arterial smooth muscle cells
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We investigated the effect of the calmodulin inhibitor and antipsychotic drug trifluoperazine on voltage-dependent K⁺ (Kv) channels. Kv currents were recorded by whole-cell configuration of patch clamp in freshly isolated rabbit coronary arterial smooth muscle cells. The amplitudes of Kv currents were reduced by trifluoperazine in a concentration-dependent manner, with an apparent IC₅₀ value of 1.58 ± 0.48 μM. The rate constants of association and dissociation by trifluoperazine were 3.73 ± 0.33 μM⁻¹s⁻¹ and 5.84 ± 1.41 s⁻¹, respectively. Application of trifluoperazine caused a positive shift in the activation curve but had no significant effect on the inactivation curve. Furthermore, trifluoperazine provoked use-dependent inhibition of the Kv current under train pulses (1 or 2 Hz). These findings suggest that trifluoperazine interacts with Kv current in a closed state and inhibits Kv current in the open state in a time- and use-dependent manner, regardless of its function as a calmodulin inhibitor and antipsychotic drug. No COI.

ABS0261
Optical mapping study of the circus movement of the excitatory waves in the rat isolated atrium preparation under the intracellular Ca²⁺ overloaded condition
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Using optical mapping methods, we have studied spatiotemporal patterns of electrical activities in the isolated rat atrial preparations without anatomical obstacles under the intracellular Ca²⁺ overloaded condition during the experimental tachyarrhythmia (tachycardia-like excitation: TE). Auricular preparations without the ostia of large vessels were dissected from adult rats. Each preparation was then stained with a fast merocyanine-rhodamine voltage-sensitive dye (NK2761). Using a multi-element (16 X 16) photodiode array, we assessed optically the spread of excitatory waves by timing the initiation of the optical action potentials. The contraction-related optical signals were suppressed by adding 2,3-butanedione monoxime (BDM: 20 mM) or Cytochalasin D (CytoD: 20 - 40 μM) to the bathing solution. Intracellular Ca²⁺ was overloaded by applying frequent stimulus (3 - 5 Hz / 10 - 20 min) in the bathing solution with high Ca²⁺ concentration (18 mM). After the loading of Ca²⁺, the tetanus stimulation (10 Hz, 3 - 5 shocks) evoked sustained anomalous tachycardia (i.e. TE). During this condition, we have optically mapped the spatiotemporal patterns of the excitation spread. In the maps, the circus movement of the excitatory waves was observed in the artificial preparation of the atrial free wall without anatomical obstacles, and this “micro re-entry” cause the repetitive excitation during TE. These findings support our hypothesis that the various anomalous patterns of the excitation spread and arrhythmias including the re-entry of the excitatory waves in isolated atrium preparations were induced by the increase and the inhomogeneity of the intracellular Ca²⁺. No COI.
**ABS0278**

**Chemerin decreases contractility and coronary flow of isolated rat hearts**

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Epicardial adipose tissue (EAT) is local energy source for the contractile activity of the heart. Chemerin is also a novel chemokine. Although chemerin and its receptor have been detected in EAT, cardiovascular effects of chemerin have not been investigated. Therefore, we studied the possible effect of chemerin on left ventricular developed pressure (LVDP; an index of cardiac contractility), maximal rate of pressure development of left ventricle (+dP/dtmax; another index of cardiac contractility), heart rate (HR), coronary flow (CF), monophasic action potential amplitude (MAPamp) and MAP duration at 90% repolarization (MAP90). The hearts were isolated under sodium thiopental (50 mg/kg) anesthesia and perfused with modified Krebs-Henseleit buffer (mK-Hb) under constant pressure conditions. After stabilization, 10, 100 and 1000 nM doses of chemerin was administered to the hearts for 30 minutes. In another group, the hearts were perfused with mK-Hb containing 10 mM L-NAME (nitric oxide synthase inhibitor) for 5 minutes before 1000 nM chemerin administration. One hundred, 100 and 100nM doses of chemerin significantly decreased LVDP and +dP/dtmax. 1000 nM dose of chemerin also decreased CF. L-NAME antagonized the contractility induced by 1000 nM chemerin. Chemerin at all doses had no effect on HR, MAPamp, MAP90. We suggest that chemerin possesses a negative inotropic action on isolated rat hearts and nitric oxide may mediate this effect. No COI.

**ABS0280**

**Has omentin a negative inotropic impact on isolated rat heart?**

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Omentin is a recently identified novel adipokine mainly expressed in epicardial adipose tissue. Although it has favorable effects on cardiovascular disease, impact of omentin on the hearts is still unknown. Hence we aimed to investigate the potential effects of omentin on the heart and to explore whether phosphoinositide 3-kinase (PI3K) signalling pathway mediates cardiac contractility induced by omentin. Therefore, we studied the possible effect of omentin on left ventricular developed pressure (LVDP; an index of cardiac contractility), maximal rate of pressure development of left ventricle (+dP/dtmax; another index of cardiac contractility), heart rate (HR), coronary flow (CF), monophasic action potential amplitude (MAPamp), MAP duration at 90% repolarization (MAP90). The hearts were isolated under sodium thiopental (50 mg/kg) anesthesia and perfused with modified Krebs-Henseleit solution (mK-Hs). After stabilization, 100, 200 and 400 ng/ml doses of omentin was administered to the hearts for 30 minutes. In another group, the hearts were perfused with mK-Hs containing LY294002 (10 µM), a PI3K inhibitor, for 5 minutes before 400 ng/ml omentin administration. One hundred, 200 and 400 ng/ml doses of omentin significantly decreased LVDP and +dP/dtmax. Omentin at all doses had no effect on HR, CF, MAPamp and MAP90. Our study is therefore the first one that demonstrates that an acute omentin treatment leads to a negative inotropic action on isolated rat heart. This effect may be mediated by PI3K-dependent mechanism. No COI.
ABS0292
The Study of the serum level of IL-4, TGF-β, IFN-γ and IL-6 in overweight patients with and without Diabetes and Hypertension
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Diabetes is one of the main reasons of mortality in the world. Overweight increases the risk of diabetes and hypertension which both expose individuals to cardiovascular diseases, renal failure and brain stroke. The cytokines are involved in the pathogenesis of these diseases. The objective of the present study is to analyze the serum levels of IL-4, TGF-β, IFN-γ, and IL-6 cytokines of overweight men with diabetes and/or hypertension so as to highlight the association of these cytokines with such diseases. Descriptive study was carried out on 164 individuals aging 20-50 years with BMI ranging 28.5-30 kg/m² participating in Kerman coronary artery disease risk factors study (KERCADRS). In this regard, 54 men lacked diabetes and hypertension (CTL group), 36 individuals had both diabetes and hypertension (DH group), 20 individuals had diabetes without hypertension (D group), and 54 persons had hypertension without diabetes (H) all of which were selected for measurement of serum cytokines through ELISA kits. Data were analyzed using Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni correction for pairwise comparisons. The results showed that the concentration of IFN-γ in DH group is significantly higher than the CTL group (p=0.002), IL-6 in DH and D groups is significantly lower than the CTL group (p=0.006, p=0.004, respectively). The serum level of TGF-β and IL-4 cytokines did not show any significant difference across the four groups. It seems that the proinflammatory cytokine IFN-γ has a significant role in the pathogenesis of hypertension in diabetes. No COI.

ABS0300
Cardiac progenitor cells that spontaneously develop into beating cardiomyocytes in the adult mouse heart
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We recently discovered a novel subpopulation of adult mouse heart cells that spontaneously develop into beating cardiomyocytes in the culture of cardiomyocyte-removed fraction cells (non-myocytes). We defined these beating cells as atypically-shaped cardiomyocytes (ACMs) based on their peculiar morphology. In cultures of the non-myocytes, some of the small cells were found to grow in size or fuse with each other to become more complexly shaped multi-nuclear beating ACMs without requiring hormones or chemicals. The ACMs then continued to beat for at least three weeks, but did not appreciably proliferate. ACMs could be identified in the neonatal heart and survive the long-term post-natal development while preserving the expression of fetal cardiac gene products, such as atrial natriuretic peptide (ANP) and T-type Ca²⁺ channel (Ca,3.2). When non-myocytes and myocytes were co-cultured, the ACMs did not fuse to ventricular myocytes, but rather independently developed into beating cells. Cellular prion protein (PrP), also known as CD230, was found to serve as a marker for ACMs that enable us to identify these cells within various types of non-myocytes, such as fibroblasts, in the culture. In combination with cardiac-specific contractile protein cardiac troponin T (cTnT), PrP was demonstrated to specifically identify native ACMs in the interstitial spaces among ventricular myocytes in the heart. These data suggest that quiescent but still functionally viable cardiac progenitors, originating from the fetal stage, exist in the adult mouse heart. No COI.
ABS0335

Human cardiac progenitor cells expressing prion protein and cardiac troponin T in the normal and infarcted heart

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The adult heart is now known to comprise cardiac stem or progenitor cells. We recently discovered a novel subpopulation of adult mouse heart cells that spontaneously develop into beating cardiomyocytes, defined as atypically-shaped cardiomyocytes (ACMs). ACMs showed more resistance to severe ischemic conditions compared to ventricular myocytes and survived the long-term post-natal development while preserving the expression of fetal cardiac gene products. In combination with cardiac-specific contractile protein cardiac troponin T (cTnT), cellular prion protein (PrP), also known as CD230, was demonstrated to specifically identify native ACMs in the adult mouse heart. According to the data obtained in the mouse heart, we examined the localization of PrP/cTnT-expressing ACMs-like cells in the human heart. Human cardiac ventricular tissues fixed within 2 h after death were selected from patients (aged 41~85 years). A small number of PrP/cTnT-positive cells were observed in the interstitial space among ventricular myocytes from the endo- to the epicardium of the normal heart tissues, indicating that these cells remain in the normal human heart throughout one’s lifetime. We also found that the PrP/cTnT-positive cells existed in the border zone of acute myocardial infarction, ~7 days after infarction, in which adjacent ventricular myocytes died. These findings suggest the possibility that the PrP/cTnT-positive ACMs can survive under pathophysiological conditions and may act as substitution for dying cardiomyocytes, although they do not appear to be able to rescue the cardiac function. No COI.

ABS0347

Acute ethanol increased whole-cell currents via an adenosine receptor in human coronary artery endothelial cells

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Ethanol (EtOH) is known to induce coronary vasorelaxation and NO release. In other cells, it can modulate several ion channels, especially Ca$^{2+}$-activated potassium channel (KCa), which can influence endothelial NO release. Additionally, EtOH-induced NO release may be mediated by adenosine receptor (AdoR) in umbilical veins endothelial cells. Therefore, we hypothesized that EtOH may affect K$^+$ channels in human coronary artery endothelial cells (HCAECs), via AdoR, leading to NO release. We investigated the effects of acute ethanol application (1-50 mM) on HCAEC whole-cell currents in response to a voltage ramp (-110 to +100 mV in 200 ms) to compare whole-cell currents with vs. without cell exposure to EtOH. Current-voltage relation was constructed for each cell. % Control of whole-cell currents at +80 mV after acute application of 1, 3, 10, 20, 30 and 50 mM EtOH were (mean±s.e.m.) 95.31±1.87 % (n=6; not significant, ns), 102.99±4.35 % (n=7, ns), 103.48±0.90 % (n=6; p<0.05), 129.11±9.99 % (n=9; p<0.05), 124.23±6.99 % (n=9; p<0.05) and 141.71±10.21 % (n=6; p<0.05), respectively. However, after preincubation with 10 µM CGS15943, a non-selective AdoR antagonist, EtOH could no longer increase HCAEC currents significantly (n=5; ns). Our data indicate that 10-50 mM EtOH may increase whole-cell currents in HCAECs via adenosine receptor activation. No COI.
ABS0362
Bilobalide-induced SKCa current increase in human coronary artery endothelial cells is mediated by 5-HT1/2 receptor and PLC
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Ginkgo biloba leaves extract and its active ingredient bilobalide have been shown to cause vasorelaxation in some vascular beds. Recently, our laboratory demonstrated in human coronary artery endothelial cells (HCAECs) that bilobalide could dose-dependently increase whole-cell currents through activation of SKCa channels, but the responsible mechanism remains to be elucidated. In this study, HCAEC currents in response to a voltage ramp (-100 to +100 mV, 200 ms) were recorded using whole-cell patch clamp technique. With external SKCa isolating solution (containing blockers of BKCa, IKCa, Kir, TRP and Cl⁻ channels), 100 µM bilobalide significantly enhanced SKCa currents by 63.62 ± 46.48 % compared to control (n=6, p<0.05), while subsequent exposure to 500 µM apamin, a specific inhibitor of SKCa channel, could significantly decrease the bilobalide-induced currents by 75.87 ± 24.86 %, (n=6, p<0.05). To investigate whether a 5-HT receptor was involved, HCAECs were pre-incubated with 1 µM methiothepin, a non-selective 5-HT1/2 receptor antagonist. The results showed that methiothepin pre-incubation could prevent HCAEC current increase in 100 µM bilobalide (n=5, p>0.05). Moreover, pre-incubation with U73122, a specific PLC blocker, also significantly decreased HCAEC currents after bilobalide exposure (n=5, p<0.05). Our results indicate that 100 µM bilobalide significantly increased SKCa currents in HCAECs via 5-HT1/2 receptors and possibly PLC activation. No COI.

ABS0337
The responses of pulmonary and systemic circulation and airway to allergic mediators in anesthetized BALB/c mice
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Systemic anaphylactic shock sometimes accompanies pulmonary vaso- and broncho-constriction. No systematic study in which the effects of anaphylactic substances were investigated on pulmonary vascular resistance (PVR) in in vivo mouse by directly measuring cardiac output and the inflow and outflow pressures in the pulmonary circulation. We determined the responses of pulmonary vascular resistance (PVR), total peripheral resistance (TPR) and airway pressure (AWP) to platelet-activating factor (PAF), histamine, serotonin, leukotriene (LT) C4, and prostaglandin (PG) D4 in anesthetized BALB/c mice. Pulmonary arterial pressure (PAP), left atrial pressure (LAP), and aortic blood flow were measured. PVR increased dose-dependently in response to consecutive administration of all vasoconstrictors with the order of maximal responsiveness being PAF>LTC4>serotonin>>histamine=PGD2. TPR decreased dose-dependently in response to PAF, serotonin, and histamine, while it showed an increase or no changes when LTC4 or PGD2 was injected, respectively. Serotonin, but not the other agents, increased airway pressure. In conclusion, PAF, LTC4 and serotonin cause substantial pulmonary vasoconstriction and PAF, serotonin, histamine vasodilatation. Serotonin induces airway constriction in anesthetized BALB/c mice. No COI.
Inflammation is regarded as a complicated pathophysiology process that is triggered by direct activation of receptors or by the secretion of inflammatory mediators. These resulted in vasodilatation, increased vascular permeability, leakage of fluid and migration of immune cells at the site of inflammation. However, if prolonged, can lead to tissue damage as well as pathogenesis of fatal diseases. Stachytarpheta jamaicensis (SJ) or locally known as Selasih Dandi in Malaysia, belongs to the family of Verbenaceae. The plant was previously proven to have high medicinal properties, nevertheless, little is known on the anti-inflammatory benefit of SJ. Thus, this study was designed to explore the effect of SJ on inflammatory properties. The cytotoxicity of methanol extract of SJ was investigated against normal (HUVECs) and cancer cell lines (RAW 246.7) using 3,(4,5 -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To determine the anti-inflammatory properties of SJ, nitric oxide (NO) production inhibitory activity was assessed in LPS-stimulated RAW 246.7 using Griess assay. The methanol extract of SJ was found to have no cytotoxicity against HUVECs and RAW 246.7 at concentrations ranging from 0.005 mg/ml – 0.1mg/ml. 45.28% maximum inhibition of NO in RAW 246.7 cells treated with methanol extract of SJ were observed at the dose of 0.1mg/ml. The MTT cell viability assay result revealed that the inhibitory effect of methanol extract was not due to cell damage (viability >85%). It is concluded that 0.1mg/ml of methanol extract of SJ could ameliorate LPS-induced inflammation by decreasing NO overproduction. Thus, these findings suggest that SJ might be a novel therapeutic strategy for treating vascular inflammation in the future. No COI.
ABS0411

Atrial natriuretic peptide-activated pGC-cGMP signaling participated in the rapid atrial pacing-induced atrial fibrillation in rabbit

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The clinical study showed that concentration of ANP increased in the blood of atrial fibrillation (AF), but its pathophysiological role is not clear. The aim of this study, we investigated the effect of ANP-activated pGC-cGMP signaling on rapid atrial pacing induced AF in rabbit. The model of rapid atrial pacing in rabbits was divided into electrically stimulating for 8 hours (P8) and control group (P0). Rapid atrial pacing increased blood ANP secretion concomitantly with decreased level of cGMP in atrial tissue, with statistical significance (P<0.01). HE staining showed that there was no obvious difference in sham and 8h pacing of rabbit. Expression of NPR-A/B was observed in sham and 8h pacing groups by IHC and Western blot, but NPR-A/B staining showed no significant between the two groups. Indirect measurement of the pGC actinity demonstrated a significant increase in 8h pacing group compared with that in sham using ELISA technique. These data suggest that altered role of ANP/pGC/cGMP signaling pathway may be involved in the cardiac dysfunction on rapid atrial pacing induced AF. This research was supported by the National Natural Science Foundation of China (81160022, 81460056). No COI.

ABS0436

Intermedin1-53 protects against cardiac hypertrophy by inhibiting endoplasmic reticulum stress via activating AMP-activated protein kinase

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Objective: Intermedin (IMD), a novel member of the calcitonin/calcitonin gene-related peptide family, is involved in maintaining circulatory homeostasis and is a protective factor of heart and vessel. Here, we investigated the effects of IMD on cardiac hypertrophy in vivo and in vitro and explored the mechanisms involved. Methods and Results: IMD1-53 (100 ng.kg-1.h-1) was systemically administered to rats with cardiac hypertrophy induced by abdominal aortic constriction (AAC) by a mini-osmotic pump the next day after surgery continuously for 4 weeks. The AAC treated rats before IMD infusion showed increased IMD content and expression of its receptors in the hearts. In vivo administration of IMD1-53 greatly attenuated the cardiac hypertrophy as shown by heart weight to body weight(HW/BW), hemodynamics, echocardiography, histological analyses, and expression of hypertrophic markers atrial natriuretic peptide (ANP) and brain natriuretic peptide(BNP) induced by AAC. IMD1-53 treatment significantly reduced the myocardial protein expression of endoplasmic reticulum stress (ERS) markers such as glucose-regulated protein 78 (GRP78), CCAAT/enhancer binding protein homologous protein (CHOP), and caspase-12, whereas the protein level of phosphorylated AMP-activated protein kinase (p-AMPK) was upregulated with IMD1-53 treatment, which was further confirmed in cultured cardiomyocytes. Concurrently, cardiomyocyte apoptosis in vivo and in vitro was ameliorated by IMD1-53 treatment. The inhibitory effects of IMD1-53 on ERS and apoptosis were eliminated on pretreatment with compound C, an AMPK inhibitor. Conclusion: IMD1-53 could exert its cardioprotective effect on cardiac hypertrophy by inhibiting myocardial ERS and apoptosis, possibly via activation of AMPK signaling. No COI.
Supraphysiological dose of testosterone induces hypertrophy of both cardiac and soleus muscles but differentially impacts on the mitochondrial functions.

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Anabolic-androgenic steroids have been abused in many athletes to increase skeletal muscle mass and strength. However, cardiac hypertrophy is also resulted and cardiac sudden death may be apparent. We have found that supraphysiological doses of testosterone time dependently induced both physiological and pathological cardiac hypertrophy. Whether mitochondrial functions are differentially altered in these two types of testosterone-induced cardiac hypertrophy as well as in the skeletal muscle were questioned. Male Sprague-Dawley rats, sham and testosterone-injected (10 mg/kg BW, 3 times/week) groups, were treated for 4 and 12 weeks to induce physiological and pathological cardiac hypertrophy, respectively. While hypertrophy of cardiac and soleus muscles were detected in every testosterone-treated groups, mitochondrial density was significantly lower (~12%) only in pathological hypertrophic hearts. Mitochondrial ATP production was also significantly lower (~17%) in pathological hypertrophic hearts without affecting ROS production. With antimycin A, ROS production was significantly enhanced in both types of hypertrophic heart compared to controls. Interestingly, mitochondrial membrane hyperpolarization was detected in pathological hypertrophic hearts without mitochondrial swelling. In contrast, prolonged hypertrophy of soleus muscle yeilded suppressed levels of antimycin A-activated ROS production. These results suggest that mitochondrial functions are differentially altered in the two types of cardiac hypertrophy as well as in the hypertrophic soleus muscle induced by testosterone. No COI.

Hesperetin-induced vesorelaxation in human umbilical vein preparation

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Hesperetin, a metabolized form of hesperidin found in citrus fruits, has been found to possess vasorelaxing effects in both experimental animals and human subjects, but the mechanisms involved have not been well characterized. This project aimed to study the mechanism of hesperetin-induced vasorelaxation in human umbilical vein (HUV) rings using isometric force measurement. HUV rings (3-5 mm) from normal pregnancy and delivery were suspended in modified Krebs solution (37 °C, pH 7.4, 95% O2/5% CO2). The effects of 10–3 to 10–5 M hesperetin were compared to corresponding DMSO concentrations, osmolality changes (using mannitol for adjustment) and time control. In endothelium-denuded HUV pre-contracted with 35 mM KCl, 10–4, 3x10–3, 10–3 M hesperetin caused significant reduction in HUV tension by 14.43 ± 4.23%, 48.52 ± 4.38% and 71.11 ± 4.19%, respectively (n=6, p<0.05), compared with exposure to corresponding DMSO concentrations, osmolality and the time control. Similarly, the same hesperetin concentrations could relax endothelium-denuded HUV pre-contraction with 10 µM 5-HT (serotonin) by 15.80 ± 1.99%, 27.64 ± 0.56 and 44.07 ± 2.60%, respectively. Moreover, the hesperetin-induced vasorelaxation was comparable in both endothelium-intact and denuded HUV rings. Our data indicate that hesperetin-induced vasorelaxation was not endothelium-dependent and could be mediated by membrane potential, and possibly ion channels and/or intracellular Ca2+ release mechanism, in vascular smooth muscle cells. No COI.
Effects of morelloflavone from Garcinia dulcis on the contraction of isolated thoracic aorta of cisplatin-treated rats

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Cisplatin is the one of the most widely used for cancer chemotherapy, however, it has been reported to cause a variety of cardiovascular side effects such as stroke, myocardial infarction, arterial and venous thromboembolism, enhancement of carotid artery intima media thickness, coronary artery dissection and hypertension. These side effects may be due to the action of cisplatin on a reduction in endothelial nitric oxide and an increase in reactive oxygen species. Morelloflavone is a biflavonoid extracted from Garcinia dulcis (Kurz) which has been shown to possess antioxidant effects and recently shown to cause vasodilation via endothelium dependent nitric oxide release. This study was aimed to investigate the protective effect of morelloflavone on contractile function and structural changes of thoracic aorta of cisplatin-treated rats. Male Wistar rats were divided into three groups including vehicle control, cisplatin and cisplatin + morelloflavone group. DMSO and 0.9% NaCl were used as solvent for morelloflavone and cisplatin, respectively. Morelloflavone (1 mg/kg, i.p.) was given twice 1 day and 10 mins before a single dose of cisplatin (7.5 mg/kg, i.p.) injection. Seven days after cisplatin injection, the contractile responses of isolated thoracic rings were performed by cumulative addition of 10⁻¹⁰ - 10⁻⁸ M phenylephrine from 1 g of resting tension. The relaxation responses were evaluated by cumulative addition of either acetylcholine or sodium nitroprusside (10⁻¹² – 10⁻⁵ M) after precontracted with 10⁻⁷ M phenylephrine. It is likely that the degree of vasoconstriction and vasorelaxation of cisplatin-treated rats were altered from control rats and morelloflavone may restore these changes as supported by histological study. No COI.
Analysis of the mechanism of type 2 diabetes involving ADAMTS9 /GON-1
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ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in transport from the ER to the Golgi. It has been reported that an ADAMTS9 gene variant is associated with type 2 diabetes. However, the molecular mechanisms of ADAMTS9 on the beta cell and peripheral tissues are unknown. First, we investigated how GON-1, the C. elegans homolog of ADAMTS9, is involved in the insulin signaling in C.elegans. Insulin-like proteins are secreted from neurons in the wild type background, whereas insulin-like proteins were accumulated in neurons by GON-1 depletion. To investigate the role of GON-1 in peripheral tissues, we examined the subcellular localization of DAF-16, the C. elegans homolog of FOXO. DAF-16/FOXO was present in both the nucleus and the cytoplasm in wild-type animals. DAF-16/FOXO was exclusively localized to the nucleus in peripheral tissues in the GON-1(tm3146) mutant background. Next, we investigated how ADAMTS9 is involved in the type 2 diabetes by using mammalian cell lines. Glucose-stimulated insulin secretion was gradually compromised after depletion of ADAMTS9 in the INS-1 cells, a glucose-sensitive pancreatic beta cell line. Depletion of ADAMTS9 decreased insulin-stimulated glucose uptake in differentiated 3T3-L1-derived adipocytes and differentiated C2C12-derived skeletal muscle cells. Insulin-stimulated translocation of GLUT4 to the plasma membrane was impaired by depletion of ADAMTS9 in differentiated 3T3-L1 cells. Our data suggest that ADAMTS9/GON-1 is involved in both mechanisms; insulin secretion from insulin secretory cells and insulin signaling at the peripheral tissues. No COI.

The role of reticulum endoplasmic stress in diabetes complication
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Diabetes mellitus complication is the prime cause of death in diabetic patients. Much biomedical research that can ameliorate its progressiveness has been conducted but little can elucidate its detail pathogenesis. Recent evidences shows that endoplasmic reticulum (ER) had involved in diabetes and many disease. Its not a passive organelle that functions merely as transporter of proteins into other parts of the cell or to be secreted into the extra cells. More than that, researchers has proved that ER has important role as quality control machine of protein. In protein synthesis process, ER responsible for protein three dimensional structure. ER process primary protein and or its secondary structure in each active cell. It has protein GRP78 that has high sensitive sensor in abnormal proteins formation called unfolded protein that disrupt cell homeostasis. Condition that result in the accumulation of unfolded proteins in the ER is well known as endoplasmic reticulum stress or unfolded protein response (UPR). Disturbance of ER will increase its three membranes bound protein namely IRE1, PERK and ATF6. Recent diabetes research indicate that cell in hyperglycemic environment has high ER stress state. ER stress involvement in diabetes mellitus can explain further some clinical paradox in diabetes mellitus such as phenomenon of the failure of sulfonylurea therapy, or insulin resistance caused by drugs protease inhibitor. Depletion of endoplasmic reticulum calcium depot induce apoptosis in many cell such in insulin producing cells or in vascular cell that promote micro and macro vaskuler complication. No COI.
ABS0255

**Analysis of biological functions and protein structure in mutated FcεRI β chain (D234A)**

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High affinity IgE Fc receptor (FcεRI) is expressed on mast cells as a tetrameric receptor composed of the IgE-binding α chain, four-fold membrane-spanning β chain, and disulfide-linked homodimer of the γ chains. The β chain contains immunoreceptor tyrosine-based activation motif (ITAM), a conserved feature of many antigen receptors that imparts signaling competence. We investigated the biological functions mutated FcεRI β chain (D234A) in mast cell activation upon FcεRI engagement and demonstrated that D234A severely impaired FcεRI-mediated IL-6 production, however, did not impair degranulation. On the other hands, previously we revealed that β chain ITAM with the replacement of tyrosine to phenylalanine (FFF) severely impaired degranulation, however, did not impair cytokine production. In addition, we investigated the structure that is part of FcεRI β chain wild type (β-WT, aa:143-235) protein and β-D234A (aa:143-235) protein by circular dichroism spectroscopy (CD). The far-UV CD spectra of β-WT and β-D234A are of an α-helical structure and β-D234A does not have any loss or collapse of α-helical content. Near-UV CD spectroscopy showed that a conformational change has not occurred for β-D234A. Thermal denaturation curve of β-D234A obtained from ellipticity at 222 nm was almost the same as that of β-WT protein. Significant differences of Gibbs free energy change (ΔG) were not shown between β-WT and β-D234A. Our results suggest that new signaling pathway through the D234 of the β chain may exist. No COI.

ABS0287

**Cytotoxic and antioxidant potential of Ipomoea Pes Caprae with different extracts methods**

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Ipomoea pes-caprae plant is a medicinal plant that used in folk medicine to treat jelly fish stings. The biological activities of medicinal plant are depending on solvent and method of extracts. This study is to evaluate the cytotoxic and antioxidant potential of the different solvent extracts from Ipomoea Pes Caprae. The dry plant was extracted by maceration with ethanol & water (A extracts), hexane (B extracts). Supercritical carbon dioxide (SCO2) + ethanol are used as the extracts solvent for polarity extracts (C extracts) and low polarity extracts (D extracts). The molecular cytotoxic assay was assessed by MTT, nuclear and mitochondria staining with DAPI and JC-1. The antioxidant assay was evaluated using DPPH and DCFH-DA. B and D extracts inhibited KB cells with IC50 of 200 ± 12.3 and 70 ± 4.2 μg/ml associated with the increasing of chromatin condensation. The cell population with loss of mitochondrial membrane potential (Δψm) increased significantly to 13.3% (B extracts) and 20.0% (D extracts) compared with control cells (2.0%) (p<0.05). Only A extracts had antioxidant activity against DPPH radical. The IC50 value of A extracts and ascorbic acid were 50 ± 4.2 and 5 ± 0.4 μg/ml, respectively. The intracellular hydrogen peroxide level was reduced to 15.1%, 9.1%, and 2.5% in KB cells following the treatment with 50, 100, and 200 μg/ml of A extract, respectively determined by DCFH-DA. In conclusion, the hexane and SCO2 low polarity extracts displayed cytotoxic activities through mitochondria-mediated pathway while the ethanol & water extracts showed antioxidant potential. No COI.
Homer proteins modulate RANKL-induced NFAT signaling in osteoclastogenesis
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Ca²⁺ signaling and NFATc1 activation are essential for RANKL-induced osteoclastogenesis through the induction of Ca²⁺ oscillation, calcineurin activation, and translocation of NFATc1 into the nucleus. Homer proteins are scaffold proteins and have been proposed to modulate multiple Ca²⁺ signaling channels and proteins, including inositol 1,4,5-triphosphate receptors, ryanodine receptors, transient receptor potential channels, and NFAT family of transcription factors in skeletal muscle cells and T cells. However, the role of Homer proteins in Ca²⁺ signaling during osteoclast differentiation is not known. In the present work, we investigated the role of Homer proteins (Homer2 and Homer3) in RANKL-induced Ca²⁺ signaling in osteoclasts using Homer2/Homer3 (Homer2/3) double-knockout (DKO) mice. Deletion of Homer2/3 markedly decreased the bone density of the tibias, resulting in bone erosion. In contrast, Homer2/3 deletion did not affect osteoblast formation and RANKL-induced Ca²⁺ oscillation. In forty-eight hours after RANKL treatment, the Homer2/3 DKO bone marrow-derived monocytes/macrophages (BMMs) had facilitated greatly osteoclast differentiation through NFATc1 protein expression and translocation of NFATc1 into the nucleus. Notably, the interaction of Homer proteins with NFATc1 was inhibited by RANKL treatment, but restored by cyclosporine A treatment to inhibit calcineurin in wild-type osteoclasts. In addition, RANKL treatment of Homer2/3 DKO BMMs significantly increased a ~3.0-fold induction of multinucleated cells formation. These results suggest that Homer2/3 interact with NFATc1 to sequester calcineurin in the cytosol and thus modulate the NFATc1 pathway in RANKL-induced osteoclastogenesis. No COI.

Osmo-mechanosensitive TRP channels facilitate an increase of Ca²⁺-mediated RANKL expression in mouse osteoblastic cells
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Mechanical stress plays an important role in the regulation of bone turnover. However, the intracellular mechanisms of mechanical stress under osteoblast differentiation and proliferation are not well understood. In this study, we investigated the effects of osmo-mechanosensitive transient receptor potential (TRP) channels-induced calcium signaling in primary mouse osteoblasts and MC3T3-E1 cells. Hypotonic stress induced significant increases of RANKL mRNA expression but not OPG. In addition, hypotonic stress-induced increases of intracellular calcium concentration ([Ca²⁺]i) and RANKL expression persisted in the presence of non-specific Ca²⁺ channel blockers or Ca²⁺-free bath solution. Furthermore, we examined hypotonic stress-induced effects on agonists and antagonists of osmo-mechanosensitive TRP channels in order to determine the cellular mechanism of hypotonic stress-mediated increases in [Ca²⁺]i and RANKL. We found that antagonists of TRPV4 and TRPM3 decreased hypotonic stress-mediated increases on [Ca²⁺], and protein expression levels of RANKL and NFATc1. We also identified that hypotonic stress-induced effects reduced by the genetic suppression of TRPV4 and TRPM3. Taken together, our results indicate that hypotonic stress activate the expression of RANKL and NFATc1 by [Ca²⁺], increases through TRPV4 and TRPM3 in osteoblasts. These effects may be important for the differentiation and proliferation of bone cells on bone remodeling that are mediated via mechanosensitive TRP channels. No COI.
ABS0313

Alkali stimulation-induced Ca\(^{2+}\) signaling in rat odontoblasts

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Calcium hydroxide that dissociates into Ca\(^{2+}\) and OH\(^-\) provides an alkaline environment in the dental pulp, and induces dentinogenesis. However, the detailed mechanisms in detection of extracellular alkaline environment in odontoblasts remain unclear. We examined alkaline stimulation-induced intracellular Ca\(^{2+}\) signaling pathway in rat odontoblasts.

Methods: Dentin sialoprotein- and nestin-positive odontoblasts were acutely isolated from rat incisors. Intracellular free calcium concentration ([Ca\(^{2+}\)]\(_i\)) was measured by fura-2 fluorescence. Extracellular solutions with high-pH (pH 8.5 – 10.5) were prepared by adding NaOH to standard Krebs solution. Results: In the presence (2.5 mM) and absence (0 mM) of extracellular Ca\(^{2+}\), application of alkaline solution increased [Ca\(^{2+}\)]\(_i\), showing dependence of [Ca\(^{2+}\)]\(_i\) on extracellular pH (pH 8.5–10.5) and Ca\(^{2+}\) concentration. Increases in [Ca\(^{2+}\)]\(_i\) induced by the alkaline solution (pH 10), in the presence of extracellular Ca\(^{2+}\), were inhibited by an antagonist of transient receptor potential ankyrin subfamily member 1 (TRPA1) channels. Conclusion: Alkaline stimuli activate the intracellular Ca\(^{2+}\) signaling pathway via Ca\(^{2+}\) influx and intracellular Ca\(^{2+}\) release in odontoblasts. These plasma membrane/intracellular high-pH-sensing mechanisms in odontoblasts may play an important role in cellular functions during dentinogenesis induced by calcium hydroxide. No COI.

ABS0326

The G\(_i/o\) coupled muscarinic receptors form complex with the G protein gated inwardly rectifying potassium channel

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The G protein gated inwardly rectifying potassium (GIRK) channel negatively regulates the excitability of neuronal and muscular cells. The GIRK channel is activated by G\(_{\beta\gamma}\) mostly released from Gi/o. On the other hand, G\(_{\beta\gamma}\) released from Gq or Gs have been reported to activate the channel when the expression level of the coupling receptors is high. Under a high surface expression condition, distance between the receptors and the channels is expected to be decreased, which may enable G\(_{\beta\gamma}\) release from Gq or Gs to activate the channel. To examine this possibility, the Gq coupled muscarinic receptor type1 (M1R) and the GIRK channel were connected with various lengths of glycine rich amino acid residues. The M1R activated the GIRK channel when the number of the linker residues was 100 or less, whereas it did not activate the channel when the number of the residues was 268 or more. In contrast, the Gi/o coupled muscarinic receptors, such as the M2R or the chimeric construct of the M1R and M2R (MC9), activated the GIRK channel even when the number of the linker residue was 541. Moreover, analyses of Förster resonance energy transfer (FRET) between the receptor-YFP and GIRK-CFP revealed that the MC9-YFP, but not M1R-YFP, stays in the proximity to the channel. These results suggested that the Gi/o coupled muscarinic receptors form complex with the GIRK channel for the efficient activation. No COI.
ABS0329
Voltage-gated proton channel Hv1/VSOP inhibits granule exocytosis in neutrophils and inflammation
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Voltage-gated proton channel is a molecule that regulates intracellular pH and membrane potential. We discovered this molecule and have been analyzing the function of this channel at cellular and animal levels using Hv1/VSOP deficient mice. We and collaborators have reported that Hv1/VSOP helps production of reactive oxygen species (ROS, especially O2- and H2O2) through the regulation of intracellular pH and membrane potential in neutrophils, where these factors are known to affect the activity of NADPH oxidase. Recently, we found that Hv1/VSOP regulates ROS production in another way: Hv1/VSOP negatively regulates HOCl production, which is made from H2O2 by myeloperoxidase, by inhibiting exocytosis of myeloperoxidase-containing granules (azurophilic granules) in neutrophils. Pharmacological analyses using zinc ion, an inhibitor for this channel, and valinomycin, a potassium ionophore, revealed that Hv1/VSOP on the plasma membrane regulates the granule release through in part inhibition of excess depolarization in neutrophils. Azurophilic granules contain many degradative enzymes that are known to be toxic for host itself. The release of elastase, one of the degradative enzymes, was enhanced in Hv1/VSOP deficient neutrophils during the oxidase activation. Relating to above in vitro phenotype, Hv1/VSOP deficient mice exhibited severer lung inflammation than wild-type mice after Candida infection. This result suggests that Hv1/VSOP is involved in suppressing inflammation on pathogen infection, which may be through the inhibition of azurophilic granule release in neutrophils. NO COI.

ABS0336
Overexpression of inducible nitric oxide synthase (iNOS) and increased intima/media thickness (IMT) ratio of common carotid artery with significant stenosis flow (SSF) during acute ischemic stroke
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Vascular inflammation caused by endothelial dysfunction induced atherosclerotic plaque is evident in ischemic stroke. Recently, in significant stenosis flow (SSF) stroke, profound low level of circulating nitric oxide (NO) and increased soluble Lectin-like Oxidized LDL Receptor – 1(sLOX-1) were found and showed a significant difference from those in non SSF group. In this study, we examine an iNOS expression of macrophage from buffy coat in SSF associated with increased IMT. Five SSF- stroke aged 60.60 ± 6.78 years old and five healthy control (aged 34.20 ± 4.23 years old) were studied as follows in detail by local ethic committee (MTU-EC-PH-6-076/55). IMT ratio and 50-75% stenosis flow were measured by Doppler ultrasound at both carotid arteries. Plasma NO was measured by electrochemistry method. iNOS expression was quantitated by PCR technique. In SSF-stroke, IMT ratio was 0.61 ± 0.05 mm and 0.62 ± 0.03 mm compared with control 0.48 ± 0.07 mm and 0.52 ± 0.03 mm at right and left carotid arteries, respectively. Plasma NO levels were significantly reduced (54.72 ± 0.03 nM/ml) in SSF-stroke compared with 77.12 ± 1.64 nM/L in control. Also, overexpression of mRNA iNOS was clearly evident (1.02 ± 0.01 fold of β -actin) compared with control ( 0.45 ± 0.01 fold of β -actin). These findings indicate activation of iNOS activity in macrophage during rapid flow through inflammatory narrow lumen of plaque vessel in ischemic stroke. Degree of iNOS activity might be vascular oxidative stress marker of plaque formation and rupture. No COI.
Enhancement of ciliary beating by Carbocystein via modulation of [Cl⁻], and pH, in bronchiolar ciliary cells in mice
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The ciliary transport is controlled by two parameters, ciliary beat angle (CBA) and ciliary beat frequency (CBF). Carbocystein (CCys), a mucolytic, is known to activate ciliary transport. In this study, we examined the effect of CCys on bronchiolar ciliary beating, CBA and CBF, in mice. Mice were sacrificed by pentobarbital-Na (70-80 mg/kg, ip), and then bronchiolar ciliary cells were isolated by an elastase treatment. Ciliary beating was observed with a high speed camera (500Hz) at 37°C. Experiments were performed in accordance with the Guidelines of the Animal Research Committee of Kyoto Prefectural University of Medicine. CCys respectively increased CBA and CBF by 30% and 5% within 15 min after its application. The CCys-induced CBA increase was inhibited by Cl⁻ channel blockers (NPPB and CFTR (inh)-172). CCys decreased cell volume and [Cl⁻]i of bronchiolar ciliary cells. Moreover, in a HCO₃⁻-free solution, CCys actions were mimicked by removal of Cl⁻ from the extracellular space, suggesting that CCys would increase CBA by decreasing [Cl⁻]i associated with cell shrinkage caused by activation of Cl⁻ channel (CFTR). On the other hand, the CCys-induced increase in CBF was not observed in a HCO₃⁻-free solution or in the presence of DIDS. Moreover, in a HCO₃⁻ containing Cl⁻-free solution, CCys still increased CBF. This suggests that CCys would increase CBF by elevating pH, caused by activation of NBC. In conclusion, CCys increases CBA by a decrease in [Cl⁻], via CFTR activation, and increases CBF by a pH, elevation via NBC activation. No COI.

VRAC channel composition determines its substrate specificity and resistance to the induction of apoptosis
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One of the first events observed during apoptosis is a decrease in cell volume due to efflux of K⁺, Cl⁻ and H₂O, a phenomenon known as apoptosis volume decrease (AVD), that later is followed by caspase activation and DNA fragmentation. Volume-regulated anion channels (VRAC) and volume sensitive organic anion channels (VSOAC) have been shown to play an important role in drug-induced AVD and apoptosis by physiological and pharmacological characterization. Thus, cell death induced by staurosporine and the anticancer drug cisplatin could be reduced by non-specific inhibitors of the volume-regulated anion channel VRAC, now known to be a LRRC8 heteromer. Disruption of the obligatory subunit LRRC8A and another subunit indeed reduced cisplatin- or staurosporine-induced caspase activation, while both drugs activated VRAC. Further analysis showed that the LRRC8 subunit composition determines VRACs selectivity for several substrates, providing evidence that LRRC8 heteromers directly form the pore of VRAC. No COI.
ABS0446
Comparative study on the redox state of HSA products for laboratory use and healthy young subjects

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Human serum albumin (HSA) is the most abundant plasma protein and is widely used both in the laboratory and clinical fields. HSA is composed of reduced form (HMA, human mercaptalbumin) and oxidized form (HNA, human non-mercaptalbumin). Furthermore, HNA is divided into two forms; reversible (HNA-1) and irreversible (HNA-2) forms. Therefore, we investigated the redox state of HSA products for laboratory use, such as recombinant HSA (rHSA) and plasma-derived HSA (pHSA) products, compared with that of healthy young male subjects (21.6 years, collegiate students of Gifu University), by our HPLC system. All products were obtained from Sigma-Aldrich Co. (USA) and product numbers used were as follows: A9731 for rHSA expressed in rice, A1653 (initial product; Cohn Fraction V) and A3782 (final purified product; fatty acid and globulin-free) for pHSA. Percentages of HMA of all HSA products were significantly lower than that of young subjects (P < 0.01). Percentages of HNA-1 and HNA-2 of all HSA products were significantly higher than that of young subjects (P < 0.01). Moreover, all HSA products had a dimer fraction (4.8% for A9731, 3.7% for A1653 and 9.3% for A3782), which cannot be observed in human subjects. From the sets of our results, it is suggested that oxidation and dimer formation of the HSA products may occur during manufacturing processes, such as storage and purification processes, and/or expression system. Thus, it is necessary to consider the heterogeneity of HSA products, when researchers use these products in their own field.
No COI.

ABS0480
The physiological role of endothelin in periodontitis

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Periodontitis is a very common oral inflammatory disease and results in the destruction of supporting connective and osseous tissues of tooth. Although the etiology is still unclear, Gram-negative Porphyromonas gingivalis in subgingival pockets has been thought as one of the major etiologic agent. It has been known that endothelin is involved in the occurrence and progress of various inflammatory process and diseases. However, functional roles of endothelin in periodontitis are still unclear. In this study, we explored cellular and molecular mechanisms of ET-1 actions in periodontitis using human gingival epithelial cells (hGECs) and human gingival fibroblasts (hGFs). ET-1 and ETA, but not ETB were abundantly expressed in both hGECs and hGFs. Stimulation of hGECs with P. gingivalis LPS increased the expression of ET-1 and ETA suggesting the activation of endothelin signaling pathway. Production of pro-inflammatory cytokines, IL-1β, IL-6, and IL-8 was significantly enhanced by exogenous ET-1 treatment in both hGECs and hGFs. Stimulation of hGECs with P. gingivalis LPS increased the expression of ET-1 and ETA suggesting the activation of endothelin signaling pathway. Production of pro-inflammatory cytokines, IL-1β, IL-6, and IL-8 was significantly enhanced by exogenous ET-1 treatment in both hGECs and hGFs. Moreover, ET-1 augmented the number of multinucleated osteoclasts implicating the acceleration of alveolar bone loss. Together, our study showed that activation of ET-1/ETA signaling pathway by P. gingivalis may exacerbate periodontitis by stimulating production of pro-inflammatory cytokines in hGECs and hGFs and provoking the alveolar bone loss through the increment of multinucleated osteoclasts at the same time. To directly examine the endothelin antagonism as a potential therapeutic approach for periodontitis, the inhibitors for ET receptors will be applied to the animal periodontitis model. Infiltration of immune cells, production of pro-inflammatory cytokines, and alveolar bone loss will be evaluated. No COI.
Quinacrine (QA) once was widely used in treatment of parasitic diseases such as malaria and giardiasis, and autoimmune diseases. Although QA has been substituted with more effective drugs, research related to this old drug has never been stopped. Microwave (MW) has been widely used in various industrial, communications, medical, and domestic applications, though its adverse effect remain unclear. However, the thermal effect is the most important characteristic of microwave. Recently, we found that QA administration could decrease the neuronal damage of hippocampus caused by MW. However, the detailed molecular mechanism remains unknown. For this purpose, here, PC12 cells were successfully induced into neuronal cells with RA. Next, the induced cells were pretreated before receiving microwave. The results showed that microwave exposure (3h and 6h group) significantly increased the neuronal apoptosis and necrosis compared with control, but QA pretreatment (low and high dose) can dramatically decrease the MW induced cell apoptosis and necrosis. Analysis based on Atomic force microscope (AFM) showed that QA pretreated cells displayed much less cell membrane damage. Further investigations demonstrated that QA may be involved in MW caused neuronal injury by increasing Hsp70 expression level. This work may further facilitate the research on thermal effect of MW, and also to discuss the new use of QA. No COI.
Optogenetic approach to control neuronal activity in rat vasopressin neuron in in vitro preparation
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Neurohypophysial hormone, arginine vasopressin (AVP) is synthesized in the magnocellular neurosecretory cells (MNCs) of the supraoptic (SON) and the paraventricular nuclei (PVN) of the hypothalamus. AVP release from the posterior pituitary (PP) into the systemic circulation is mainly regulated by neuronal activity of MNCs that project their axon terminals to the PP. In the present study, we have generated a transgenic rat that expresses the AVP and channelrhodopsin 2 (ChR2)-eGFP fusion gene to regulate the neuronal activities of AVP MNCs by blue light-activated ion channel (ChR2). The eGFP that indicates the expression of the ChR2 gene was observed in the SON, the magnocellular divisions of the PVN that are known to localize AVP MNCs. Confocal laser scanning microscopic observation revealed that ChR2-eGFP was mainly localized in the membrane of MNCs. The intensities of eGFP in the SON and the PVN were markedly increased after chronic salt loading. Using whole cell patch-clamp recordings in in vitro preparations such as a single cell isolated from the SON and brain slice including the SON from the transgenic rats, it was found that repeated blue light evoked action potentials repetitively in a current clamp mode, and caused inward currents in a voltage clamp mode. Thus, optogenetic approach is a powerful tool to regulate neuronal activity of AVP MNCs. No COI.

Age and sex differences in monosodium glutamate obesity
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Aim: This study aimed to assess the impact of age and sex differences on the effects of monosodium glutamate (MSG) induced obesity as regard body mass index (BMI), lef Index (LI), body weight gain, and percentage gain of body weight, fasting plasma glucose and glucose uptake by diaphragm, plasma insulin, corticosterone, testosterone and estradiol in rats. Methods: Adult and old Wistar rats of both sexes were divided into: Control groups, receiving control diet; Monosodium glutamate (MSG) obese groups, receiving control diet and MSG. At the end of the experiment, BMI, LI, body weight gain and, percentage gain of body weight were assessed. Blood samples were collected for plasma glucose and hormonal assays. The diaphragms were dissected for determination of glucose uptake by the muscles. Results: MSG obese rats showed increased values of BMI, and LI. The percentage gain of body weight was more enhanced in the adult groups compared with the old groups. MSG obese groups showed significant decrease in energy expenditure, which was more apparent in the old obese male group, with significantly decreased food intake. Fasting plasma glucose was significantly increased, while plasma insulin and glucose uptake by diaphragm were significantly decreased in the MSG obese groups. Plasma testosterone level was significantly decreased in MSG male obese groups, but insignificant changes in plasma estradiol and corticosterone were observed. Conclusion: MSG had been shown to have adverse effects on body weight, energy expenditure, glucose homeostasis of the rats, as well as, on plasma insulin and testosterone levels. Age and sex influenced some of these effects. No COI.
Rosiglitazone improved hepatic circadian-clock gene expression in the insulin resistance and diabetes of mice
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Circadian rhythms are exhibited in the physiological and behavioral processes of all mammals, and are generated by a set of circadian-clock genes which includes Bmal1 (brain and muscle Arnt-like protein-1), Clock (circadian locomotor output cycles kaput), Per1 (period 1), Per2, Per3, Cry1 (cryptochrome 1) and Cry2. Previous evidence suggests a strong link between circadian rhythms and energy metabolism; however, the underlying mechanisms remain uncertain. In the present study, neonatal streptozotocin (STZ)-treated mice were used to model the molecular and physiological progress from insulin resistance to diabetes. Two-day-old male C57BL/6 mice received a single injection of STZ to induce the non-obese, hyperglycemic and hyperinsulinemic conditions in the early stage, insulin resistance in the middle stage, and diabetes in the late stage. Levels of the hepatic circadian-clock gene expression were examined by real-time quantitative PCR. Most components of the hepatic circadian-clock gene expression, such as the mRNAs of Bmal1, Per2 and Cry1, were elevated, and circadian patterns were retained in the early and middle stages of insulin-resistant conditions. Rosiglitazone, an insulin sensitizer, returns the physiological and molecular changes associated with the diabetic phenotype to normal levels through peroxisome proliferator-activated receptor γ (PPARγ) rather than PPARα. Early and chronic treatment with rosiglitazone has been shown to be effective to counter the diabetic condition. Thus, these results support an essential role for the hepatic circadian-clock system in the coordinated regulation and/or response of metabolic pathways. No COI.

Nocturnal dipping behaviour of blood pressure and diurnal urinary sodium excretion in normotensive adolescents in response to changes in salt intake
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Nocturnal nondipping of blood pressure (BP) is associated with salt intake especially in hypertensive individuals but nocturnal dipping behaviour of BP in normotensive adolescents gain less attention. The aim of the study was to determine the effect of salt intake on nocturnal dipping behaviour of BP and diurnal urinary sodium excretion in normotensive adolescents. Subjects (n = 36) were maintained on high salt intake (200.8 ± 56.3 mmol/day) for one week followed by one week washout and then salt reduction (86.2 ± 22.3 mmol/day) for another one week. During high salt intake, 19 subjects (52.8%) were classified as non-dippers and 17 subjects (47.2%) as dippers. During salt reduction, 13 out of 19 previously non-dippers (68.4%) changed to dippers. A significant increase in nighttime urinary sodium excretion rate was observed in non-dippers during high salt intake (p<0.05) and salt reduction (p<0.01). These results demonstrated that high salt intake would induce attenuated nocturnal dipping of BP with concomitant changes in enhanced nighttime natriuresis, and modest salt reduction would restore normal dipping pattern of BP and normal pattern of urinary sodium excretion. It can be concluded that nocturnal dipping behaviour of BP could change as a consequence of salt intake even in healthy normotensive adolescents. No COI.
ABS0186
Tyrosine hydroxylase expression in CD4+ T cells is associated with joint inflammatory alleviation in collagen type II-induced arthritis
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We have recently reported that CD4+ T cells synthesize and secrete catecholamines that facilitate a shift of T helper 1 (Th1)/Th2 balance toward Th2 polarization. In this study, we used an animal model of human rheumatoid arthritis (RA), and collagen type II-induced arthritis (CIA), to explore relationship between catecholamine production in CD4+ T cells and Th1/Th2-mediated joint inflammation. Histopathological observation of ankle joints of CIA mice displayed an evident inflammatory change on day 35 and a major damage to bones on day 55 post-immunization. Expressions of Th1 specific transcription factor, T-bet, and cytokines, IL-2 and IFN-γ, and Th2 specific transcription factor, GATA-3, and cytokines, IL-4 and IL-10, were all upregulated on days 35 and 55 post-immunization, but the elevated Th1 response tended to decrease and the enhanced Th2 response tended to increase with the CIA progression. Expression of tyrosine hydroxylase (TH), a rate-limiting enzyme for synthesis of catecholamines, dramatically increased in ankle joints of CIA mice, although this increase was reduced on day 55 relative to day 35 post-immunization. In synovial tissue of CIA ankle joints but not normal joints, CD4-, T-bet-, GATA-3- and TH-immunoreactive cells were found. Importantly, co-expressed cells with CD4 and TH, T-bet and TH, and GATA-3 and TH were observed in synovial tissue of CIA ankle joints. These results suggest that an increase in catecholamine production occurs in inflamed joints of CIA. The catecholamines are, at least in part, from Th1 and Th2 cells, and they may be related to joint inflammatory alleviation in CIA progression. No COI.

ABS0203
Hyperhomocysteinemia activates Aryl hydrocarbon receptor-CD36 pathway to promote hepatic steatosis in mice liver.
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Hyperhomocysteinemia (HHcy) is associated with liver diseases, such as fatty liver and hepatic fibrosis. However, the underlying mechanism is largely unknown. The current study aims to explore the signaling pathway involved in HHcy-induced hepatic steatosis. First, we performed a meta-analysis of studies involving 2,057 subjects and found that plasma Hcy levels was associated with an increased risk of nonalcoholic fatty liver disease (WMD = 2.56, 95% CI: 1.17, 3.38, P = 0.011). Next, C57Bl/6 mice were fed a high-methionine diet (HMD) (2%, wt/wt) for 8 weeks to establish a HHcy mouse model with hepatic steatosis and elevated CD36 gene expression in liver. The increased CD36 expression was associated with activation of aryl hydrocarbon receptor (AHR). Furthermore, mass spectrometry analysis showed that hepatic content of lipoxin 4A, a well-known ligand of AHR, was significantly elevated in HHcy mice. In primary hepatocytes, the Hcy-induced CD36 expression and subsequent lipid uptake were significantly attenuated by AHR siRNA. Transient transfection assays showed that the activity of AHR response element was dramatically increased in a ligand-dependent manner by Hcy. In addition, Hcy treatment promoted the binding of AHR to CD36 promoter. Finally, AHR antagonist CH223191 reversed lipid accumulation caused by HHcy by inhibiting AhR-CD36 pathway. In conclusion, HHcy activated AHR-CD36 pathway by increasing hepatic lipoxin 4A content, which resulted in hepatic steatosis. No COI.
ABS0302
20-hydroxyecdysone alleviates hypertension and improves glucose tolerance in a rat model of metabolic syndrome
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Ecdysteroids are polyhydroxylated steroids present in plants and invertebrates. In mammals, anabolic effects on skeletal muscle cells and metabolic effects on glucose metabolism of hepatic cells of ecdysteroids have been reported. Although the favorable metabolic effects including hypoglycemic and cholesterol-lowering properties have been shown in obese rodents, no study thus far has evaluated the effects of ecdysteroids on metabolic syndrome. Thus, this study was designed to investigate how chronic treatment with a phytoecdysteroid 20-hydroxyecdysone (20HE) would affect the phenotypic characteristics of metabolic syndrome in rat model induced by high caloric diet. Female Sprague–Dawley rats were ovariectomized and fed with high-fat high-fructose diet (OVX+HFFD), and received daily oral administration of either vehicle or 20HE (5 mg, 10 mg, or 20 mg/kg body weight) for 8 weeks. Body weight, visceral fat weight, blood pressure, serum triglyceride, glucose tolerance test, and insulin action on skeletal muscle glucose transport activity were determined. 20HE treatment tended to decrease body weight and significantly reduced visceral fat content and blood pressure by 20% and 12-15%, respectively. Whole-body insulin sensitivity was increased 38% (P < 0.05) in the 20HE-treated groups. However, no significant improvements were observed for insulin-stimulated glucose transport activity in the soleus muscle of 20HE-treated animals. These data indicate that 20HE can alleviate the development of hypertension in OVX+HFFD rats and the improvement in whole-body insulin sensitivity in 20HE-treated rats occur independently of modulation of the insulin action on skeletal muscle glucose transport activity. No COI.

ABS0402
Protective effect of chronic intermittent hypobaric hypoxia on Diabetes Mellitus rats
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Accumulating evidence demonstrate that chronic intermittent hypobaric hypoxia (CIHH) has various, beneficial effects on the body, such as cardiac protection, brain protection, anti-hypertension effect, metabolic improvement, and immune regulation effect. We propose a hypothesis that CIHH could have anti-diabetes effects. Adult male Sprague–Dawley rats were randomly divided into 4 groups: control group (CON), type-2 diabetic group (DM, induced by high-fat combined with low-dose streptozotocin), CIHH treatment group (CIHH, simulating 5000 m altitude, 6 h per day for 28 days), and diabetes plus CIHH treatment group (DM+CIHH). Histopathology of liver, arterial blood pressure, blood biochemicals, glucose and insulin tolerance were determined. The expression of proteins associated with insulin signaling as well as hypoxia induced factors were assayed. The DM rats showed impaired glucose tolerance, dyslipidemia, hepatic steatosis and hepatic insulin resistance in addition to an increase in blood pressure. However, the arterial blood pressure, serum triglyceride and cholesterol were decreased, and hepatic steatosis and insulin resistance were improved in CIHH-treated DM rats. Furthermore, expression of glucokinase (GCK), insulin receptor substrate 1 (IRS-1) and 2 (IRS-2) were significantly increased, while the expression of phosphoenolpyruvate carboxykinase (PEPCK), hypoxia-inducible factors (HIF1α and HIF2α) was markedly reduced in CIHH-treated diabetic rats. It suggests that CIHH treatment has anti-diabetes effect through ameliorating insulin resistance via hepatic HIF-Insulin signaling pathway in type-2 diabetic rat. No COI.
ABS0424

M2 muscarinic receptor mediates arginine-vasopressin secretion in the mouse supraoptic nuclei

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Arginine-vasopressin (AVP) is synthesized by magnocellular neurosecretory cells in supraoptic nuclei (SON) and paraventricular nuclei (PVN) of the hypothalamus and released from the neurohypophysis into the blood. AVP maintains body fluids by altering water reabsorption in the kidney. Recently, it has been reported that M2 subtype of muscarinic receptor is abundant in the hypothalamus. However, the mechanisms of controlling the AVP secretion via M2 receptor have not been elucidated. We tested a hypothesis that M2 receptor may participate to regulate AVP secretion and thereby maintain body fluids in M2 knockout (M2KO) and wild-type (WT) mice. Immunohistochemistry was conducted to count the number of AVP-immunoreactivity positive cells. Plasma AVP concentration, the amount of drinking, the voiding volume and the urination frequency were measured. The expression of V2 vasopressin receptor and the reactivity to V2 receptor agonist desmopressin in the kidney were estimated. In M2KO mice, the number of AVP positive cells in SON was smaller than that in WT mice, although there was no significant difference in PVN. Plasma AVP concentration was significantly decreased, and the amount of drinking, the voiding volume and the urination frequency were significantly increased in M2KO mice. The expression and reactivity of V2 receptor were not significantly different between these strains. These results suggest that M2 receptor is involved in maintenance of body fluids by regulating AVP synthesis or secretion in SON of the hypothalamus. No COI.

ABS0434

Effects of heart-specific disruption of the circadian clock on systemic glucose metabolism in mice

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The mammalian circadian clock system not only generates molecular and behavioral rhythms but also coordinates various physiological processes, including nutrient and cellular homeostasis. It has been reported that glucose homeostasis can be disrupted in mice without clock gene function. Clock genes are found in nearly all tissues in the body and to regulate glucose metabolism in major metabolic tissues such as liver and pancreas. However, roles of clock genes in other non-metabolic tissues in the maintenance of systemic glucose metabolism are largely unknown. Here, we find that mice with a heart-specific deletion of Bmal1, a core clock gene, develop hyperglycemia with age besides chronic heart failure. In addition, insulin tolerance test reveals the existence of insulin resistance in heart-specific Bmal1 knockout animals. Insulin tolerance test also shows low response of the expression of genes associated with hepatic gluconeogenesis against insulin injection in mice without heart Bmal1 function. Further, we find that these hyperglycemic and insulin resistant phenotypes become more apparent when these knockout animals are fed a high-fat diet. In summary, our findings suggest that, in addition to major metabolic tissues, the heart is also an important organ in which the function of the molecular clock is linked to systemic glucose homeostasis in mammals. No COI.
Anti apoptotic and anti oxidative effects of salvianolic acid B in multiple low-dose streptozotocin-induced diabetes

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Type 1 diabetes (T1D) develops as a consequence of pancreatic β-cell destruction and results in insulin deficiency. In this study we investigated the effect of salvianolic acid B (SalB) on insulin producing function in diabetes induced by multiple low-dose streptozotocin (mlds). Rats were divided into control, diabetic and Sal B treated diabetic groups. Sal B was daily administered for 3 weeks, starting on the third day post-STZ injection. Then pancreas samples were determined for GSH level, GPx activity, apoptosis by TUNEL staining and insulin content by immunohistochemistry method. Result showed that Sal B lowered the apoptotic reactivity, raised GSH level and GPx activity and increased the islets insulin content in diabetic rats. In conclusion, acting as an antioxidant and anti apoptotic agent, Sal B protected rats from insulin deficiency in mlds model. No COI.
The effect of exercise and its stability on interleukin-17, melatonin and cortisol concentrations in serum and lymphocytes/whole blood culture in non-athletic and healthy subjects

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There are the controversial reports about moderate and chronic exercise effect on serum cortisol, melatonin and interleukin-17 (IL-17) and also in the total blood and lymphocytes culture. This study was to investigate the effect of regular and moderate exercise and the stability of the exercise on level of IL-17, melatonin and cortisol in serum and lymphocytes/whole blood culture in non-athletic and healthy subjects. Thirteen non-athletic and healthy men participated in an exercise program. Moderate exercise intensity was measured by Karvonen formula. The blood sample was taken from each subject during three phases: pre-exercise, two-month-exercise and then two-month-silence. A complete blood count test was done. The lymphocytes were dissociated by ficoll and then they and whole blood were cultured. The cortisol, melatonin and IL-17 levels were measured. Data were analyzed by statistical methods. The decrease of melatonin in lymphocytes culture after two-month silence was not only significantly seen but also the decrease of monocytes in whole blood culture after two months exercise was detected in comparison with prior to exercise. The decrease of the white blood cells in whole blood culture in two-month silence was significant in comparison with after exercise. The serum and cultural concentrations of cortisol and IL-17 after two months exercise and then two-month silence were not significant. The cortisol and IL-17 were not changed by the moderate exercise. The moderate exercise decreased melatonin in lymphocytes supernatant culture and also monocytes and white blood cells in whole blood supernatant culture. No COI.

Sustained vocalizations during kendo exercises suppress expiration of carbon dioxide

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One distinctive trait of kendo, the traditional Japanese martial art of fencing, is the execution of sustained, high-effort vocalizations during actions. The purpose of this study was to determine the effect of these vocalizations on breathing patterns and respiratory functions. Respiratory indicators of eight university kendo athletes were analyzed using a portable breath gas analyzer during the most intensive kendo exercise, kakari-keiko, with and without vocalization. Sustained vocalization was found to prolong expiratory time, and suppress breathing frequency and expired minute ventilation. Analysis of exhaled gases revealed no effect of sustained vocalization on oxygen uptake, but did reveal reduced carbon dioxide output (VCO2) and increased fraction of end-tidal carbon dioxide (FetCO2) during exercise session and enhanced VCO2 in recovery periods. Thus, we conclude that the sustained high-effort vocalizations greatly affect expiration breathing patterns in kendo. Moreover, repetition of kakari-keiko caused a reduction in VCO2 and an increase in CO2 storage and FetCO2. We hypothesize that these vocalizations of kendo may increase cerebral blood flow in athletes. No COI.
ABS0354
The possibility of increase in both the local oxygen consumption and blood flow of the skeletal muscle by the forearm immersion to artificial CO$_2$ water
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Clinical observations of CO$_2$-hot spring (CO$_2$ ~1000 ppm) immersion revealed the effects, e.g. an immersed part reddening, skin blood flow improvements, etc. In this study, whether O$_2$ consumption (MO$_2$) and blood flow (BFmuscle) in the local muscle of forearm were influenced by immersion of the forearm into water containing high concentration-CO$_2$ (CO$_2$-water) was investigated by using near-infrared spectroscopy (NIRS). Six healthy female volunteers (21-22 years) seated in upright position were instrumented on the right forearm with a laser Doppler flowmetry probe for recording skin blood flow (BFskin), and a NIRS-probe for recording muscle hemoglobin (Hb) contents (oxy-Hb, total-Hb). Blood flow of the forearm was restricted by comprising the inflatable cuff on the upper arm to perform three consecutive venous occlusions (<50 mmHg for 20 s) followed by an arterial occlusion (<280 mmHg, 50 s). MO$_2$ was determined by evaluating the slope of linear regression line of oxy-Hb decreases during arterial occlusion, and BFmuscle was determined by the rate of increase of total-Hb during venous occlusion. Bath water (30 °C) was exchanged for another, i.e. tap water (CO$_2$<20 ppm) for CO$_2$ water (860-990 ppm) or vice versa, every about 20 min. All subjects reported that CO$_2$-water was warmer than the tap water. The BFskin was significantly 3.6 times larger during CO$_2$-water immersion than tap water immersion. MO$_2$ and BFmuscle during CO$_2$-water immersion was respectively 15 % and 32 % larger than during tap-water immersion. Results suggest that the bathing with artificial CO$_2$-water may stimulate the muscle metabolism hence increased muscle-blood flow, as well as skin blood flow. No COI.

ABS0355
The role of brain serotonin levels and its related gene expressions in regulating amount of daily spontaneous physical activity
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Decreasing levels of amount of daily physical activity is considerable health concern that has to be solved all over the world. Thus, we have investigated a novel methodological target to prevent decreasing levels of daily physical activity using rat models. Our previous studies have shown that there is a close relationship between daily low spontaneous physical activity and brain serotonin levels. In this study, we examined the gene expressions related to serotonergic systems in order to elucidate the neuronal mechanisms in regulating amount of daily spontaneous physical activity. In addition, we also examined the effects of changing levels of brain serotonin on amount of daily spontaneous activity. Male rats were housed individually in cages with or without an attached running wheel. Physically active rats were allowed voluntary access to their wheels for 4 weeks. The rats were screened into high runner (HR) or low runner (LR) based on the calculated daily running distance. After 4 weeks from the start of running, we assessed the gene expressions related to brain serotonergic systems in HR and LR using cDNA microarray. The results of microarray analysis showed that gene expression related to serotonin transporter in LR were significantly higher than that in HR. Furthermore, the analysis of serotonin precursor injections, which enhanced the levels of brain serotonin, revealed that changing levels of brain serotonin actually decreased amount of spontaneous physical activity. These results refine the role of brain serotonergic system as a neuronal mechanism in regulating amount of daily spontaneous physical activity. No COI.
ABS0378

Negative rebound in hippocampal neurogenesis following exercise cessation
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Exercise is known to enhance brain function, whereas effects of exercise cessation (ExC) are largely unknown. We reported that ExC impaired hippocampal neurogenesis in mice (Nishijima et al., 2013). This study aimed to elucidate the detailed time-course profile of hippocampal neurogenesis following ExC. Male C57BL/6 mice were randomly assigned to either a control (Con) or an ExC group. ExC mice were reared in a cage with a running wheel for 8 weeks and subsequently placed in a standard cage to cease the exercise. Con mice were reared in a standard cage throughout the experiment. BrdU (50 mg/kg BW, i.p.) was injected on the last two days of the exercise period. At 0, 1, 2, 5, and 8 weeks after ExC, both Con and ExC mice were sacrificed for immunohistochemical examination of hippocampal neurogenesis (n = 5 at each time point). Exercise significantly increased the density of doublecortin (DCX)-positive immature neurons in the dentate gyrus (at week 0). Following ExC, the density of DCX in ExC decreased and was identical to that of Con at 2 weeks after ExC. The density decreased further and was significantly lower than that in Con at 5 and 8 weeks after ExC, indicating that ExC leads to negative rebound in hippocampal neurogenesis. Density of Ki-67-positive cells did not differ between groups. The survival of BrdU-positive cells in ExC was slightly lower than that in Con group at 5 and 8 weeks after ExC. These results indicate that negative rebound in neurogenesis is in part caused by suppression of cell survival. This study suggests that ExC or a decrease in physical activity is associated with an increased risk of impaired hippocampal function, which might increase vulnerability to stress-induced mood disorders. No COI.

ABS0445

Lactate threshold during exercise in a cool environment is decreased by whole body surface cooling prior to exercise with an enhanced sympathetic nerve activity
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Lowered skin temperature by whole body surface cooling results in an enhanced sympathetic nerve activity as well as a lowered plasma volume associated with the cold diuresis. We hypothesized that these physiological responses performed a graded maximal cycling exercise after pre-conditioning for 60 min under three different conditions. Ambient temperature (climate chamber) and whole body surface temperature (water-perfusion suits) were regulated at 10 °C and 10 °C in Cool-Cool (CC), 25 °C and 10 °C in Mild-Cool (MC), and 25 °C and 34 °C in Mild-Neutral (MN). Esophageal and skin temperatures (Tes and Tsk, respectively), plasma lactate ([Lac]p) and noradrenaline ([Nord]p) concentrations, hemoglobin, and hematocrit were measured before and after pre-conditioning and during and immediately after exercise. LT was determined and relative change in plasma volume (% ΔPV) was evaluated. Tes was not different among conditions while Tsk in CC and MC was lower than in MN. [Lac]p during exercise was higher while LT was lower in CC than the other two conditions (p<0.05). [Nord]p during exercise was also higher in CC than the other two conditions (p<0.05). % ΔPV during exercise was lower in CC than in MN (p<0.05). LT was significantly correlated with [Nord]p during exercise (p<0.05) while not with % ΔPV. In conclusion, whole body surface cooling prior to exercise in cool environment decreases LT via an enhanced sympathetic nerve activity. No COI.
ABS0473

Functional capacity and maximal oxygen consumption in obese, overweight and normal weight young adults and the effects of arm swing exercise training: A preliminary study
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Low-intensity exercise was shown to effectively burn fat. Obesity is associated with reduced functional capacity (FC) and maximal oxygen consumption (VO2max). Arm swing exercise (ASE) is low-intensity exercise. Therefore, we investigated preliminary effects of obesity and ASE training on FC and VO2max. Four obese and 6 overweight (OW group) and 25 normal weight (NW group) young adults, aged 19-31 years, participated in this study. Before and after ASE training for 8 weeks (30 min/day, 3 days/week), FC and VO2max were assessed in all subjects. FC was measured by 6-minute walk test (6MWT) and VO2max was calculated from 6MWT distance, body weight, resting heart rate, age, and body mass index. Before ASE training, OW group showed significantly lower VO2max than NW group (37.73±4 vs. 43.39±3.24 ml/kg/min; p=0.000) without any difference in FC between both groups (540.5±29.45 vs. 566.2±36.22 m.; p=0.055). After ASE training, both groups had tendency to increased in FC (OW group: 540.5±29.45 vs. 561.6±37.84 m.; p=0.065 and NW group: 566.2±36.22 vs. 584.88±43.63 m.; p=0.056) without any significant difference between groups. There was no significant effect of the ASE on VO2max in both groups (OW group: 37.73±4 vs. 38.41±3.83 ml/kg/min; p=0.505 and NW group: 43.39±3.24 vs. 43.52±3.73 ml/kg/min; p=0.818). Moreover, VO2max remained lower in OW than NW group (38.41±3.83 vs. 43.52±3.73 ml/kg/min; p=0.001). This study shows that ASE training for 8 weeks did not alter FC and VO2max in OW and NW groups. No COI.

ABS0516

Effects of gender and intensity of exercise on immunity during exercise in patients with diabetes type 2
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The effects of gender and intensity of exercise on immunity during exercise in patients with type 2 diabetes mellitus (T2DM) are unclear. This study aimed to determine the effects of gender and intensity of exercise on immunity during exercise in patients with T2DM. Six women and 6 men with T2DM (aged between 30 and 60 years) cycled on ergometer at a target workload equal to 25 (low), 65 (moderate) and 85 (high) % of peak oxygen consumption for 10, 10 and 5 minutes, respectively with at least 7 days apart. Blood samples were taken to count their immune cells (white blood cells (WBCs), neutrophils (NE), lymphocytes (LY) and monocytes (MO)) before and immediately after the exercise. Peak oxygen consumption of male and female subjects were 25.6±6.4 and 19.0±7.5 ml/kg body mass/min. Men had significantly increased WBCs and LY after the exercise at high intensity, whereas women had significantly increased WBCs and LY after the exercise at moderate and high intensity. In addition, men had significantly increased in NE after the exercise at moderate and high intensity. However, women had significantly increased NE immediately after the exercise at every intensity. At moderate intensity, women had significantly higher NE and LY after the exercise than men. MO was not significantly affected by the intensity and gender. The present study suggests that there was the effect of intensity of exercise on WBCs, NE and LY. No COI.
Effects of ezrin knockdown on the architecture of gastric glandular epithelia
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Ezrin, an adaptor protein that cross-links plasma membrane-associated proteins with actin cytoskeleton, is concentrated on apical surface of epithelial cells especially in microvilli of small intestines and stomachs. In stomachs, ezrin is predominantly expressed on apical canalicular membrane of parietal cells. It is involved in remodeling of apical surface membrane and in gastric acid secretion. The ezrin knockdown (Vil2kd/kd) mice showed achlorhydria due to the impairment of membrane fusion between tubulovesicles and apical membrane. However, the effects of knockdown of ezrin expression on the architecture of gastric epithelia have not been studied yet. Here, we studied the architecture of gastric epithelia of the Vil2kd/kd mice by immunohistochemistry, and newly found that the mice showed hypergastrinemia and foveolar hyperplasia in the gastric fundic region. Dilation of fundic glands was observed with the percentage of parietal and chief cells being decreased, and that of mucous-secreting cells being increased. The parietal cells of the Vil2kd/kd mice contained dilated tubulovesicles and abnormal mitochondria, and subsets of these cells contained abnormal vacuoles and multilamellar structures. Therefore, ablation of ezrin causes not only achlorhydria and hypergastrinemia, but also changes in the architecture of gastric gland with severe perturbations in the secretory membranes of the parietal cells. No COI.

Effects of dietary zinc supplementation on the intestinal epithelial histomorphology in Bama miniature pig
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Zinc (Zn) is an essential micronutrient for pigs that plays a critical role in numerous enzyme reactions. Following oral exposure, Zn is primarily excreted via the gastrointestinal tract and eliminated in the feces with approximately 70-80% of an ingested dose. In this study, we aimed to evaluate the effects of dietary Zn supplementation on small intestine. 18 Bama miniature pigs were randomly allocated into three groups and treated with three levels of supplemental Zn (0, 15 or 1500 mg/kg, as Zn sulfate), respectively. After 38 days of dietary treatment, pigs were euthanized and the small intestinal epithelial tissue was excised to determine the effects of dietary Zn supplementation on Zn concentration, and morphological examination of small intestine. Immunohistochemistry and western blot detection of cleaved caspase-3 were performed to assess the status of caspase-3-mediated apoptosis in the small intestinal epithelium. Zinc concentration of small intestine of pigs in group with dietary Zn supplementation at 1500 mg/kg was higher than those in group with dietary Zn supplementation at 0 mg/kg (P<0.05). Dietary Zn supplementation at 1500 mg/kg caused the marked damage to small intestinal epithelium of pig, particularly in the jejunum. Results of immunohistochemistry and western blot analysis showed that expression of cleaved caspase-3 was elevated in the intestinal epithelium of pigs in group with dietary Zn supplementation at 1500 mg/kg (P<0.05). These results indicated that dietary Zn supplementation at high dose could increase the intestinal Zn accumulation and up-regulate the expression of cleaved caspase-3 in intestinal epithelium, which induce the apoptosis and damage in small intestinal epithelium in Bama miniature pig. No COI.
ABS0333

**Inhibition of cholesterol absorption by coffee pulp extract in intestinal Caco-2 cells**

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Coffee pulp (CP), a by-product of coffee processing, has been found to have antioxidant capacity. Among its major constituents including chlorogenic acid, epicatechin, and anthocyanin, chlorogenic acid has been shown to decrease total cholesterol and low-density lipoprotein, and also increase high-density lipoprotein leading to improve atherogenic index and cardiovascular risk factors in both obese and type 2 diabetes mellitus in vivo. Thus, CP-enriched chlorogenic acid could also have a potential for cholesterol lowering effects. However, it remains unknown whether CP aqueous extract—rich in chlorogenic acid has anti-hyperlipidemic effects. Therefore, the aim of this study was to investigate the effect of CP aqueous extract (CPE) on cholesterol transport and its possible mechanism involved in intestinal Caco-2 cells. The uptake of [3H]-cholesterol into the intestinal Caco-2 cells was determined. In addition, cholesterol solubility and micelles particle size were investigated in vitro. The results showed that 100 mg/ml of CPE inhibited [3H]-cholesterol uptake in Caco-2 cells by approximately 50% compared to that of control. The doses of CPE also correlated with an increase of cholesterol micelle particle sizes. Moreover, CPE tended to reduce cholesterol solubility in micelles in vitro. Therefore, these findings indicated that CPE might be a potential nutraceutical product for dyslipidemia. Nonetheless, a further study in vivo is required to elucidate the mechanism of anti-lipidemic action. This study is supported by the Research and Researcher for Industry (RRI) by Thailand Research Fund (TRF) and Hillkoff Co., Ltd (Chiang Mai, Thailand).

ABS0343

**Antiulcerogenic effects of Momordica Charantia L. essential oil on hcl/ethanol-induced gastric ulcer in rats**

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Introduction: Bitter gourd or Momordica charantia L. (MC), a cucurbitaceae family plant is a plant native to the semi-tropical climate of Thailand, India, Asia and Africa and has been traditionally used as a folk remedy and best known for its anti-diabetic, anti-inflammatory, anti-microbial, anti-ulcer and anthelmintic properties. Objective: This study was conducted to evaluate the anti-ulcerogenic effect of MC essential oil on HCl/ethanol-induced gastric ulcer in rats. Method: Five groups of Spraque Dawley rats (n = 6) were given treatment orally for 7 consecutive days. These group consist of I=negative control, II = positive control, III = 10 mg/kg MC, IV = 50 mg/kg MC and V = 100mg/kg MC. On the 7th day, after 1 hour of treatment administration, the rats were induced with HCl/ethanol to produce ulcers and were kept for 6 hours. Thereafter, the rats were sacrificed and their stomachs were removed to measure the ulcer index and percentage of inhibition Results: At the dosage of 10, 50 and 100 mg/kg, MCEO significantly (p < 0.05) reduced the total area of gastric lesion (mm²) from 59.7 ± 7.4 to 18 ± 4.0, 7.8 ± 3.4 and 5.0 ± 1.9 respectively. Ranitidine reduced the total area of gastric lesion from 59.7 ± 7.4 to 40.5 ± 3.2. Inhibition was well shown in the 100 mg/kg MC (91.6%) concentration followed by 50 mg/kg (86.9%) and 10 mg/kg (69.8%) after induced by HCl/ethanol. All inhibiting effects obtained were compared with ranitidine, 100 mg/kg (32.1%). Conclusion: Based on the results obtained, MC essential oil has the properties to reduce the severity of gastric ulcer in rats. No COI.
Effects of Xenin on spontaneous circular muscle contraction in rat distal colon

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In search of a mammalian counterpart for the octapeptide xenopsin of amphibian skin was detected in the mucosa of the upper gastrointestinal tract of humans and various mammals. This peptide was named Xenin25 (Xen) which is a 25 amino acid neurotensin-related peptide produced by GIP-producing K cells in the small intestine and peptide sequence is highly conserved during evolution. We have shown that Xen functions as a mediator on the ion transport in the rat colon [J. Physiol. Sci., 65 (Suppl.1):S195, 2015]. Previous morphological and functional studies support the concept that xenin represents a new member of regulatory peptide. To support the concept, in the present study we have evaluated the effect of Xen on smooth muscle activity in the colon. Xen was synthesized by a solid-phase methodology with Fmoc-strategy using an automated peptide synthesizer (Model Pioneer; Life Technologies, CA, USA). For the mechanical experiments, full-thickness circular muscle strips (approximately 2 mm wide and 8~10 mm long) were attached to isometric transducer under a constant load of 3~8 mN in 15 mL oxygenated (95%O2 and 5% CO2) Krebs solution maintained at 37 °C. An amplifier (Quad Bridge Amp.) and a PowerLab system (ML846: ADInstruments, Bella Vista, NSW, Australia) were used to record circular muscle activity. The tissues were allowed to equilibrate for at least 2 h to develop spontaneous contractions of varying amplitude. Xen concentration-dependently inhibited spontaneous circular muscle contractions in rat distal colon. These results indicate that Xen may contribute to an inhibitory modulator in colonic circular smooth muscle. No COI.

Effects of ε-viniferin, a dehydrodimer of resveratrol, on ion transport and ion permeability in the rat intestinal epithelia

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ε-Viniferin is a dehydrodimer of resveratrol. Resveratrol is reported to have a variety of health beneficial functions including antioxidant, anti-inflammatory, and anti-mutagenic effects, etc. However, ε-viniferin is reported to be little absorbed in the intestine in contrast to resveratrol. In the present study, we found the mucosal, but not serosal, ε-viniferin-evoked secretory response and change in ion permeability in the rat intestinal mucosa. Mucosa-submucosa preparations of rat small and large intestines were mounted on Ussing chambers, and short-circuit current (Isc) and tissue conductance (Gt) were continuously recorded. In cecum, more than 10-5 M of ε-viniferin and > 10-4M of resveratrol concentration-dependently evoked an increase in Isc and transient decrease in Gt followed by an increase in Gt. The mucosal viniferin (10-4M)-evoked Isc response, but not Gt response, was attenuated by the pretreatment with a selective COX-1 inhibitor, SC-560, or an EP4 PGE2 receptor antagonist, ONO-AE3-208, but not TTX, atropine, or hexamethonium. These results indicate that mucosal ε-viniferin stimulates cecal mucosa inducing a fluid secretion mediated via prostaglandin production and EP4 receptor activation. In addition, mucosal ε-viniferin concentration-dependently attenuated the mucosal propionate (10-3M)-evoked increase in Isc. In immunohistochemistry, COX-1-immunoreactive epithelial cells were detected in the cecal crypts. In conclusion, the present study suggests that ε-viniferin has a potential developing intestinal function-regulating medicines or supplements by modulating mucosal functions in the large intestine. No COI.
Agonists and antagonists for the free fatty acid receptor 3, FFA3 (GPR41) – Short-chain fatty acid-evoked anion secretion in the mice and rat intestinal mucosa

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Short-chain fatty acids (SCFAs) produced by intestinal microbiota were known to evoke anion/fluid secretion in intestine. Although this phenomenon was considered to be mediated via specific receptors for SCFAs, free-fatty acid receptor 2, FFA2 (GPR43) and/or FFA3 (GPR41), there had been no direct evidence. In the present study, we investigated the effects of SCFAs on transepithelial ion transport in the cecal mucosa-submucosa preparations of FFA2- or FFA3-KO mice. Transepithelial ion transport was measured by the Ussing chamber technique. In WT mice, mucosal propionate concentration-dependently evoked a phasic increase in Isc (E C50 = 1.6×10⁻⁴ M; Emax = 183.3 µA/cm²). In FFA2-KO mice, 10⁻³ M of mucosal propionate also evoked an increase in Isc as much as WT mice, but the effect was the almost completely abolished in FFA3-KO. This indicates that the mucosal propionate-evoked Isc response is due to FFA3 activation, and the measurement of Isc is useful in a search for agonists and antagonists of FFA3. In rat distal colon, mucosal propionate also concentration-dependently evoked phasic increase in Isc (EC50 = 4.8×10⁻⁴ M; Emax = 232.4 µA/cm²). However, the propionate (10⁻³ M)-evoked increase in Isc was attenuated in the presence of 3-chloropropionate (IC50: 2.5×10⁻⁵ M) or crotonate (IC50: 1.3×10⁻⁴ M) in concentration-dependent manners. The Ki values were calculated to be 8.0×10⁻⁶ M in 6-chloropropionate and 4.2×10⁻⁵ M in crotonate. These compounds are suggested to be useful for physiological studies of SCFAs. No COI.

Detailed analyses of gastrointestinal motility and brain-gut peptides in liver stagnation and spleen deficiency syndrome with functional dyspepsia in rats

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Functional dyspepsia (FD), a functional gastrointestinal disorder and a typical psychosomatic disease, is characterized by the presence of one or more chronic dyspepsia symptoms in the absence of any organic disease. The present study was to establish an adult rat model of liver stagnation and spleen deficiency syndrome with functional dyspepsia and to investigate the changes of gastrointestinal motility and brain-gut peptide levels in rat serum and gastrointestinal tissue, which aimed to elucidate the mechanisms of gastrointestinal motility disorder. Male Wistar rats were divided into control group (Con) and FD model group. FD model was established by stimulating semi-starvation rats via tail damping, provocation and forced exercise fatigue until gastrointestinal motility disorder appeared, and then the levels of motilin, leptin, cholecystokinin (CCK), vasoactive intestinal peptide (VIP) and P substance were detected in serum by Elisa and in gastrointestinal tissues by RT-PCR, Western blotting and Immunohistochemistry. The results showed the rates of gastric emptying and intestinal propulsion slowed down markedly as compared to Con (P<0.05) and gastrointestinal myoelectric activity attenuated, interdigestive myoelectric activity complex (IDMEC) interrupted and instead of irregular myoelectric activity. The level of leptin and VIP markedly increased as compared to Con (P<0.05 in serum. The expression of leptin, P substance, VIP increased, motilin and CCK decreased as compared to Con (P<0.05) in gastrointestinal tissues. The data suggest parasecretion of brain-gut peptides is the main reason to cause gastrointestinal motility disorder in FD rats. No COI.
ABS0433
Colokineti
The monoamine neurotransmitter serotonin is released from spinal terminals of nucleus raphe magnus (NRM) neurons. One of major serotonergic effects in the spinal cord is to depress ascending nociceptive transmission. Since pattern of release from NRM neurons uses volume transmission, we hypothesized that serotonin diffuses and influences the defecation center in the lumbosacral spinal cord. In the present study, therefore, we examined effects of 5-HT receptor stimulation in the lumbosacral defecation center on colorectal motility in rats. Rats were anesthetized with α-chloralose and ketamine, and colorectal intraluminal pressure and expelled liquid volume were recorded in vivo. Intrathecal administration of serotonin into the L6-S1 spinal cord caused propulsive contractions of the colorectum. This serotonergic colokinetic effect remained unaffected even after disconnecting from supraspinal regions by severing the T10 spinal cord. On the other hands, transection of the pelvic nerves prevented the serotonin-induced enhancement of colorectal motility. Pharmacological experiments revealed that the effect of serotonin is mediated by 5-HT2 and 5-HT3 receptors. In conclusion, lumbosacral serotonin acting on 5-HT2 and 5-HT3 receptors promotes propulsive colorectal motility via the pelvic nerves. Considering that visceral pain activates descending inhibitory pain modulation pathways including NRM neurons, our results provide a rationale for the concurrence of abdominal pain and colonic motility disorder such as irritable bowel syndrome. No COI.

ABS0443
Inflammation-associated intestinal barrier disruption caused by the current circulating strain of Vibrio cholerae

Vibrio cholerae is known to cause non-inflammatory diarrhea. However, inflammatory responses were detected in the intestinal mucosa of cholera patients, suggesting that intestinal inflammation may play roles in pathogenesis of cholera, especially by enhancing severity of cholera diarrhea as a result of intestinal barrier disruption. In this study, the effect of a current circulating strain of V.cholerae known to cause severe diarrheas, V.cholerae O1 El Tor variant, on intestinal barrier function was investigated in comparison with the O1 classical strain of V.cholerae. The two strains of V.cholerae were inoculated into the mouse closed ileal loops and, 12 hours later, intestinal barrier integrity was evaluated by determining trans-intestinal flux of 4-kDa dextran tagged with fluorescein thiocyanate (FITC). V. cholerae O1 classical strain did not induce intestinal barrier leakage. In contrast, V.cholerae O1 El Tor variant significantly promoted intestinal barrier leakage compared with control. Interestingly, NF-κB activity and mRNA levels of proinflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8 were dramatically increased in V.cholerae O1 El Tor variant-infected group compared with control and the classical strain. Indeed, inhibition of NF-κB activation by NF-κB inhibitor, BAY 11-7082, diminished intestinal barrier leakage and expression of proinflammatory cytokines in the V.cholerae O1 El Tor variant-infected group. The present study reveals that the circulating strain of V.cholerae O1 El Tor variant induces intestinal barrier disruption through NF-κB-mediated inflammatory responses. This mechanism may be accountable for the more severe diarrheas observed in patients infected with the current circulating strain of V.cholerae. No COI.
Gastroparesis, a delay in gastric emptying rate, is a complication found more common in diabetes. However, accelerated gastric emptying has also been reported in both type 1 and type 2 diabetes. There are few studies about gastric emptying changes during the progression of diabetes in the same animal. Moreover, gastric emptying study in human is very expensive and exposed to radioactive substance. We aimed to determine the alteration of gastric emptying rate during the progression of diabetes in rats. The rats were divided into control group (CON) and diabetic group (DM; streptozotocin (STZ), i.p. 60 mg/kg BW). Fasting blood glucose (FBG) levels were measured weekly in blood from tail vein. The [13C] acetic acid breath test was used to evaluate the gastric emptying changes at the early diabetic state (12 weeks of diabetes) and long-term diabetes (20 and 28 weeks of diabetes). The results showed that the FBG of DM was significantly increased as compared to CON (P<0.05) whereas the body weight of DM was gradually decreased as compared to CON (P<0.05). Interestingly, the gastric emptying rate was accelerated at 12 weeks of diabetes and returned to normal at 20 and 28 weeks of diabetes. These data suggest that in STZ-induced diabetic rats, the gastric emptying was accelerated in early state of diabetes and then declined to normal. The delayed gastric emptying was not found up to 28 week of diabetes. The effect of long-standing diabetes (> 28 weeks) needs to be further investigated. No COI.

Effect of Tiliacora triandra (Colebr.) diels on cholesterol synthesis and absorption in vitro.
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Tiliacora triandra (Colebr.) Diets (TT) or Yanang, in Thai is the native plant of Southeast Asia and widely used in northeastern Thai cuisines. In vivo and in vitro studies, TT demonstrated anti-pyretic, anti-inflammatory, anticancer, antioxidant, and anti-dyslipidemia effects. However, the nutraceutical effects of TT on cholesterol synthesis and absorption are limited. Therefore, this study aimed to investigate the direct effects of TT aqueous extract (TTE) on cholesterol synthesis and absorption using human intestinal Caco-2 cells. Cholesterol synthesis was examined using the HMG-CoA reductase (HMGR) assay and cholesterol absorption, represented by the physicochemical property of cholesterol micelles size and solubility formations, was also investigated. Moreover, the effect of TTE on [3H]-cholesterol transport into human intestinal Caco-2 cells was determined. The results showed that TTE inhibited the activity of HMGR, a key enzyme for liver cholesterol synthesis, in a concentration-dependent manner. In addition, marked decreases in micellar solubility of cholesterol and significant increases in micelle size by TTE were observed in a dose-dependent. Consistently, TTE significantly reduced [3H]-cholesterol uptake into human intestinal Caco-2 cells compared to that of control. Thus, these findings suggest that TTE exhibits lipid lowering action by decreasing both liver synthesis and intestinal absorption of cholesterol. Further in vivo studies are required to elucidate the mechanisms involvement of anti-dyslipidemia effects by TTE. This study is supported by the Research and Researcher for Industry (RRI) by Thailand Research Fund (TRF) and Ampol Food Processing Co., Ltd (Bangkok, Thailand).
**ABS0481**  
*The effects of furosemide on ileal motility in male mice.*  
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Furosemide is a loop diuretic that acts on the Na+-K+-2Cl– co-transporter in the thick ascending of Henle's loop to inhibit Na+ and Cl– reabsorption resulting in increased urine production. Furosemide also has non-diuretic effects on the gastrointestinal tract. Associated with the side effects of patients taking furosemide have diarrhea or constipation probably resulting from gastrointestinal motility disorder. Therefore, the aim of this study was to investigate the effect of furosemide on the longitudinal smooth muscle contractions in male ICR mice ileum by using in vitro organ bath study. After 20 minutes equilibration, the isolated ileal tissue is exposed to DMSO (vehicle control), 10, 50, 100, 500 µM or 1 mM furosemide added directly into the tissue chamber containing 37°C oxygenated Krebs solution. The amplitude and frequency of smooth muscle contraction were recorded every 2 minutes throughout the 20 minutes experiment. It was found that at 50, 100, 500 µM and 1 mM furosemide, the contractile amplitude was significantly decreased (P<0.001, P<0.01, P<0.001 and P<0.05), but had no impact on the frequency. Time-course studies showed that optimal period for furosemide inducing intestinal smooth muscle contractile inhibition was 18 minutes post-incubation. These studies showed that furosemide inhibited ileal smooth muscle motility, which might be possible to base on pharmacological considerations for applying to severe diarrhea patients. Further studies should demonstrate the mechanisms of furosemide on gastrointestinal motility. No COI.

**ABS0483**  
*Effect of morelloflavone on duodenal contraction of cisplatin-treated rats*  
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Cisplatin is one of the most effective cancer therapeutic drug, however, its adverse effect may involve gastrointestinal irritation. The adverse mechanism of cisplatin action may be due to the production of free radicals or reactive oxygen species (ROS). Morelloflavone is a biflavonoid purified from Garcinia dulcis Kurz has shown antioxidant effect. This study aimed to investigate the protective effect of morelloflavone on duodenal contraction of cisplatin-treated rats. Rats were divided into three groups including vehicle control, cisplatin, and cisplatin + morelloflavone group. DMSO and 0.9% NaCl were used as morelloflavone and cisplatin solvent, respectively. Morelloflavone (1 mg/kg, i.p.) was given twice, 1 day and 10 mins before a single dose of cisplatin (7.5mg/kg, i.p.) injection. Rat duodenum was cut into 4 pieces and hung in organ bath, containing Tyrode’s solution aerated with carbogen gas. The resting tension was set at 1 g. After equilibration, cumulative dose response to acetylcholine (10-12-10-3 M) was performed. Duodenal tension, amplitude and frequency were averaged within 10 min after each dose of acetylcholine added and presented as the percentage from control values. It is likely that duodenal tension of cisplatin treated-rat responded less to acetylcholine when compared to vehicle control while the amplitude and frequency were not different. Treatment with morelloflavone may show the protective effect against cisplatin-induced gastrointestinal toxicity. No COI.
ABS0505

Effect of genistein attenuates NSAIDs-induced gastropathy in rats
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Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed worldwide and known to induce gastric injury from multiple mechanisms. Genistein, one of the flavonoid compounds, isoflavone, is found mostly in soybean. Previous studies show that flavonoid compounds have good outcomes with gastritis, ulcer, and cancer. The aim of this study was to investigate the anti-inflammation effect of genistein on NSAIDs-induced gastropathy. Male Sprague-Dawley rats were randomly divided into three groups (n = 6, each). Group 1 (control) was fed with distilled water. Group 2 (NSAIDs) was fed with 150 mg/kg indomethacin dissolved in 5% NaHCO3 at time 0th and 4th h. Group 3 (NSAIDs + Gen) was fed with 150 mg/kg indomethacin as previously described and 100 mg/kg genistein dissolved in 0.1% DMSO at time 0th and 4th h. Stomach was removed to study histopathology 8 h after treatment. Serum was collected to determine TNF-α and PGE2 using ELISA technique. In NSAIDs group, serum TNF-α was significantly increased and PGE2 was significantly decreased when compared to control group (210.28 ± 0.10 vs 126.40 ± 0.13 pg/mL and 152.83 ± 0.01 vs 303.33 ± 2.16 pg/mL, P = 0.000, respectively). These significantly attenuated in NSAIDs + Gen group when compared to NSAIDs group (156.59 ± 0.10 vs 210.28 ± 0.10 pg/mL and 247.65 ± 0.01 vs 152.83 ± 0.01 pg/mL, P = 0.000, respectively). In NSAIDs group, rats developed moderate to severe gastric inflammation, erosion, and ulcer. In the NSAIDs + Gen group, histopathology was improved when compared to the NSAIDs group. Genistein attenuated NSAIDs-induced gastropathy by increased PGE2, reduced inflammatory cytokines, and improved histopathology. No COI.

ABS0517

Protective effects of manassantin A against ethanol-induced gastric ulcer in rats
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We examined the protective effect of manassantin A on the ethanol-induced gastric ulcer in rats. Gastric injury was induced by intragastric administration of 5 mL/kg body weight of absolute ethanol to each rat. The positive control group and the manassantin A group were given oral doses of omeprazole (50 mg/kg) or manassantin A (200 mg/kg), respectively, 2 h prior to the administration of absolute ethanol. The stomach of each animal was excised and examined for gastric mucosal lesions. To confirm the protective effects of manassantin A, we evaluated the degree of lipid peroxidation, the level of reduced glutathione (GSH), and the activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). In addition, Prostaglandin E2 (PGE2) and iNOS level were measured. Furthermore, we conducted an acute toxicity study to evaluate the safety of manassantin A according to OECD guideline. Manassantin A reduced ethanol-induced hemorrhage, hyperemia, and loss of epithelial cell in the gastric mucosa. Manassantin A reduced the increased lipid peroxidation associated with ethanol-induced acute gastric lesions, and increased the mucosal GSH content and the activities of antioxidant enzymes. In addition, manassantin A pretreatment prevented the ethanol-induced decrease of Prostaglandin E2 and decreased iNOS level in gastric mucosa. Furthermore, manassantin A did not cause any adverse effects. These results indicate that manassantin A protects the gastric mucosa against ethanol-induced gastric injury by increasing the antioxidant status. We suggest that manassantin A could be developed as an effective drug for the treatment of gastric injury caused by alcohol intake. No COI.
ABS0146
Role of KCC2 down-regulation on motor functional recovery after sciatic nerve injury
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The K⁺-Cl⁻ co-transporter (KCC2) is a major Cl⁻ efflux pathway in neurons necessary for hyperpolarizing GABAA and Glycine-receptor mediated inhibitory responses. Down-regulation of KCC2 expression occurs in several pathological conditions and results in a conversion of GABA responses from the inhibitory to the excitatory. Excitatory GABA signaling at post-injury has been proposed to facilitate the recovery of neuronal circuits and appropriate firing patterns. To directly test this hypothesis we manipulated KCC2 expression using a tetracycline-inducible transgenic mouse and examined motor function recovery following peripheral nerve injury. In wild type control mice, KCC2 mRNA expression in the ventral horn ipsilateral to the nerve injury was significantly decreased 3 days after injury, as compared to the contralateral side, and recovered to control levels by 42 days after injury. Motor function, assessed by the rotor rod test, was markedly decreased in WT mice following injury and recovered to control levels by 28 days. In contrast, in mice with overexpression of KCC2 motor function recovery was delayed, and was still incomplete by 42 days post injury. Furthermore the expression of the GABA synthesizing enzymes GAD65 and GAD67 were decreased at 42 days post-injury in WT mice but not in KCC2 overexpressing mice. Hence injury induced loss of KCC2 and subsequent excitatory GABA and reduced GABA-ergic signaling is beneficial for neuronal recovery after injury. Down-regulation of GABA inhibitory signaling may enhance excitability of neural circuits and contribute to promoting recovery of motor function. No COI.

ABS0174
P2Y6 purinoceptor mediates the effect of H5N1 avian influenza virus on cytokine production in human respiratory epithelium
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Important pathogenesis of H5N1 infection is the cytokine storm and severe respiratory distress. Recent reports suggested that H5N1 may trigger cytokine production via unknown infection-independent mechanisms. We investigated the mechanism underlying the effect of β-propiolactone (BPL)-treated, non-replicated, H5N1 on cytokine production in 16HBE14o- human respiratory epithelial cells. We found that BPL-H5N1 increased mRNA expression of IL-6 and IL-8, which was inhibited by suramin, MRS2578, and apyrase. The effect of H5N1 was attenuated by α-2,3 sialidase, suggesting that an interaction between H5 hemagglutinin and α-2,3 sialic acid receptors of the host cells is essential for inducing the effect. In addition, an inhibitory effect of apyrase suggests that the effect of H5N1 is mediated by nucleotides released from the epithelium. Furthermore, PLC, PKC, intracellular Ca²⁺, NF-κB, ERK1/2 and p38 MAPK were found to be involved in the H5N1-mediated effect. Taken together, our findings suggest that the infection-independent effect of H5N1 is mediated by one of the viral structural proteins that triggers nucleotide releases from the respiratory cells. Consequently, activation of P2Y6R signaling leads to increasing transcription of IL-6 and IL-8 genes. Our data also suggest that pharmacological inhibitors of PLC, PKC and MAPK may be useful therapeutic tools for delaying an onset of hypercytokinemia known to be the major cause of fatality in H5N1 infected patients. No COI.
Inhibition of the extrinsic aging-related ion channels TRPV1 and ORAI1 by constituents of Cyperus rotundus rhizome
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Exposure to solar ultraviolet (UV) radiation is the major cause of extrinsic skin aging (photoaging), which is clinically characterized by deep skin wrinkling and pigmentation. These phenomena are caused by an increase in matrix metalloproteinase-1 (MMP-1) expression in keratinocytes and tyrosinase activation in melanocytes. In a recent study, it was reported that two Ca2+ channels, transient receptor potential vanilloid type-1 (TRPV1) and calcium release-activated calcium channel protein 1 (ORAI1), are respectively involved in MMP-1 expression and tyrosinase activity induced by exposure to UV radiation. In the present study, we evaluated whether an extract of Cyperus rotundus rhizomes has inhibitory effects on TRPV1 and ORAI1 by using the whole-cell patch-clamp technique and measuring intracellular Ca2+ levels. In our electrophysiological study, the hexane fraction of the Cyperus rotundus extract was found to strongly block capsaicin-induced TRPV1 and ORAI1 currents in HEK293T cells overexpressing TRPV1 or a combination of ORAI1 and STIM1. Furthermore, of the 5 compounds isolated from the hexane fractions, valencene had inhibitory effects on both ORAI1 (95 ± 5% at 90 μM) and TRPV1 (69 ± 15% at 90 μM) activation. Our findings suggest that the C. rotundus extract and its constituents provide a novel approach for treating and preventing UV-induced skin aging. No COI.

Enhanced expression of epithelial sodium channel (ENaC)-α, β and γ in kidneys of orchidectomized rats by testosterone
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Previous studies documented sexual dimorphism in blood pressure. In males, higher testosterone level may contribute to this effect. We hypothesized that testosterone effect could involve changes in renal sodium handling associated with changes in expression of epithelial sodium channel (ENaC). In the present study, we investigated changes in expression of gene and protein for ENaC-α, β and γ subunits in kidneys under testosterone influences. Male rats were first divided into 8 groups and underwent orchidectomy. Orchidectomised rats received peanut oil (control), testosterone (125 μg/kg/day and 250 μg/kg/day) with or without flutamide (8 mg/kg/day) an androgen receptor blocker or finasteride (5 mg/kg/day), a 5α-reductase inhibitor for seven consecutive days. At the end of experiment, animals were sacrificed and kidneys were harvested and subjected for realtime PCR and Western blotting. Immunohistochemistry was performed to identify distribution of ENaC-α, β and γ in kidneys. Results: Orchidectomy accompanied by loss of testosterone decreased ENaC-α, β and γ mRNA and proteins expression level in kidneys. Testosterone replacement resulted in significant increase in expression of mRNA and proteins for ENaC-α, β and γ. Immunoperoxidase revealed the present of ENaC-α, β and γ in epithelium lining the distal tubules and collecting ducts. Expressions of these proteins were reduced following co-administration of flutamide and finasteride with testosterone. In conclusions, the findings demonstrated the enhanced ENaC-α, β and γ expressions in kidney distal tubules and collecting ducts by testosterone could affect renal sodium handling, thus affecting the blood pressure. This finding could at least in part explain the higher blood pressure in males compared to females. No COI.
ABS0301

Anti-carcinogenic effects of isoamericanol A from Jatropha curcas seeds on the human breast cancer cell, MCF-7 by cell cycle arrest

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The extract of Jatropha curcas (J. curcas) seed has been used as biodiesel in Asian counties such as Thailand. However, this process results in a great amount of waste byproduct. Therefore, possible medicinal usage from this waste has been investigated. One of the extracts from the seeds of J. curcas, isoamericanol A (IAA) has been successfully achieved by the Faculty of Agriculture, Kagawa University, Japan. While almost all parts of J. curcas have been used for both biological and medicinal purposes, this study is the first to report on an anti-carcinogenic activity of IAA as J. curcas seed extract. In our experiment, IAA was tested for an inhibitory effect of cell proliferation in a series of human cancer cell lines: MCF-7 (human breast cancer), MDA-MB231 (human breast cancer), HuH-7 (human hepatocellular carcinoma), and HeLa (human cervical cancer). Cell proliferation assay proved IAA exhibits anti-carcinogenic activity starting at a dose of 25 µg/ml (p<0.05). The molecular mechanisms of IAA on MCF-7 were investigated by DNA-microarray analysis, flow cytometry, TUNEL assay, western blot, and quantitative real-time PCR. The results showed increased expression of BTG2 (B-cell translocation gene 2, p<0.05), p21 (p21WAF1/CIP1, p<0.05), and GADD45A (growth arrest and DNA-damage-inducible, alpha, p<0.001), in addition to decreased expression of CDK1 (cyclin-dependent kinase 1, p<0.05) and cyclins B1 (p<0.001) and B2 (p<0.001), changes which all resulted in G2/M cell cycle arrest (p<0.001). These findings suggest that IAA has great potential as a future therapeutic reagent for breast cancer as well as other types of cancer. No COI.

ABS0361

In vivo two-photon laser ablation of neural processes within cortical layer V of mouse brain under an optimized condition

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A laser ablation using two-photon microscopy (TPLA) enables to destruct a minute region around the focal point of objective lens in living body and has recently applied to living mouse brain to sever axon or dendrite of neurons in vivo. However, TPLA in living mouse brains was available only in a superficial cortical region so far. In general, to facilitate TPLA, the focal spot size of the excitation laser should be minimized because of the non-linearity of two-photon excitation, although the size tended to increase in the deeper region of the living brain, probably due to several optical aberrations caused mainly by refractive index mismatches between the immersion liquid and the specimen. In this study, we examined several optical properties and the procedure in order to sever neural processes in deeper regions of living mouse brains. For experiments, Thy1-eYFP-H mice were operated with “Open-skull” methods. In vivo imaging and TPLA were performed by a two-photon microscope system. We first examined effects of various refractive indexes in the immersion liquid on the laser focal size in the deeper layers. Next, by optimizing the procedure of TPLA, we succeeded in severing neural processes in the cerebral cortex layer IV reproducibly, and also sometimes in the layer V when the open-skull resulted as favorable. TPLA of axons induced a dissimilar response compared to that in the case of dendrites. In summary, improving the focusing property successfully enhanced the depth limit of the in vivo TPLA. Here, we weren’t required to modify the basic design of the microscope system. We hope this method would be helpful for functional connectomics. No COI.
ABS0413

Postural change-induced modification of thermal sensation during mild-hyperthermia is disappeared in elderly men

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We have observed that whole body thermal sensation was enhanced while heat dissipative responses were attenuated with postural change from supine to sitting in young subjects. With normal aging, heat dissipative responses are attenuated while whole body thermal sensation is blunted during mild-hyperthermia. In this study, we assessed whether whole body thermal sensation was also modified with postural change from supine (SUP) to sitting (SIT) during mild-hyperthermia (HT) in elderly subjects. Methods: Seventeen young (21 ± 1.6 yrs, mean ± SD) and 12 elderly (71 ± 3.0 yrs) healthy men underwent measurements of whole body thermal sensation (VAS) in random order of SUP and SIT in normothermia (NT; Tes, 36.6 ± 0.0 °C and 36.4 ± 0.2 °C, respectively, mean ± SE) and mild-hyperthermia (HT; Tes, 37.3 ± 0.0 °C and 37.4 ± 0.2°C, respectively; lower legs immersion in 42 °C water). Tes and Tsk were measured continuously. Results: Whole body thermal sensation was lower in elderly than young men under all conditions (P<0.05). During HT, whole body thermal sensation was increased in SIT compared with SUP in young men while it remained unchanged in elderly men. Change in whole body thermal sensation with the postural change during HT was lower in elderly than young men (P < 0.05). Conclusions: Whole body thermal sensation during mild-hyperthermia was blunted regardless of body position in elderly compared with young men, furthermore the response of whole body thermal sensation with postural change during mild-hyperthermia was disappeared in elderly men. No COI.

ABS0454

Enhancement adipose-derived stem cells chondrogenesis for cartilage regeneration by using thermo-responsive HA-modified poly(N-isopropylacrylamide) hydrogels

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Injectable thermo-responsive hydrogels are advantageous cell delivery-biomaterials for applications in the minimally invasive tissue engineering. We previously demonstrated that a hyaluronic acid (HA)-enriched microenvironment enhances the chondrogenesis of adipose-derived stem cells (ADSCs). In this study, we investigated the chondrogenic potential of two newly developed HA-modified thermo-responsive poly(N-isopropylacrylamide) (PNIPAAm) hydrogels in vitro and in vivo in neo-cartilage formation combined with rabbit ADSCs (rADSCs) for articular cartilage tissue engineering. The thermo-responsive HA-mixed PNIPAAm (HA-PNIPAAm-CP) and HA-cross-linked PNIPAAm (HA-PNIPAAm-CL) were fabricated using physical interaction and chemical cross-linking methods, respectively. The in vitro results showed that, compared to unmodified PNIPAAm, both HA-modified hydrogels significantly increased the cell viability, expression of chondrogenic marker genes (collagen type II and aggrecan) and formation of sulfated glycosaminoglycan (sGAG) in embedded rADSCs. The chondrogenic effects of HA-modified hydrogels on rADSC were confirmed in vivo by intra-articular injection of rADSC/hydrogels constructs into rabbit joint cavities for 3 weeks, in which embedded-rADSCs in hydrogels were traced using CM-Dil labeling. The in vivo results showed that implanted rADSC/HA-PNIPAAm-CL constructs had markedly more neo-cartilage formation than that of rADSC/HA-PNIPAAm-CP and rADSC/PNIPAAm constructs. These results indicated that the HA-PNIPAAm-CL hydrogel provides a suitable microenvironment to enhance ADSC chondrogenesis for cartilage tissue engineering applications. No COI.
Apelin-13 impaired acquisition but not consolidation or expression of contextual fear in rats

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Recently apelin-13 was demonstrated to be involved in the processes that contribute to learning and memory. While contextual fear conditioning is a form of associative learning that has been used to elucidate mechanisms that are involved in aversive memory processes. At present study, we tested the effects of exogenous apelin-13 (1.0, 2.0 and 4.0 µg/rat) on contextual fear conditioning (experiment 1), fear consolidation (experiment 2) and fear expression (experiment 3) in rats. Behavior procedure involved three training phases: habituation, fear conditioning and test, each separated by 24 h. Apelin-13 was injected i.c.v. 10 min before conditioning, immediately after conditioning and 10 min before testing. Percent freezing time on each time block during which no footshock was presented was scored. We found that apelin-13 administrations had no effect on freezing in experiment 2 and 3 but produced a decrease in experiment 1[F (3,28) = 3.494, P < 0.05]. Apelin-13 (2.0 µg/rat) group in experiment 1 presented a significant lower freezing during both fear conditioning (time block 4, P < 0.05; 5 and 6, P < 0.01) and test phases (P < 0.01). Further results indicated that the decreased freezing in experiment 1 was not attributed to nonspecific changes of locomotor activity. These results showed that apelin-13 impaired fear acquisition but not fear consolidation or expression, which extend previous research on the apelin-13 effects on learning and memory. (This work was supported by grants #81270065, #81370116 from NSFC, grant#2013JJ4030, #2015JJ2147 from Hunan Natural Science Foundation, and grants #14C0128 from Scientific Research Fund of Hunan Provincial Education Department). No COI.
Comparison between MRI findings and motor development in the cases with neonatal HIE
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In the cases with moderate to severe neonatal hypoxic-ischemic-encephalopathy (HIE), it is well known that those MRI findings are one of the most sensitive predictor of neurological prognosis of these patients. However, there are some cases show differences between MRI findings and their development. We investigated correlative of 43 cases with moderate to severe HIE, comparing MRI findings at six to twelve months old with neurological prognosis at one and a half year old. No COI.
MEMBRANE AND EPITHELIAL TRANSPORT

ABS0047

Microcirculation and paracellular fluid secretion in the perfused submandibular gland
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The raised hydrostatic pressure could function as a main driving force for paracellular fluid secretion by the isolated perfused submandibular gland from rat. The fluorescent dyes, Lucifer Yellow and Sulforhodamin B, is sized around 500 dalton and cannot enter the cell. Then their transfer from the circulation to the saliva indicates paracellular transport. We found that the transfer of Lucifer Yellow depends on the perfusion pressure indicating that the solvent drag is the main mechanism for paracellular transport of Lucifer Yellow. Whereas the perfusion pressure decreased upon cholinergic stimulation in the gland perfused at the constant rate, indicating vasodilatation of arterioles and/or relaxation of precapillary sphincters to open the microcirculation, thus increasing the area and hydrostatic pressure of vascular bed surrounding the acinar cells. We also found that Danshen, Chinese herb, decreased the arteriovenous pressure immediately but the paracellular secretion was delayed around 5 min after start of stimulation. This finding was concluded that the onset of paracellular transport requires not only the increase in microcirculation but also the increase in the permeability across the tight junction. No COI.

ABS0056

Role of TRPM6 and TRPM7 in maternal-fetal calcium transport
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The placenta is required to transport calcium from mother to fetus during fetal bone mineralization. However, the molecular mechanism and regulation of this transport has not been determined. In an attempt to clarify the molecular basis of calcium entry for this transport, we identified TRPM6 as a candidate. TRPM6 mRNA increased during the last 4 days of pregnancy in mice, coinciding with fetal bone mineralization. TRPM6 mRNA and protein was localized in the trophoblasts in labyrinth and intraplacental yolk sac, both of which are known to play a role in maternal-fetal calcium transport in rodents. Moreover, in patch-clamp recordings in mouse trophoblasts, TRPM6/TRPM7 heteromer-like currents were observed after starting fetal bone mineralization but not before mineralization. Plasma membrane calcium permeability in HEK293T cells indicated that TRPM6/TRPM7 heteromers were actually functional under physiological magnesium and ATP concentration but TRPM6 or TRPM7 homomers were not. These results suggest that TRPM6 is implicated in maternal-fetal calcium transport forming a complex with TRPM7, which might enable to sustain calcium uptake in mouse trophoblasts during fetal bone mineralization. No COI.
In vitro study of organic cation drug transport mediated by human organic cation transporter 1 (SLC22A1) and 3 (SLC22A3) in hepatocellular carcinoma (HepG2) cells

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Human organic cation transporter 1 (hOCT1) is known as liver transporter that is highly expressed at the sinusoidal membrane of hepatocytes while isoform 3 (hOCT3) is also found at the low level. Previous study has revealed that hOCT1 was responsible for the uptake of anti-neoplastic paclitaxel, cationic drug, which exhibited its action in lymphoma cells. The hepatocellular carcinoma (HepG2) cell line has been widely used for drugs screening test. However, the cellular uptake of cationic drugs by hOCT1 and 3 functions in HepG2 cells has not been investigated yet. Therefore, this study aimed to clarify the cationic drug transport function mediated by hOCT1 and 3 in HepG2 cells using fluorescence 4-(4-(dimethylamino)styryl)-N-methylpyridinium (ASP+). Human OCTs protein expressions and cell viability were also determined. The results showed that ASP+ was transported into HepG2 cells and its uptake was significantly reduced by tetrapentylammonium, a potent OCTs inhibitor, in a dose-dependent. In addition, the accumulation of ASP+ in HepG2 cells by hOCT1 and 3 functions was corresponded with their protein expressions. Moreover, cytotoxic action of paclitaxel in HepG2 cells at a dose of 10 µM significantly reversed by metformin, an anti-diabetic cationic drug. These findings suggest that organic cation transport function mediated by endogenous hOCT1 and 3 in HepG2 cells exists and this could be a beneficial tool for screening organic cation drug that exerts its action in hepatocellular carcinoma. This work was supported by the Faculty of Medicine Research Fund, Chiang Mai University, Thailand, and the National Research Council of Thailand. No COI.

The effect of oxidative and nitrosative stresses on renal organic anion transporter 3 function in type 2 diabetic rats.

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Oxidative and nitrosative stresses have been shown to be major mediators in the progression of diabetic nephropathy. Significant reductions of renal organic anion transporter 1 (Oat1) and 3 (Oat3) mRNA expressions, and urinary organic anion metabolite, a homovanillic acid, were reported in diabetic nephropathy patients. However, the significant role of oxidative and nitrosative stresses on Oat3 function in Type 2 diabetic (T2DM) condition has not been investigated yet. This study was aimed to determine the effects of oxidative stress and nitric oxide productions on renal Oat3 function in experimental T2DM rats. Diabetic rats were induced by a combination of high-fat diet and a single dose of streptozotocin (35 mg/kg BW). General characteristics of T2DM and renal oxidative stress markers were investigated. The renal transport function was examined by the uptakes of para-aminohippurate (PAH) and estrone sulfate (ES) mediated rOat3 function using renal cortical slices. The results showed that T2DM rats had significantly developed hyperglycemia, hypertriglyceridemia, insulin resistance, and renal oxidative stress. Although, the uptakes of PAH and ES by Oat3 were not different among experimental groups, sodium nitroprusside-induced nitrosative stress impaired PAH and ES transports in T2DM rat kidneys which related to the levels of renal nitric oxide production. These findings indicate that T2DM condition impaired renal Oat3 function through oxidative/nitrosative stress pathways that contribute to a consequence of diabetic nephropathy. This work was supported by the Faculty of Medicine Research Fund, Chiang Mai University, Thailand, and the Researchers and Research for Industry Grants: Master Research Grants by Thailand Research Fund (RRI-MAG). No COI.
ABS0375

Properties of volume-sensitive anion channels in butyrate-triggered apoptosis of murine colonic epithelial cells
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Ulcerative colitis is one of inflammatory bowel diseases of unknown etiology. Recent studies have demonstrated that the disease is associated with an increase in apoptosis of colonic epithelial cells. Although butyrate is an energy source for colonic epithelium, excess amount of butyrate has been reported to induce apoptosis in the epithelial cells. Since the activation of volume-sensitive outwardly rectifying (VSOR) anion channels has been demonstrated to requisite for apoptotic induction, we investigated whether VSOR anion channels participate in apoptosis induced by butyrate in murine colonic epithelial MCE301 cells. In whole-cell patch-clamp recordings, hypotonic cell swelling increased membrane currents exhibiting outward rectification and a time-dependent inactivation at depolarized potentials. The swelling-activated currents were anion selective (I− > Br− > Cl− > F−) and inhibited by VSOR anion channel blockers, DCPIB (2.5 µM) and NPPB (10 µM). These results suggest that VSOR anion channels are functionally expressed in MCE301 cells. Flow cytometry revealed that MCE301 cells exposed to sodium butyrate (8 mM) for two days were stained by apoptosis markers, annexin V-FITC and propidium iodide. In addition, caspase 3/7 activation was observed in the cells treated with sodium butyrate for 16 h. Importantly, DCPIB (2.5 µM) and NPPB (10 µM) significantly inhibited these apoptotic events. We therefore suggest that sodium butyrate causes apoptosis via activation of VSOR anion channels in MCE301 cells. No COI.

ABS0405

Human organic anion transporter 10 (OAT10) transports urate and Ketone bodies
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Kidneys play an important role for the reabsorption of filtered urate. Two renal urate/anion exchangers have been described in brush-border membrane vesicles in human: one for which urate has more affinity than lactate (high urate affinity exchanger), and another for which lactate has more affinity than urate (low urate affinity exchanger) (Roch-Ramel et al., 1996). Previously, we identified URAT1 (SLC22A12) as a high urate affinity exchanger based on its transport properties (Enomoto et al., 2002). Recently, we found that one of the transporter genes in SLC22 family, named OAT10, seems to be another urate/anion exchanger in Xenopus oocyte expression system. OAT10 showed time- and concentration-dependent transport of urate with low affinity and in Na+-independent manner. We further characterized transport properties of these two urate/anion exchangers. Similar to URAT1, OAT10-mediated urate transport was trans-stimulated by aromatic monocarboxylates such as nicotinate and pyrazinoate. In contrast, the trans-stimulated urate transport by various aliphatic monocarboxylates such as lactate and ketone bodies as well as dicarboxylates, not tricarboxylate in Krebs’ cycle could be observed in OAT10 but not in URAT1. Inhibition profiles by several anionic compounds on OAT10-mediated urate transport were different from those of URAT1. These results indicate that OAT10 has characteristics compatible to low urate affinity exchanger and it might be another promising drug target for treatment of hyperuricemia. This study was supported in part by the Grant-in-Aid from Japan Society for the Promotion of Science (JSPS) # 23590647. No COI.
Evidence for a modulatory role of annexin A2 in the maxi-anion channel activity

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The maxi-anion channel (Maxi-Cl) is characterized by its large unitary single-channel conductance (about 300-400 pS), and its functional expression has been detected in a large variety of mammalian cells and tissues. The Maxi-Cl has attracted significant attention because of its permeability to anionic forms of organic metabolites, like ATP and glutamate, and thereby plays an essential role in the cell-to-cell purinergic and glutamatergic signal transduction. We have been putting effort to elucidate the still unidentified molecular nature of this channel. Based on pharmacology, RNAi gene silencing, over-expression, ATP release assay and other necessary experiments we so far excluded several possible candidates including pannexin and connexin 43 hemichannels, plasmalemmal VDAC, a tweezy homolog TTYH and adenine nucleotide translocator (ANT). Based on microarray analysis, we found that several members of annexin (Anx) family are differentially expressed between Maxi-Cl-rich C127 cells and Maxi-Cl-deficient C1300 cells. siRNA-mediated gene silencing of Anxa4 and Anxa6 did not exert any suppressive effect on the Maxi-Cl activity in C127 cells. However, using both siRNA- and miRNA-mediated transient gene knockdown strategies, the Maxi-Cl currents recorded in the inside-out patch-clamp mode were found significantly reduced in Anxa2-silenced C127 cells as compared to the mock-transfected cells. Maxi-Cl activity was also partially inhibited by anti-Anxa2 antibody. However, when annexin A2 was overexpressed in C1300 cells, the Maxi-Cl activity could not be retrieved. We conclude that annexin A2 functions as a modulator rather than channel molecule itself. No COI.

New strategy for atopic dermatitis therapy via modulation of calcium ion channels by topical botanical products

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Intracellular Ca²⁺ signaling via various calcium channels has been shown to directly modulate skin inflammation. Ca²⁺ influx through these channels eventually generates intracellular Ca²⁺ signaling that results in different outcomes dependent on the individual Ca²⁺ channel type, for example, immune cell modulation through Orai1, epidermal barrier formation and keratinocyte differentiation through TRPA1, and keratinocyte cornification through TRPV3. Therefore, a specific agonist/antagonist for each calcium channel is required for maintaining skin barrier homeostasis and for the treatment of dermatological diseases. We prepared 70% MeOH extracts of 30 medicinal herbs, performed bioassay-guided fractionation of the active extracts, and then isolated and identified the bioactive constituents. By performing the combination of automated and conventional whole-cell patch clamp studies, we found eight medicinal herb fractions for Orai1, four for TRPV1, two for TRPA1, and one for TRPV3 that showed >50% inhibition rates at 30 μg/mL. We also found three fractions with TRPA1 agonist activity. Further, we also identified chemical constituents that inhibit Orai1 (compound V: 95 ± 5% inhibition at 90 μM) and TRPV1 (compound M: 93.9 ± 2.45% inhibition at 90 μM). Considering that most regional plants have not been investigated chemically or pharmaceutically, they remain as untapped potential sources of topical agents for drugs and other application. We found major active components and chemical constituents of plant extracts for the modulation of various calcium ion channels, which may have potential clinical applications for abnormal skin barrier functions such as atopic dermatitis. No COI.
Selenium-induced autophagy is associated with decreased mitochondrial marker and contributes to regulation of oxidative fiber proportion in L6 myoblasts
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Selenium is a trace element that is naturally present in many foods. It appears to be necessary for normal muscle function and homeostasis. Hypo- or hyperselenium is associated with several muscular diseases affecting both cardiac and skeletal muscles in both cattle and humans, and has led to the identification of a new clinical syndrome called nutritional muscular dystrophy. Indeed, excess selenium intake has been reported to be associated with muscle weakness and peripheral neuropathy, whereas selenium deficiency caused dilated cardiomyopathy (keshan disease). Unfortunately, there is limited information about how selenium maintains muscle function. Therefore, we studied the effects of selenium on mitochondria content and fiber type arrangement and autophagy induction in a L6-rat myoblast derived cell line (L6). L6 cells were seeded into a 6/12-well plate with cell density of 4 × 10^6 cells/well and cultured for 3 days until 80-90% confluency. 24h before cell harvesting, the myoblasts were treated with different concentrations of Selenium (1, 10, 100 µM). We observed that 10 µM selenium significantly decreased LC3II (autophagosome marker) and p62 protein levels indicative of increased autophagic flux. Furthermore, 10 µM selenium increased phosphorylation of AMPK and ULK1 (S555), and decreased mTOR signaling, two key pathways that regulate autophagy. Interestingly, we also observed that PPAR1A (PGC1α), COX IV and HSP60 protein levels were decreased by selenium treatment. Moreover, selenium also reduced significantly the expression of the oxidative muscle fiber marker, βMHC. Taken together, selenium induced autophagy and decreased mitochondrial content to reduce oxidative fiber ratio and potentially may lead to suboptimal muscle performance. No COI.

Expression level of importin and exportin of mouse skeletal muscle in response to unloading with or without reloading
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Loading is a regulatory factor for skeletal muscle mass as well as function. However, the molecular mechanisms of loading-associated gene expressions in skeletal muscle remain unclear. Recently, it has been suggested that an accumulation of proteins, such as muscleblind-like 1 (MBNL1), in nuclei plays an important role in skeletal muscle atrophy in myotonic dystrophy (DM1). In the present study, therefore, we investigated the response of the nuclear transport proteins, importin b and exportin, to unloading in mouse skeletal muscle. Mice (C57BL/6J) were randomly divided into two groups, untreated control and hindlimb suspension (HS) groups. The mice of HS group were subjected to continuous hindlimb suspension for 2 weeks with or without 2-week ambulatory recovery. The medial gastrocnemius (MGAS) and plantaris muscles of mice in both groups were dissected bilaterally immediately after the suspension and/or 2 weeks of recovery. Significant reduction of muscle mass was observed in MGAS, but not plantaris. Although the expression level of importin was not changed in both muscles, the expression level of exportin in plantaris was upregulated by unloading, but not in MGAS. Therefore, the accumulation level of proteins in nuclei might be a loading-sensitive regulatory factor of skeletal muscle. No COI.
Activation of cannabinoid system in nucleus accumbens affects cost-benefit decision making
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Ability to choose goals based on usefulness or the time of reaching goals are the important aspects of decision-making. Previous evidence showed that decision making altered in cannabis abusers but, the role of the cannabinoid system in decision-making circuits has not been investigated exactly. In the current study, we examined the effects of cannabinoid modulation during cost-benefit decision making in the nucleus accumbens (NAc). We trained different groups of rats in a delay-based and an effort-based form of cost-benefit T-maze decision-making task. During test days, the rats received local injections of either vehicle or win 55,212-2, a cannabinoid receptor agonist, (0.125, 0.25, 0.5, 1 and 2 mM/0.5 μl DMSO) in the NAc bilaterally. We measured spontaneous locomotor activity following the same treatments. Then, AM251, a cannabinoid antagonist, (250 μM/0.5 μl DMSO) was injected in the NAc, 5 min before the administration of the most effective dose of win 55,212-2 (2 mM). The results showed that activation of cannabinoid receptor in the NAc impaired decision making such that rats were less willing to invest physical effort to gain high reward. Similarly, cannabinoid receptor activation in this area induced impulsive pattern of choice such that rats preferred small immediate rewards to large delayed rewards. Control tasks ensured that the effects were specific for differential cost-benefit tasks. These finding revealed that the cannabinoid system in the NAc plays a critical role in regulating cost-benefit decision making. No COI.

Wnt signaling regulates blood pressure by downregulating a GSK-3b–mediated pathway to enhance insulin signaling in the central nervous system
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Aberrant Wnt signaling appears to play an important role in the onset of diabetes. Moreover, the insulin signaling pathway is defective in the nucleus tractus solitarii (NTS) of spontaneously hypertensive rats (SHRs) and fructose-fed rats. Nevertheless, the relationships between Wnt signaling and the insulin pathway, and the related modulation of blood pressure (BP) in the central nervous system, have yet to be established. The aim of this study was to investigate the potential signaling pathways involved in Wnt-mediated BP regulation in the NTS. Pretreatment with the LDL receptor–related protein (LRP) antagonist Dickkopf-1 (DKK1) significantly attenuated the Wnt3a-induced depressor effect and nitric oxide production. Additionally, the inhibition of LRP6 activity using DKK1 significantly abolished Wnt3a-induced glycogen synthase kinase 3b (GSK-3b) S9, extracellular signal-regulated kinases 1/2T202/Y204, ribosomal protein S6 kinaseT359/S363, and Akt S473 phosphorylation; and increased insulin receptor substrate 1 (IRS1) S332 phosphorylation. GSK-3b was also found to bind directly to IRS1 and to induce the phosphorylation of IRS1 at Serine 332 in the NTS. By contrast, administration of the GSK-3b inhibitor TWS119 into the brain decreased the BP of hypertensive rats by enhancing IRS1 activity. Taken together, these results suggest that the GSK-3b-IRS1 pathway may play a significant role in Wnt-mediated central BP regulation. No COI.
**ABS0043**

**Endoplasmic reticulum stress in RVLM mediates neurogenic hypertension through activation of PI3K/Akt pathway**

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Endoplasmic reticulum (ER) stress has been implicated in the rostral ventrolateral medulla (RVLM), where the sympathetic premotor neurons for maintenance of basal vasomotor tone are located, and contributed a role in pathogenesis of neurogenic hypertension. The underlying mechanism is, however, unknown. In comparison to normotensive Wistar-Kyoto rats, expressions of phosphatidylinositol 3-kinase (PI3K) and phospho-Akt, were significant greater in RVLM of the spontaneously hypertensive rats (SHR). Inhibition in PI3K expression and dephosphorylation of Akt in the RVLM of SHR by microinjection bilaterally into the nucleus of a PI3K inhibitor, LY294002, caused a significant decrease in systolic arterial pressure (SAP), alongside suppression of the augmented GRP78 expression, an ER stress marker, but the ROS was not decreased. Moreover, ER stress inhibitors, Salubrinal or an intracellular calcium stabilizer, 3,4,5-Trimethoxybenzoic acid 8-(Diethylamino) octyl ester (TMB-8), both could not affect PI3K or AKT activity. However, the superoxide dismutase mimetic, Tempol, does not only inhibit the ROS, but also decrease the expression of PI3K-Akt and ER stress. Collectively these results suggest that ER stress is the new important factor in the manifestation of neurogenic hypertension via redox-sensitive activation of PI3K/Akt pathway in the RVLM of SHR. No COI.

**ABS0051**

**Synchronous oscillatory network activity is driven by cholinergic system in the slug olfactory center**

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Synchronous oscillatory activity in a laminar structure is common in the olfactory nervous system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. We recently found that oscillatory neuronal network was formed from dispersed cell culture of PC neurons. Increases in neurite arborization, neurite connection and cell aggregation were observed with time in culture. Calcium imaging for each PC neurons showed that acetylcholinesterase inhibitor or nicotine increased the number of spontaneous calcium transients and induced synchronous oscillatory activity. On the other hand, histamine increased the number of calcium transients without synchronous oscillatory activity in a smaller number of PC neurons. These results suggest that acetylcholine can function as an excitatory modulator on the synchronous oscillatory activity of the PC neuron network via nicotinic acetylcholine receptors activation. No COI.
ABS0052
Spinal IL-33/ST2 signaling contributes to neuropathic pain via neuronal CaMKII-CREB and astroglial JAK2-STAT3 cascades in mice
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Nerve damage-initiated neuroinflammation and immune response contribute to the development of neuropathic pain. This study investigates the role of spinal interleukin (IL)-33 and its receptor ST2 in the spared nerve injury (SNI)-induced neuropathic pain. Intrathecal administration of ST2-neutralising antibody or ST2 gene knockout (ST2-/-) significantly attenuated the SNI-induced mechanical and cold allodynia. The expression of spinal IL-33 and ST2 were substantially elevated by 255.8±27.3% and 266.4±83.5% (mean±SD) on the 7th day following SNI, respectively. Mechanistic studies showed that the increased expression of spinal NR1 subunit of N-methyl-D-aspartate (NMDA) receptor after SNI was reduced by ST2 antibody administration or ST2-/- . The induction of noiceptive behaviours in naïve mice by rIL-33 was reversed by the non-competitive NMDA receptor antagonist MK-801. ST2 antibody administration or ST2-/- markedly inhibited the increased activation of the astroglial janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) cascade and the neuronal calcium-calmodulin dependent kinase II (CaMKII)-cAMP response element-binding protein (CREB) cascade after SNI. Moreover, intrathecal pretreatment with the CaMKII inhibitor KN-93 or the JAK2-STAT3 cascade inhibitor AG490 attenuated the rIL-33 induced noiceptive behaviours and NR1 up-regulation in naïve mice. The results demonstrate that spinal IL-33/ST2 signaling contributes to neuropathic pain by activating astroglial JAK2-STAT3 cascade and neuronal CaMKII-CREB cascade. No COI.

ABS0070
Anxious behavioural responses in male Formosan wood mice
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Most of wood mice studies focused on the reproductive and/or ecological fields. Formosan wood mice (Apodemus semotus), is the Taiwan native rodent and dominates in the Taiwan mountains, which the ranges of heights are from 1500 to 3500 meters. Whether the emotional responses in Formosan wood mice contributed the dominant events was not well known. The present study used couples of emotional behavioural tests for anxious responses to examine this hypothesis. The emotional behavioural tests in this study contained the light/dark exploratory test, marble burying test, elevated plus maze and open field exploratory test. Male Formosan wood mice had the higher durations in the light component during the light/dark exploration test compared to those in male common laboratory C57BL/6 mice. In the marble burying test, the numbers of marble burying in male Formosan wood mice were higher than those in male C57BL/6 mice. The rearing duration in marble burying test was also higher in Formosan wood mice. Higher moving distance in the both open and close arms of elevated plus maze in male Formosan wood mice had been found. Finally male Formosan wood mice showed higher distance of movement in the central zone or total distance of open field exploratory test than male C57BL/6 mice. Taken together, these results showed higher activity and exploratory behaviours in male Formosan wood mice. In summary, Formosan wood mice might have these behavioural responses to get the surviving advantages in Taiwan Mountains. The authors declare that they have no conflict of interests related to this work. No COI.
ABS0071

Behavioural responses in depression and performance of learning and memory in male Formosan wood mouse
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Formosan wood mice (Apodemus semotus) is the prevalent and Taiwan native rodent in the intermediate altitude of Taiwan mountains. Ecological and/or reproductive studies in Formosan wood mice have been reported; however, the behavioural responses in depression test and the learning and memory ones are few. This study focused on the performance of the learning and memory, and depressive behavioural responses in male Formosan wood mice. Porsolt forced swimming, tail suspension, hot plate passive avoidance and novel object recognition tests were used to examine the behavioural responses in male adult Formosan wood mice and C57BL/6 mice, the common laboratory mice. Male Formosan wood mice showed higher latency and lower duration in the immobilization of forced swimming test than that in male C57BL/6 mice. Male C57BL/6 mice also exhibited the lower latency and higher duration in the immobilization of tail suspension test. Additionally male Formosan wood mice revealed higher number of step down and lower latency to hot plate in the hot plate test compared to those in male C57BL/6 mice. In the novel object recognition test, both male C57BL/6 mice and male Formosan wood mice revealed the similar responses in the performance of the learning and memory. Taken together, these results in the present study showed that male Formosan wood mice did not have depressive-like behavioural responses. Male Formosan wood mice also did not have lower performance of learning and memory. The current study indicated that Formosan wood mice in the laboratory environment still kept the instinctive behaviours as the wild natural environment. No conflict of interests related to this work. No COI.

ABS0072

The effect of estrogen on iron metabolism in astrocytes and neurons
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Estrogen affects iron metabolism in peripheral tissues, and what is the role of estrogen on iron metabolism in neurons and astrocytes in midbrain? In this study, we investigated the effect of estrogen on the iron transport proteins as well as its mechanisms. The results were as follows: Iron exporter ferroportin1 (FPN1) and iron importer divalent metal transporter 1 (DMT1) was up-regulated after estrogen was treated for 12h in primary cultured astrocytes. Hypoxia inducible factor-1alpha (HIF-1α) was up-regulated, but hypoxia inducible factor 2 alpha (HIF-2α) remained unchanged after estrogen was treated for 12 h in primary cultured astrocytes. In neurons, DMT1 was decreased but FPN1 was up-regulated after estrogen was treated for 12h in primary cultured neurons. IRP1 was down-regulated while HIF-1α and HIF-2α remained unchanged after estrogen was treated in primary cultured neurons. The results suggest that the regulations of estrogen on astrocytes and neurons are different. Estrogen can increase FPN1 and DMT1 expressions by elevating HIF-1α in astrocytes. However, the decreased expression of IRP1 may account for the decreased DMT1 and increased FPN1 expressions in neurons. No COI.
Rosmarinic acid protects SK-N-SH cells by inhibiting iron induced α-synuclein aggregation
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Rosmarinic acid (RA) is a naturally occurring polyphenolic compound, and is composed of caffeic acid and danshensu. Our previous studies have confirmed RA could protect against 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl pyridine cation (MPP+) induced cell injury. Improving evidence showed iron-induced α-synuclein aggregation played important roles in the etiology of Parkinson's disease (PD). However, whether RA could protect dopaminergic neurons through inhibiting the aggregation of α-synuclein in PD is unclear and the regulation mechanisms underlying this inhibition were not elucidated. Therefore, the experiment proposed to explore the effects of rosmarinic acid against iron-induced α-synuclein aggregation in dopaminergic cells and elucidate the possible mechanisms in the SK-N-SH cells. Results showed that iron could reduce the mitochondrial transmembrane potential (ΔΨm) and induce α-synuclein aggregation in the SK-N-SH cells. In accordance with iron responsive element/iron regulatory protein (IRE/IRP) system, iron could increase the mRNA levels of α-synuclein. Results also showed that RA pretreatment could restore the ΔΨm reduction induced by iron and alleviate iron induced α-synuclein aggregation. Further results showed that RA pretreatment could inhibit iron induced α-synuclein aggregation by up-regulating hemeoxygenase-1 (HO-1). In addition, RA pretreatment could decrease the mRNA levels of α-synuclein via decreasing the protein levels of IRP1. These results provide new findings and new strategies for the prevention and treatment of PD. No COI.

Interleukin 6 regulates iron related proteins through c-Jun N-terminal kinase activation in BV2 microglial cell lines
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Parkinson’s disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons in the substantia nigra (SN) and the subsequent DA depletion in the striatum. Extensive studies have demonstrated that microglia activation and nigral iron accumulation play a key role in the pathogenesis of PD. The activated microglia showed amplified levels of iron deposit, however, the relationship between microglia activation and iron accumulation was not fully elucidated. In the present study, we aimed to investigate how the iron level affects interleukin-6 (IL-6) synthesis in BV2 microglia, as well as the effect of IL-6 on cellular iron metabolism in BV2 microglia. The results were as follows: IL-6 mRNA was up-regulated after FAC treatment for 12 h in BV2 cells. Iron regulatory protein 1 (IRP1) was up-regulated and iron exporter ferroportin1 (FPN1) was down-regulated after IL-6 was treated for 24 h in BV2 cells. Phosphorylated c-Jun N-terminal kinase (JNK) increased significantly after IL-6 was treated in BV2 cells for 1 h compared with the control. Pretreatment with JNK inhibitor SP600125 attenuated the up-regulation of IRP1 and down-regulation of FPN1 compared with IL-6 treated group in BV2 cells. The results suggest that iron load can increase IL-6 mRNA expression in BV2 cells. IL-6 up-regulates IRP1 expression and down-regulates FPN1 expression in BV2 microglial cells through JNK signaling pathways. No COI.
The neuronal and glial reorganization in the intact hemisphere contributes functional remodeling after focal stroke.

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In spite of the dramatic development of medical care system, the number of patients who suffer from serious sequelae such as aphasia and/or paralysis after a focal brain stroke has not markedly decreased throughout the world. Thus, basic study for the stroke is necessary to apply the result in clinical setting aiming the improvement of the quality of life for patients. In human case, stroke may cause functional disturbance such as aphasia; however, such disturbances can be sometimes recovered using intact contralateral hemisphere. In this presentation, we focus on functional compensation achieved by the intact region contralateral to the stroke. Using in vivo imaging techniques combined with electrophysiology and behavior tests, we found that the functional recovery was achieved through the specific synaptic (neuronal circuit) remodeling at the region contralateral to the focal stroke region one week after the stroke. In vivo microdialysis studies have revealed that the astrocytes play a critical role in reducing the accumulation of synaptic released glutamate, which may otherwise cause excitotoxicity. These findings indicate that the contralateral intact hemisphere after stroke can potentially achieve bilateral functions even in adults if proper remodeling of neuronal circuits occurs. These findings also indicated that activating the intact hemisphere may become a new therapeutic strategy for stroke patients. The author has no conflict of interest to disclose with respect to this presentation. No COI.

Long-lasting sound-evoked afterdischarge in the auditory midbrain

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We have discovered a previously unobserved phenomenon where excitatory and inhibitory neurons in the auditory midbrain exhibit a long-lasting sound-evoked afterdischarge (LSA) that continues long after sound stimulation ceases. To evoke LSA, we used long duration, 30 – 60 s, one-octave noise (60 dB) for 44 GABAergic neurons and 48 presumed glutamatergic neurons. We found that 20% of GABAergic and 17% of glutamatergic neurons continued to fire after the sound termination. The discharge after sound was stronger when the response during sound (RDS) was higher and the sound duration was longer. The minimum sound duration required to induce LSA was around 30 s. The RDS had to be sustained to evoke the LSA since a LSA was not seen when the RDS was transient. The number of spikes in the LSA and RDS responses were positively correlated (R = 0.51). LSA+ neurons had less adaptive firing during sound than LSA- neurons. In response to 30 s sound, both GABAergic and nonGABAergic LSA+ neurons showed more sustained firing during sound than LSA- neurons. Some LSA+ neurons had build-up firing which was not seen in LSA- neurons. The time course of LSA was variable. A peak firing rate occurred 1.0 - 50.1 s after the sound termination. There was no correlation between the peak times and the number of LSA spikes (R = 0.29), but the decay of the LSA was strongly correlated with the number of LSA spikes. The decays ranged from 0.4 - 235.6 s. LSA was also evoked by discontinuous sound (1 s noise bursts presented every 2 s, 50 repetitions). Interestingly, there was a gradual increase in interstimulus spikes not seen in neurons lacking LSA. These results suggested a form potentiation that might allow LSA neurons to overcome synaptic adaptation during long duration sounds. No COI.
ABS0260

The estrous cycle modulates voltage-gated ion channels in TG neurons

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Migraines typically occur more frequently in women than men because of the effects of estrogen on both the frequency and severity of migraine attacks. Many women suffer from migraine attacks during menstruation, which are known as menstrual migraines. The pathophysiology of menstrual migraines can be explored by using the rat estrous cycle, which shows a cyclical fluctuation of estrogen levels that resembles the menstrual cycle. The aim of this study was to investigate whether each stage of the estrous cycle is involved in migraine development by comparing the susceptibility of trigeminal ganglion (TG) neurons in each stage of the estrous cycle by using action potential (AP) parameter assessments. The stages of the estrous cycle were identified by a vaginal smear and measuring the estrogen levels in collected blood. The proestrus and estrus stages had higher estrogen levels compared with the diestrus and metestrus stages. Whole-cell patch clamp recordings demonstrated that TG neurons in the proestrus and estrus stage had lower AP thresholds, decreased rheobases, enhanced AP heights, shorter falling times of AP and deeper after-hyperpolarization (AHP) depth. Our results revealed that the high level of estrogen in the proestrus and estrus stage alters the AP properties of TG neurons. Estrogen may increase membrane sensitivity and the summation of cellular responses, which alters the AP properties. The alterations of the AP properties in the proestrus and estrus stage are due to a modification of voltage-gated ion channels in TG neurons, which may be a pathogenesis for menstrual migraine. No COI.

ABS0263

Tannic acid inhibits hyperpolarization-activated current (Ih) in DRG neurons

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (Ih) has a key role in controlling the rhythmic activity in cardiac pacemaker cells and spontaneously firing neurons. Tannic acid, which is water soluble polyphenol and is widely distributed in fruits and food, has many biological function and activity such as anti-mutagenic, anti-carcinogenic, anti-oxidant activities, anti-histamine, anti-inflammatory, anti-bacterial, and anti-viral activities. In this study, we examined the effect of tannic acid on Ih in mouse dorsal root ganglia (DRG) neurons using the whole-cell patch clamp technique. Tannic acid (5 µM) irreversibly decreased the amplitude of Ih to 58.9 ± 4.2% in DRG neurons. In addition, tannic acid decreased Ih at all voltage range (-120 ~ -50 mV) in a dose-dependent manner (0.05 ~ 50 µM) and IC50 of tannic acid was 0.9 µM. In conclusion, tannic acid might directly block Ih in DRG neurons in a dose-dependent manner. We suggest that HCN channel could be a molecular target for tannic acid in the modulation of nociceptive information. No COI.
The effect of N-acetyl-L-cysteine on β-amyloid induced Alzheimer disease model in rat: A behavioral and electrophysiological study

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by a decline in cognitive function due to the accumulation of beta-amyloid peptide (Aβ) in extracellular space of neurons. Aβ stimulates oxidative stress and neuronal death. The purpose of this study was to evaluate the protective effect of N-acetyl-L-cysteine (NAC) with its potent antioxidant activity, on learning and memory impairment induced by Aβ induced Alzheimer disease model in adult Wistar male rats. Thirty five rats were divided into five groups including: control, sham-operated, intra-hippocampal receiving Aβ, Aβ +NAC (1-14 days), Aβ +NAC (14-28 days) groups. After the treatments, learning and memory was evaluated by passive avoidance test and hippocampal long term potentiation (LTP). Results indicated intra-hippocampal Aβ injection reduces step-through latency in passive avoidance test and also decreased amplitude of hippocampal population spikes (PS) and slope of excitatory post synaptic potentials (EPSP) in the Aβ group compared to the sham and control groups. Administration of NAC in rats receiving Aβ increased the mentioned variables in compared to the Aβ group. Systemic administration of NAC decline behavioral and electrophysiological symptoms of Aβ induced memory impairment. NAC treatment has more prominent effect than NAC pre-treatment. Part of this effect may due to antioxidant activity and eliminating free radicals by NAC. The results of the study suggest that NAC can be considered for Alzheimer disease treatment. No COI.

The effect of combined administration of estrogen and angiotensin receptor (AT1R) blocker on intracranial pressure after traumatic brain injury in female rats

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The neuroprotective effect of alone administration of estrogen and candesartan (angiotensin receptor blocker) has reported in traumatic brain injury (TBI). In this study we evaluated the effect of combined administration of estrogen and candesartan (AT1R blocker) on intracranial pressure after TBI. Material and Methods: The ovarectomized (OVX) female rats were divided to 7 group as follows: 1- sham, 2-TBI, 3-TBI+ estrogen, 4-TBI+OIL, 5-TBI+estrogen+low dose of candesartan, 6-TBI+estrogen+high dose of candesartan, 7-TBI+OIL+VEH. The diffuse TBI was induced by Marmarou method. Intracranial pressure (ICP) was evaluated in -1, 1, 4, 24 hours after TBI. Results: The ICP was higher in group 2 compared to group 1 in all times. Estrogen decreased ICP compared to TBI in all times. Although the combined administration of estrogen and high dose of candesartan wasn’t effective on ICP in the first hour after TBI, but the effect of estrogen on ICP was inversed by this group at 4 hour after TBI. Conclusion: The results of this study indicated that the combined administration of estrogen and high dose of candesartan after TBI eliminated the neuroprotective effect of estrogen on ICP. No COI.
ABS0274
Epigenetic regulation of Nrf2 transcription in oxidative stress-associated hypertension induced by angiotensin II
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The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a principal player in cellular antioxidant defense mechanisms via regulation of transcription of antioxidants bearing the antioxidant response element (AREs) in their promoter regions. Downregulation of many Nrf2-regulated antioxidant enzymes is known to be involved in the pathogenesis of redox-associated cardiovascular disease, including hypertension. The Nrf2 is subjected to epigenome control. The present study investigated the significance of epigenetic regulation of Nrf2 in angiotensin II (Ang II)-induced hypertension. Exposure with Ang II (0.01, 0.1 or 1 μM) to the N2a cells resulted in a decrease in Nrf2 expression, decreased expressions of the ARE-regulated antioxidants, including peroxisome proliferator-activated receptor gamma (PPARγ), heme oxygenase-1 (HO-1), superoxide dismutase 1 (SOD1), SOD2 and Kelch-like ECH-associated protein 1 (Keap1), increased protein expression of histone deacetylases (HADC) 1-5, an upregulation in trimethyl-histone H3-Lysine 9 and a downregulation of acetylated-histone H3-Lysine 9 expression. Chromatin immunoprecipitation results further showed a reduction in binding affinity of H3K9-Ac to promoter region in the Nrf2 gene. Pyrosequencing for the evaluation of methylation of the CpG islands in promoter regions of Nrf2 gene, however, showed no significant change following Ang II treatment. In normotensive WKY rats, Ang II-induced pressor response was augmented in rats subjected to treatment with Nrf2 siRNA. In contrast, the gene transfer of lentivirus encoding Nrf2 to SHR resulted in a moderate decrease in arterial pressure. Together our results indicate that Ang II-dependent epigenetic downregulation of Nrf2 expression may be involved in the pressor response to Ang II. No COI.

ABS0304
Nrf2 mediated mitochondrial biogenesis deficit in neuron of rostral ventrolateral medulla led to systemic inflammation-associated hypertension
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Neuroinflammation in rostral ventrolateral medulla (RVLM) leading to raised blood pressure has been demonstrated in our sustained systemic inflammatory model. Here, we went further to dissect the connection between neuroinflammation and pressor response. Evolving concept pinpoints that inflammation damages mitochondria, the center of cellular metabolism. Mitochondrial biogenesis plays critical role in maintenance of mitochondrial mass. Here, we demonstrated that deficit of mitochondrial biogenesis at the levels of mitochondrial DNA copy number and mitochondrial transcription factor A (TFAM) in RVLM was critical in triggering pressor response. Preventing the deficit by Coenzyme Q10 effectively reversed the increased blood pressure. Activation of nuclear factor (erythroid-derived 2)-like 2 (phospho-Nrf2; p-Nrf2) accounted for TFAM expression in this model. Both acute and sustained evidence indicated that interleukine-1β (IL-1β) down-regulated the levels of total Nrf2 and of nuclear p-Nrf2. Central blockade of IL-1beta significantly prevented the decrease of nuclear p-Nrf2 and pressor response. In vitro studies of chromatin immunoprecipitation (ChIP) further demonstrated that suppression of p-Nrf2 interaction with tfam promoter turn down neural mitochondrial biogenesis. Together, our studies suggested that IL-1beta accumulation depressed Nrf2-mediated nuclear-mitochondrial interaction leading to deficit of mitochondrial biogenesis in RVLM resulting in neurogenic pressor response under sustained systemic inflammation. No COI.
ABS0314

**Antinociceptive effects of Rhus coriaria L. extract in male rats**

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It is well known that the tendency toward the medicinal plants is increasing in recent years. They have low side-effects and high varieties of efficient components. This study was designed to investigate the analgesic effect of hydro alcoholic leaf extract of Rhus coriaria (HRCLE) in a rat model. For this purpose, 42 adult male rats were divided into 7 groups: control, HRCLE (80, 100 and 300 mg/kg, i.p.), morphine (1 mg/kg, i.p.), aspirin (1 mg/kg, i.p.), and HRCLE 300 mg/kg plus naloxone (1mg/kg, i.p.). The analgesic effects of HRCLE were assessed with writhing, tail flick and formalin tests. The data were compared with control by one-way ANOVA and Tukey post hoc test. All dose levels of HRCLE inhibited the number of contractions induced by acetic acid in the writhing test significantly. None of the dose levels of HRCE have been shown to have antinociceptive activity in the formalin test except the dose of 100 mg/kg (at chronic phase) and the dose of 300 mg/kg (at chronic– acute phase). In the tail flick model, the highest effect was at the dose of 300 mg/kg of HRCLE (P<0.01). Utilization of naloxone plus extract inhibited the antinociceptive effect of HRCLE. In this study, our findings suggest that analgesic effect for the HRCLE may be mediated via both peripheral and central mechanisms. The presence of flavonoids might be responsible for the antinociceptive activity of this plant. No COI.

ABS0318

**Cognitive and neurogenesis deficits in a MPTP-induced Parkinson's disease rat model**

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Previous studies have shown cognitive deficits and cell loss in the hippocampal CA1, striatum, and substantia nigra pars compacta (SNc) in MPTP-induced Parkinson’s disease (PD) rat model, which is similar to that seen in patients with PD. The hippocampus is involved in working memory and recognition. Hippocampal dentate gyrus (DG) shows neurogenesis in adult animals. However, whether MPTP lesion affects neurogenesis and changes in cognition are not clear. The aim of this study was to investigate cognitive function and neurogenesis in MPTP-induced PD rat model. MPTP was stereotaxically injected into the SNc of male Wistar rats. Then, the rats underwent the bar-test, T-maze test, and object recognition test. Immunohistochemistry was used to detect new born (BrdU+) cells in the hippocampal DG. MPTP-lesion rats showed impairments of working memory and recognition. Moreover, neurogenesis in the DG was lower than that in the control group. In conclusion, these data suggest that impairment of neurogenesis in the hippocampus may underlie cognitive deficits in PD. This provides a new view for feature PD research. No COI.
**ABS0319**

**EEG burst activities evoked with sensory stimuli in sevoflurane-anesthetized rats**

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Electroencephalographic (EEG) field potentials of a burst suppression pattern have been reported under deep anesthesia in human beings and animals. The aim of the present study was to clarify physiological properties of EEG burst activities evoked with sensory stimuli in sevoflurane-anesthetized rats. EEG was recorded simultaneously at four loci in the cerebral cortex with two silver ball electrodes arranged anteroposteriorly in each hemisphere or four electrodes positioned anteroposteriorly in the right hemisphere. Somatosensory (electric) stimuli applied to the sole and flash visual stimuli evoked burst activities at all recording sites with a burst shape similar to that of spontaneous bursts. Latencies of evoked bursts decreased as stimulus intensity increased. Burst activity evoked with somatosensory or visual stimuli had shorter latencies in the somatosensory or visual area, respectively, while spontaneous bursts generated simultaneously in all recording sites. Intracortical electrical stimuli delivered to several cortical sites evoked bursts at all recording sites. When the left hemisphere was depressed with cortical spreading depression, electric stimulation of the left sole evoked bursts in both hemispheres. However, stimulation of the right sole did not evoke bursts in any recording sites. Visual stimuli applied to the right and left eyes evoked bursts at all four recording sites during depression. Together with anatomical facts that somatosensory information is sent contralaterally to the cerebral cortex while visual one is sent to both hemispheres (90% contralaterally and 10% ipsilaterally), the present findings suggest that the primary sensory responses may play important role for generation of burst activity induced with sensory stimuli. No COI.

**ABS0322**

**Developmental exposure to diesel engine exhaust origin secondary organic aerosol on social behavior in adult mice**

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Secondary organic aerosol (SOA) is a component of particulate matter (PM) 2.5 and formed in the atmosphere by oxidation of products from volatile organic compounds. Recently, we have reported that inhalation exposure to diesel exhaust origin SOA (DE-SOA) may affect novel object recognition ability and impair maternal behavior using an adult mouse model. However, it has not been cleared whether developmental exposure to SOA would affect social behavior in later life. In the present study, we aimed to investigate the effects of gestational and lactational exposure to DE-SOA on social behavior and related gene expression in mature mice. Male mice were exposed to clean air (control), DE (100 µg/m\(^3\)), DE-SOA (114 µg/m\(^3\)) and gas without particles from gestational day 14 to postnatal day 21 in the inhalation chambers (5 h/day, 5 days/week). Social behaviors were examined at the age of 13 week by a sociability and social novelty preference, social interaction with a juvenile mouse and light dark transition test using behavioral scoring software (ANY-maze, Muromachi Kikai Co., Ltd). Moreover, social behavior-related gene expressions in the hypothalamus were detected by real-time RT-PCR. Sociability, social novelty preference and social interaction were remarkably impaired and mRNA expressions of estrogen receptor-alpha and oxytocin receptor were significantly decreased in mice exposed to DE-SOA during gestational and lactational periods. This is the first study and our results suggest that the constituent(s) of DE-SOA may trigger late-onset neurotoxicity after early life exposure and may affect social behavior and related gene expressions in the hypothalamus of mice. No COI.
**Effect of developmental exposure to acetamiprid on behavioral profiles in mice**

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There is growing concern that certain environmental chemicals interfere with development. Especially, neonicotinoid pesticides have been concerned about their hazardous actions on not only ecosystem but also mammalian neural development. In the present study, we focused on examining the effect of developmental exposure to acetamiprid, a neonicotinoid, on murine behaviors. To carry on the developmental exposure, we administered acetamiprid dissolved in water at doses of 0 mg/kg bw/day (Control group), 1 mg/kg bw/day (Low dose group) or 10 mg/kg bw/day (High dose group) by oral gavage to dams from gestational day 6 to postnatal day 21 and performed behavioral tests of offspring in adulthood. We found that the exposure at low dose affected male sexual and aggressive behaviors while the exposure at high dose did not alter them. The exposure at both low and high doses prolongs the time spent in light place in light-dark transition test in males, suggesting the possibility of reduction in anxiety under stressed condition. On the other hand, no impairment in behavioral flexibility was found in both sexes developmentally exposed to acetamiprid. Our results suggest that acetamiprid affects socio-sexual and emotional behaviors in a male-specific manner. To know the causes of these behavioral alterations, further experiments examining the mechanism how acetamiprid impairs brain function are required. No COI.

**Role of parabrachial monosynaptic inputs in the central amygdala nucleus network**

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The central nucleus of the amygdala (CeA) is a key structure underlying nociception-induced emotional responses. Nociceptive information is conveyed from the superficial layer of the dorsal horn to the capsular part of the CeA (CeC) via the lateral parabrachial nucleus (LPB). It has been demonstrated that LPB-CeC synaptic transmission is enhanced in various pain models by using electrical stimulation of the fibers arising from the LPB in brain slices. However, it is difficult to selectively stimulate monosynaptically projecting fibers without activating local neurons and passing fibers. To overcome this limitation and elucidate the role of LPB inputs in CeA network activities, we transfected AAV vector for channelrodopsin (ChR2) expression to the LPB in rats and prepared brain slices containing the amygdala 5-7 weeks after transfection. We found that blue light illumination on the CeC, but not the surrounding areas, resulted in monosynaptic EPSCs with very small latency fluctuation in CeC neurons regardless of the firing pattern type. These EPSCs were followed by large polysynaptic IPSCs. This feedforward inhibition rapidly brought the membrane potential back to the resting state level after depolarization. Moreover, intraplantar formalin injection made 24 hours before the slice preparation resulted in a significantly larger EPSC amplitude than those with saline injection only in the CeC neurons showing late-firing pattern. These results indicate that direct monosynaptic inputs from the LPB not only excite the CeC neurons but also regulate the CeA network excitability through robust feedforward inhibition, which is under plastic modulation in response to persistent inflammatory pain. YKS is a JSPS Research Fellow. No COI.
ABS0341
Area MT neurons at the population level represent binocular disparity in a manner between correlation-based and match-based representation
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Binocular disparity is a cue for stereopsis. Psychophysical evidence suggests that the visual system represents binocular disparity based on both binocular-correlation and binocular-match signals. We previously showed that area V4 employs a match-based representation of disparity in responses pooled across neurons. As a step towards identifying the brain areas that represent disparity with binocular-correlation signals, we examined the disparity representation of MT, a counterpart of V4 in the dorsal visual pathway. We recorded single-unit activity of MT neurons from a monkey performing a fixation task and analyzed the pooled-average of the resulting tuning curves. We then manipulated the level of binocular correlation in random-dot stereograms (RDSs) by reversing the luminance contrast of a varying proportion of dots in one eye (graded anti-correlation). The amplitude of pooled disparity-tuning curve gradually decreased as the level of correlation was decreased from 100% (normal RDSs) to 0% (RDSs with half of the dots contrast-reversed). At 0% correlation, the tuning curve became completely flat. With further decrease from 0% to ~100% (anti-correlated RDSs), response modulation by disparity reappeared, gradually grew, and exhibited an inverted tuning-curve shape. The tuning amplitude at ~100% correlation was approximately 40% of the amplitude at 100% correlation. These changes in disparity tuning functions fall between correlation-based and match-based representation of binocular disparity. MT thus represents disparity in a more correlation-based manner than V4, whose representation is completely match-based at the population level. No COI.

ABS0344
Evaluation of spatial resolution in “in-vivo” two-photon microscopy by fluorescent microbeads injected into living mouse brain cortex
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Observation of dendritic spines in living brain is usually performed by in vivo two-photon microscopy, because of its high spatial resolution and deep imaging capability. Previously, we found that penetration depth could be improved by changing the diameter of the irradiation excitation laser beam. However, how the diameter affected the spatial resolution was not quantitatively examined, because the focal spot size of the excitation light was not measured precisely. The resolution of a laser scanning microscope is in principle correlated with the focal spot size that is determined by optical conditions. The resolution often deteriorates owing to several aberrations caused by refractive index mismatches between the immersion liquid and the specimen. In this study, we estimated the resolutions by measuring FWHM from single-bead images that injected into the cortex, and then examined how the resolutions depended on the laser diameter and refractive index of the immersion liquid. The results showed that FWHM on narrower beam diameter was larger than that on full-filled condition. However, degradation of resolution was not remarkable for imaging of cortical neurons. Furthermore, to increase the refractive index of the immersion liquid, we achieved higher resolution at deeper regions of living mouse brain. Thus, adjustment of the observation conditions to match the optical properties of the brain improves resolution without requiring the use of a special device. We hope that this technique will be applicable to investigations of various neural functions, including the morphological changes undergone by neurons during physiological phenomena. No COI.
Non-selective NSAIDs do not affect long term synaptic plasticity but induce memory impairment

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The effect of Non-steroidal anti-inflammatory drugs on different processes such as inflammation and pain is mediated by inhibiting cyclooxygenase (COX). There is evidence that prostaglandin-E2, a COX product, plays a critical role in synaptic plasticity, a cellular mechanism underlying learning and memory. In the present study the effects of non-selective NSAIDs (aspirin and sodium salicylate) on memory and synaptic plasticity in the hippocampus were examined. Methods: Spatial memory and long term potentiation (LTP) were assessed by water maze performance and field potential recording, respectively. Field excitatory post synaptic potentials (fEPSP) were recorded from CA1 following Schaffer collateral stimulation. Sodium salicylate (SS) was administered with high dose (300 mg/kg, twice-daily, i.p.) for three consecutive days prior to the tests. In other group, rats received aspirin (2 mg/ml) in drinking water for 6 weeks. Results: Aspirin-treated rats showed a slower rate of memory acquisition (Two Way ANOVA; p<0.001). Further analysis disclosed a significant increase in escape latency and swimming distance on the first day in animals treated with aspirin but not with SS. In probe test, rats treated with both drugs spent less time (unpaired t-test; P<0.01) in target quadrant zone. Neither aspirin nor SS has effect on LTP induction. They could not suppress LTP induction when compared with control animals (p>0.05, unpaired t-test). Conclusion: In summary, the present results revealed that although in vivo aspirin and salicylate did slightly impair spatial memory in the Morris water maze task, they do not affect synaptic plasticity. No COI.

The effect of GABA on serotonergic neuron CGC modulated by taste aversive conditioning in pond snail

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Gamma-aminobutyric acid (GABA) is well known as neurotransmitter for various animals, including the pond snail Lymnaea stagnalis. The pond snail can acquire a long-term memory of taste aversive conditioning learning. Central giant cell (CGC) in the pond snail plays key role in the feeding system of the CNS, and exhibits spontaneous oscillation of action potentials. Previous studies have shown that CGC in taste aversion-trained snails regulated feeding motor neurons. In this study, we investigated the effect of GABA on activity of CGC in taste aversion-trained snail. We performed taste aversive conditioning with sucrose as the conditional stimulus and potassium chloride as the unconditional stimulus. From the result of memory test 24 h after the training, we decided whether the snail was “good” learner or “poor” learner. We performed intracellular recording of CGC in the isolated CNS to reveal the characteristic of the oscillatory activity in the trained snail. In naïve snails (non-trained snails), the spontaneous firing rate of CGC was 30.9 ± 6.5 spikes/min. The CGC firing rate of good learner decreased to 8.3 ± 3.0 spikes/min, while that of poor learner did not appear to decrease significantly (21.3 ± 1.9 spikes/min). Application of GABA to the isolated CNS induced a significant decrease of firing rate in both “good” learner by 7% and “poor” learner by 56%. In naïve snails, application of GABA did not change the firing rate of CGC. These results indicate that taste aversive conditioning can produce the change of GABAergic-like neurons to connect to CGC. No COI.
ABS0356

The effect of individual and group housing on voluntary exercise and brain monoamine levels in laboratory rats

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Voluntary wheel running with living alone has been widely used as a common physical exercise model to enhance physiological and psychological health in rodents. However, numerous studies have suggested that individual housing, which is an unusual condition for rodents, influences behavioral and physiological aspects. Thus, we cannot rule out a possibility of negative influences of living alone on health effects of exercise. In this study, we investigated the effect of housing conditions on individual wheel running activity in laboratory rats using radio frequency identification technology. Additionally, we assessed the effect of the voluntary exercise on psychological aspect by measuring brain monoamine levels. Male Wistar rats were implanted with electronic identification devices “microchips” subcutaneously. Animals were housed either individual or group housing conditions with running wheel for 4 weeks. Each cage was equipped to monitor an individual animal’s access to running wheel using microchip-scale system. Daily wheel revolutions in each cage were recorded digitally from counters attached to the running wheel, and individual running distance estimated to be calculated by multiplying wheel circumference by the number of revolutions based sequential data of individual access behavior. The result from our original calculation showed no significant difference in average daily running distance between individual and group housing conditions. In the several brain regions, dopamine and serotonin levels were different between housing conditions. Taken together with previous reports that voluntary exercise alters brain monoamine levels, these results suggest the possibility of synergistic effect between housing conditions and voluntary exercise. No COI.

ABS0358

A sex difference in green fluorescent protein expression under the control of the estrogen receptor-α promoter in the hypothalamus of mice

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Estradiol, which originates from testicular testosterone and binds to estrogen receptor-α (ERα) during the perinatal period, is necessary for organization of the male-type brain in mice. In this study, we examined transgenic mice, in which ERα-expressing cells of the brain can be visualized by green fluorescence protein (GFP) under the control of the ERα promoter, to determine whether the transgenic mice are useful for the study of the sexual differentiation of the brain. Fluorescence microscopy of the brain sections obtained from adult transgenic mice showed the existence of many GFP-expressing cells in the medial preoptic area, medial preoptic nucleus, bed nucleus of the stria terminalis (BNST), striohypothalamic nucleus (StHy), and anterior hypothalamic area in both sexes. Most GFP-expressing cells in the hypothalamic brain expressed neuronal nuclear antigen, a neuronal marker. Analysis of GFP-expression area in adult female transgenic mice was decreased by postnatal treatment with testosterone or estradiol. These findings indicate that a sex difference in ERα-expressing neurons of the hypothalamus can be visualized by GFP. The transgenic mice may be useful for the analysis of the sexual differentiation of the brain. No COI.
Effect of Rosa damacena mill hydroalcoholic extract on passive avoidance learning and memory in high fat diet adult rats

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The high fat diet can threat brain function by reactive oxygen species generation and causes memory defects. This study was to investigate the effect of hydroalcoholic extract of Rosa Damascena Mill on learning and memory in male rats that received high fat diet. Forty male Wistar rats were used and received high fat diet for 3 months and hydroalcoholic extract of Rosa (1000 mg/kg) for 1 month. At the end of the study, the shuttle box apparatus for assessing passive avoidance learning and Morris water maze were used for spatial memory measurement. The groups included: high fat diet, high fat diet plus extract, extract, control. We measured trials, step-through latency in the acquisition and in the retention test, time spent into the dark compartment. Results showed that high fat diet and extract groups significantly increased time spent into the dark compartment compared to control group. Extract group significantly decreased step-through latency in the retention test compared to the control group. There was no significant difference between trials and step-through latency in the acquisition in groups. Results suggest that hydroalcoholic extract of Rosa in extract group lead to memory deficit because Rosa extract eliminate a lot of reactive oxygen species that can be important in memory cell signaling. On the other hand, high fat diet causes reactive oxygen species generation and lead to memory deficit. In this study, high fat diet plus extract had no useful effect on memory probably due to the high dose of extract. No COI.

Disruption of balance between excitation and inhibition in the primary somatosensory cortex contributes to chronic pain

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Neurons in the primary somatosensory cortex (S1) receive nociceptive information from peripheral nerve and code location, intensity, and duration of pain. Under chronic pain conditions, brain activities of S1 increase and recent study demonstrates that S1 has critical roles in chronic pain. Under chronic pain conditions, S1 excitatory neuronal activities are enhanced and these hyperactivities contribute to chronic pain. Inhibitory GABAergic neurons in the S1 project to S1 excitatory neurons and can attenuate excessive excitation of excitatory neurons. However, little is known about how inhibitory neurons in the S1 modulate excitatory neuronal activities and pain behavior under chronic pain conditions. Using two-photon calcium imaging and electrophysiological methods, we found that inhibitory neuronal activities increased in S1 in inflammatory chronic pain. Local application of a GABAA receptor blocker further enhanced excitatory neuronal activities in S1 and pain behavior in chronic pain. This suggests that enhanced inhibitory neuronal activities in S1 contribute to reduce exaggerated cortical excitatory neuronal activities and pain behavior. However, reduction of K-Cl cotransporter expression in S1 excitatory neurons resulted in inhibition being less efficacious. Thus, although there is a net increase in inhibition within S1 cortical circuit, it is not enough to balance the enhanced excitatory neuronal activities and prevent chronic pain behavior. No COI.
ABS0383
Male-biased sexually dimorphic nuclei found in the brain of Suncus murinicus
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The brain of mammals contains sexually dimorphic nuclei (SDNs) that underlie sex-biased physiological functions. In mice (Mus musculus) belong to Rodentia, the calbindin-sexually dimorphic nucleus (Calb-SDN) of the preoptic area and the principal nucleus of the bed nucleus of the stria terminalis (BNSTp) are SDNs that abundantly express calbindin, a calcium-binding protein. Both the SDNs exhibit male-biased sex differences in the volume and neuron number. Suncus (Suncus murinicus), which belongs to Insectivore, is the useful model for physiological study. However, the existence of SDNs in suncus is largely unknown. This study was aimed to determine the existence of SDNs in suncus. Coronal brain sections obtained from adult suncus of both sexes were subjected to Nissl-staining and calbindin-immunohistochemistry. In the results of histological analysis, we found a cluster of calbindin-immunoreactive cells in the preoptic area. This cluster of males was larger and contained more calbindin-immunoreactive cells compared to females, suggesting that this is a homologue of the Calb-SDN. The BNSTp of suncus showed male-biased sex difference in the volume and Nissl-stained neurons, although few calbindin-immunoreactive cells were in the BNSTp of suncus. These results indicate that male-biased SDNs that may be homologues of the Calb-SDN and BNSTp of mice exist in suncus, although there may be a species difference in the BNSTp. No COI.

ABS0384
Analgesic effects of Bee venom derived phospholipase A2 in a mouse model of oxaliplatin-induced neuropathic pain
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Oxaliplatin, a chemotherapy drug used to treat colorectal cancer, induces specific sensory neurotoxicity signs that are aggravated by cold/mechanical stimuli. Bee Venom (BV) has been used in Korea to treat various pain symptoms. Our previous study demonstrated that BV alleviates oxaliplatin-induced cold allodynia in rats, via noradrenergic and serotonergic analgesic pathways. Here we examined the analgesic effect of BV derived phospholipase A2 in a mouse model of oxaliplatin-induced neuropathic pain. The behavioral signs of cold and mechanical allodynia were evaluated by acetone and von Frey hair test on the hind paw, respectively. The significant allodynia signs were observed from one day after an oxaliplatin injection (6 mg/kg, i.p.). Daily administration of bvPLA2 (0.2 mg/kg, i.p.) for five days markedly attenuated cold and mechanical allodynia, which was more potent than BV effect (1 mg/kg, i.p.). The depletion of noradrenaline by an injection of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP4, 50 mg/kg, i.p.) blocked the analgesic effect of bvPLA2, whereas the depletion of serotonin by injecting DL-p-chlorophenylalanine (PCPA, 150 mg/kg, i.p.) for three days did not. Furthermore, idazoxan (α2-adrenergic antagonist, 1 mg/kg, i.p.) completely blocked bvPLA2-induced anti-allodynic action, whereas prazosin (α1-adrenergic antagonist, 10 mg/kg, i.p.) did not. These results suggest that bvPLA2 strongly alleviates oxaliplatin-induced cold and mechanical allodynia in mice through the activation of α2-adrenergic receptors. This work was supported by a grant of Korea Health Technology R&D Project through KHIDI funded by Ministry of Health & Welfare (HI14C0738) and a grant of National Research Foundation funded by Korea government (NRF-2013R1A1A1012403).
CEF recovers neuronal density and activity changes in an MPTP-induced Parkinson’s disease rat model: an MEMRI study

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CEF, a beta-lactam antibiotic, has been shown neuroprotective effect in a Parkinson’s disease (PD) rat model. We measured neurohistology and neuronal activity changes after treatment with CEF (100 mg/kg/day, i.p., for 14 days) in a PD rat model that was induced by microinjection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the substantia nigra pars compacta (SNc). On day 13 after MPTP lesioning, the rats received injection with MnCl2 (20 mg/kg/day, i.p.). On day 14, rats were scanned with manganese-enhanced magnetic resonance imaging (MEMRI) for detecting brain activity. The brain was taken for histological assay on day 15. Densities of dopaminergic neurons and terminals, stained by tyrosine hydroxylase, in the SNc and striatum, respectively, were decreased in PD rats. Cell loss, determined by Nissl stain, was observed in the areas of hippocampal CA1, CA3, and dentate gyrus. However, hyperactivity was found in the subthalamic nucleus. Neuronal activity, measured by MEMRI, showed the same changing profile. All the above neuronal alterations were recovered to control level by the treatment with CEF. More interestingly, CEF enhanced neurogenesis in the DG of hippocampus. We suggested that CEF may be a potential treatment for PD. No COI.

Visualization of spontaneous brain activity in chronic pain model with manganese-enhanced magnetic resonance imaging (MEMRI)

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Persistent activation of brain regions involved in emotion and self-consciousness is the signature of the chronic pain in human patients (Apkarian et al., 2013). To understand the neuronal process for development of chronic pain, we employed manganese-enhanced MRI (MEMRI), a brain imaging technique based on visualization of activity-dependent accumulation of Mn2+ (Aoki et al., 2004) to follow the changes in MEMRI during the chronification process after formalin-induced inflammation. MnCl2 was injected intravenously to mice and formalin solution or saline was administered into the left hind paw at 2, 6 or 24 h before MRI acquisition with ultra-high field scanner under anesthesia at 23-24 h after MnCl2 injection. Two distinct types of methods, region of interest- (ROI-) and voxel-based analyses were performed to evaluate changes after formalin injection. The ROI-based analysis revealed an early activation of the right CeA (6 h) and latent activation of the bilateral CeA, left BLA and right dentate gyrus (24 h). The voxel-based analysis revealed wide-spread brain activities in addition to the amygdala in 6- and 24-h inflammation groups. MEMRI combined with ultra-high field scanner is powerful and useful in identifying wide-spread brain areas spontaneously activated in the course of pain chronification in animal model of inflammatory pain. Identification of neural networks and molecular basis underlying sequential activation of these brain areas would provide new targets of pain neuroscience. No COI.
ABS0410

**Basal ganglia and cerebellar control of thalamocortical activity**

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Both the basal ganglia and cerebellum receive inputs from the cerebral cortices and project back to the original cortices via the thalamus, and control voluntary movements. To understand how these structures modulate thalamocortical activity, we recorded thalamic neurons projecting to the motor cortices of macaque monkeys under awake states. We identified thalamocortical neurons by antidromic responses to motor cortical stimulation, and then examined responses to stimulation of the cerebellar nucleus (CN) and the internal segment of the globus pallidus (GPi), major output nuclei of the cerebellum and basal ganglia, respectively. Thalamocortical neurons with CN inputs were found in the posterior part of the motor thalamus, while those with GPi inputs were located in the anterior part, and only a few neurons responded to both CN and GPi stimulation. During repetitive CN stimulation at 50 or 100 Hz, a train of biphasic responses composed of short-latency brief excitation and following inhibition was observed corresponding to each stimulus pulse. On the other hand, each stimulus pulse evoked short-latency inhibition and following firings during repetitive GPi stimulation at 50 or 100 Hz. Local injection of GABA-A receptor antagonist abolished both the inhibition and following firings induced by GPi stimulation without significant changes in spontaneous activity. Thus, the following firings can be considered as postinhibitory rebound excitation. These results suggest that basal ganglia and cerebellum control thalamocortical activity in different manner: Cerebellar outputs convey information through excitation immediately followed by inhibition, whereas basal ganglia outputs convey information through inhibition with rebound excitation. No COI.

ABS0412

**Functions of the cortico-subthalamic hyperdirect pathway investigated by a photodynamic technique**

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The subthalamic nucleus (STN) receives direct cortical inputs and projects to the substantia nigra pars reticulata (SNr), the output station of the basal ganglia (BG), and thus forms the cortico-STN-SNr hyperdirect pathway. We observed BG activity and motor behaviors in mice before and after the selective elimination of the cortico-STN projection by a photodynamic technique. We injected retrogradely transportable microspheres conjugated with chlorin e6 into the STN. Then, we irradiated the motor cortex with a near-infrared laser to activate chlorin e6 and selectively induce apoptosis in the labeled cortico-STN neurons. We recorded the activity of globus pallidus (GP) and SNr neurons to stimulation of the forelimb motor cortex in awake mice by constructing peri-stimulus time histograms. Before the elimination, a triphasic response composed of early excitation, followed by inhibition and late excitation was the most common. After the elimination, early excitation diminished in both GP and SNr neurons. We also examined locomotor activity of mice before and after the bilateral cortico-STN elimination. The locomotor activity gradually increased after one week from the laser irradiation and became significantly higher than that of control mice in 2–4 weeks. These results suggest that the hyperdirect pathway conveys fast excitatory signals from the motor cortex to the SNr, inhibits the thalamic and cortical activity and suppresses motor behaviors. No COI.
ANGIOIENSIN II facilitates GABAergic neurotransmission at postsynaptic sites in rat central amygdala neurons
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The central nucleus of the amygdala (CeA) in the forebrain is a critical brain site for the regulation of sodium appetite. Angiotensin II type-1 (AT1) receptors and angiotensin II (ANG II) terminals are present in the CeA. However, there is no clear evidence for the role of ANG II in the CeA. In this study, we determined the effect of ANG II on the excitatory and inhibitory synaptic inputs to the CeA neurons. Whole-cell patch-clamp recordings were performed on CeA neurons in brain slices. Application of 0.1–5 μM ANG II significantly potentiated the amplitude of spontaneous GABAergic inhibitory postsynaptic currents (IPSCs) in a concentration-dependent manner. Also, 2 μM ANG II increased the amplitude of miniature IPSCs from 22.86 ± 2.54 to 29.45 ± 3.32 pA (P < 0.05, n=11), without affecting the frequency. The effect of ANG II on miniature IPSCs was blocked by the AT1 receptor antagonist losartan, but not by the AT2 receptor antagonist PD123319. Furthermore, inclusion of a G protein inhibitor, GDP-β-s, in the pipette internal solution attenuated the facilitatory effect of ANG II on GABAergic transmission. By contrast, ANG II had no effect on the spontaneous glutamatergic excitatory postsynaptic currents (EPSCs) and did not alter the frequency and amplitude of miniature EPSCs at concentrations that facilitated IPSCs. Thus, this study provides substantial new evidence that ANG II facilitates GABAergic synaptic inputs through activation of postsynaptic AT1 receptors in the CeA. No COI.

Effect of valproate sodium on electrical activity of Helix aspersa F1 neuron in a pentylenetetrazol-induced epileptic model using intracellular recording system
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Epilepsy is a common disorder of the brain and valproate sodium (VPA) is one of the most important drugs in this regard. Paroxysmal depolarization shift (PDS) is the most important physiological properties of epileptiform activity. In this study, the effect of VPA in epileptic and non-epileptic conditions on PDS and action potential (AP) parameters were assessed. Intracellular recordings were made under current clamp condition and the effect of VPA on epileptic and non-epileptic conditions were assessed. The results demonstrated that valproate sodium application caused suppression of PDS-induced PTZ application. Valproate sodium decreased firing frequency of action potential significantly and increased afterhyperpolarization (AHP) amplitude and resting membrane potential and prevented depolarization of resting membrane potential (RMP) induced by PTZ. Pre-exposure with VPA did not have similar influences. Based on our results, VPA could not prevent complications due PTZ and may be able to exert curative effects against induction of epileptiform activity. No COI.
Complexity of autonomic modulation and neurovascular oxidative stress during hypertensive response in acute stroke

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Stroke is closely related with vascular oxidative stress in atherosclerosis. During acute large artery ischemic stroke, variations of high blood pressure and heart rate have been reported. In this study, we investigated autonomic control and neurovascular oxidative stress during hypertensive response in large artery ischemic stroke. Sixty five patients were studied as follows local ethic committee (MTU-EC-IM-018154). Three groups of systolic blood pressure (SBP) were classified as follows: Gr I, SBP< 139 mmHg (n, 23 aged 59.45 ± 2.34); Gr II, SBP =140-159 mmHg (n, 21 aged 63.09 ± 2.79); and Gr III, SBP > 160 mmHg (n, 21 aged 62.38 ± 2.86). Plasma nitric oxide (NO) and hydrogen peroxide (H₂O₂), neurovascular oxidative stress marker, were recorded by electrochemistry technique. Autonomic control was assessed by Lead II ECG - short term heart rate variability (HRV) using Kubios Program. Carotid stenosis by means of flow and intima/media thickness (IMT) ratio was assessed using Doppler ultrasound. Gr III had greater IMT ratio than in Gr I and Gr II and differed significantly. Profound lower NO and higher (H₂O₂) were also presented. In all groups by SD2, SD1 Poincare plot, sympathetic drive gradually increased whereas parasympathetic activity decreased dramatically. Significant correlation of SBP and SD2/SD1 was evident (r = 0.81). Surprisingly, less scatter plot and fractal scaling exponent by α1/α2 were shown in Gr III. These findings indicate that reorganized complexity of autonomic modulation plays a vital role in hypertensive response. No COI.

The effects of paclitaxel in regard to NaCl and sucrose preference in rats

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The alteration in taste (dysgeusia) is a common and severe side effect in approximately 60% of cancer patients undergoing chemotherapy. However, its symptoms remain unclear. Dysgeusia causes malnutrition in a majority of the patients and affects their quality of life. However, research on this problem is lacking. The aim of the present experiments was to investigate the effects of paclitaxel (Taxol®) on voluntary NaCl intake using twenty-eight rats and a two bottle choice. For this purpose, the rats were presented with a NaCl solution (0.1 M) and water, and their consumption was measured daily. The rats received two cycles of paclitaxel chemotherapy. The cycles were repeated at 14-day intervals. Paclitaxel was administered for five consecutive days in rats. We did not observe any effects of paclitaxel on NaCl intake and preference such as the effect on sucrose. We have reported that paclitaxel decreased sucrose intake and preference in a few days after its administration and sucrose preference improved within several days after we stopped administering it. These results suggest that paclitaxel alters sucrose, but not NaCl, preference and there are different side effects of paclitaxel on the preference of NaCl and sucrose in rats. These findings indicate that paclitaxel modifies the specific taste function. No COI.
ABS0439
Opt-fMRI imaging of somatosensory cortical activity using transgenic rat which expresses channelrhodopsin-2 in the peripheral mechanoreceptive neurons
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The rodent whisker-barrel cortical system has been a model to reveal somatosensory representation in the brain. Optogenetics would facilitate this with high spatiotemporal resolutions. Recently, we have identified the expression of ChR2 in the mechanoreceptive neurons in the trigeminal ganglion (TG) in one of thy1.2-channelrhodopsin 2 (ChR2)-Venus transgenic rat lines, W-TChR2V4 (Honjo et al., 2014). Each whisker follicles were also richly innervated by the ChR2-positive nerve endings. The whiskers of a ChR2-expressing rat were attached with plastic optic fibers in array of 4x4 in the awake state. Another end of the optic fiber was connected to blue LED, individual of which was turned on and off by a computer-generated pulse. Therefore, the whiskers could be stimulated with various spatiotemporal patterns (optogenetic tactile pattern, OTP). The functional magnetic resonance imaging (fMRI) responses of barrel cortex were investigated using OTP under 7T-MRI system. The whisker irradiation induced a change of blood oxygenation level-dependent (BOLD) responses in the barrel field of contralateral somatosensory cortex in a manner dependent on time. The response to a single whisker irradiation was more regional than the simultaneous 16-whiskers irradiation. It is suggested that the OTP was accompanied with specific spatiotemporal changes of BOLD response. Our OTP, in combination with fMRI, would facilitate to study how the spatiotemporal pattern of the whisker mechanoreception would be represented in the cortex. All animal procedures were conducted in accordance with the guiding principles of Physiological Society of Japan and NIH. No COI.

ABS0440
Aldosterone induces rapid sodium intake by a nongenomic mechanism in the nucleus tractus solitaries
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The purpose of this study was to determine whether aldosterone has a rapid action in the nucleus tractus solitarius (NTS) that increases sodium intake, and to examine whether this effect of aldosterone, if present, is mediated by G protein-coupled estrogen receptor (GPER). Adult male Sprague-Dawley rats (250-300 g) with a stainless-steel cannula in the fourth ventricular (4th V) were used. Aldosterone was injected into the 4th V at the doses of 5, 50, 100, and 200 ng 0.5 μl⁻¹. A rapid dose-related increase of 0.3 M NaCl intake was induced within 30 min and this increase was not suppressed by the mineralocorticoid receptor (MR) antagonist eplerenone (100 ng 0.5 μl⁻¹). Water intake was not affected by aldosterone. The G protein-coupled estrogen receptor (GPER) agonist G-1 (50 ng 0.5 μl⁻¹) produced a parallel and significant increase in sodium intake, while pre-treatment with GPER antagonist G15 (100 ng 0.5 μl⁻¹) blocked the G-1 or aldosterone-induced rapid sodium intake. In addition, sodium intake induced by sodium depletion or low-sodium diet fell within 30 min after injection into the 4th V of the MR antagonist eplerenone, while G15 had no effect. Our results confirm previous reports, and support the hypothesis that aldosterone evokes rapid sodium intake through a non-genomic mechanism involving GPER in NTS. No COI.
**ABS0452**

**Blockade of Smad4 SUMOylation impairs spatial learning and memory in rats**

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We have previously found that the protein inhibitor of activated STAT1 (pias1) gene is differentially expressed between fast learners and slow learners from the water maze learning task with fast learners showing a higher expression level of pias1 in hippocampal neurons. Spatial training also increases PIAS1 expression in rat CA1 area. Further, overexpression of PIAS1 in CA1 neurons enhances whereas knockdown of PIAS1 impairs spatial learning and memory performance in rats. Because PIAS1 is a transcriptional regulator that possesses small ubiquitin-like modifier (SUMO) E3 ligase activity, in this study, we aimed to examine the mechanism underlying PIAS1-mediated learning and memory facilitation. Smad4 is a downstream target of transforming growth factor-beta (TGF-beta) signaling. Smad4 has been well studied in the immune system, but its role in the brain has been rarely examined. In this study we examined whether Smad4 could be SUMO-modified by PIAS1 in the brain and whether Smad4 SUMOylation plays a role in spatial learning and memory formation. Our results showed that Smad4 could be SUMO-modified by PIAS1 at Lys-113 and Lys-159 both in cell lines and in rat hippocampus in vivo. Further, water maze training increased the level of Smad4 SUMOylation in CA1 neurons compared with the swim controls. Smad4 SUMOylation is also induced by neuronal activation. On the other hand, transduction of the lenti-Smad4 WT vector did not apparently affect spatial learning and memory, but transduction of the lenti-Smad4 sumo-mutant vector (Smad4K113R K159R) significantly impaired spatial learning and memory performance. In future studies, we will identify the downstream genes that are regulated by Smad4 SUMOylation and are involved in spatial learning and memory formation. No COI.

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**ABS0459**

**Evaluation of adopted weight drop device to induce contusive spinal cord injury in rats: behavioral and histopathological studies**

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Recently, many experimental devices have been designed to construct standardized animal spinal cord injury (SCI) models, because electromagnetic SCI devices are expensive. To evaluate adopted weight drop device inducing contusive SCI, the fixed weight was used and dropped down from varied heights, then followed by behavioral and histopathological studies. Fifteen adult male Sprague Dawley rats were divided into laminectomy (L), moderate injury (MI) and severe injury (SI) groups. The C5 hemicontusion injury was performed and resulted in the right side hemiplegia and forepaw deficits. Both MI and SI rats showed the clubbing forepaw at 24 h after injury. The skilled locomotion using the horizontal ladder test was analyzed. The SI showed a significant increase in error scores, percentage of total rungs used and decrease in percentage of correct placement when compared to L group, p<0.05. The normal recovered placement (type II) was shown at day 7 after injury but higher numbers in MI than SI group. The somatosensory function using sticker removal test was also analyzed. The SI group showed a significant somatosensory deficit at day 3, 7 when compared to L group, p<0.05. Behavioral deficits were related to histopathological study using H&E counterstained with luxol fast blue staining, the higher degree of injury, the larger area of lesion. The lesion was mostly in lateral funiculus related to rubrospinal and lateral corticospinal tract involving skilled movement of forepaw. Results indicate that this more suitable rat SCI model should be simple, reliable and induced as moderate injury for allowing significant recovery of function. No COI.
Delta X, a new synthetic compound that promotes adult neurogenesis
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There are currently two known pools of cells in the adult brain that can continuously generate new neurons. One of these pools of cells is located in the subgranular zone (SGZ) of the hippocampus. Here we report a new synthetic compound “Delta X” that is capable of promoting proliferation of neural progenitor cells (NPC) from SGZ of the hippocampus in vivo. Previously, we have demonstrated that Delta X can promote the proliferation of adult rat hippocampus derived neural progenitor cells in primary culture. The average number of neurospheres in the Delta X treated group was 2.3 fold higher than that in the control group. The observed effect of Delta X treatment on cultured cells is dose-dependent and this information was used to identify a working concentration for in vivo treatment. In a rat model, adrenalectomy (ADX, removal of adrenal glands) and the resulting corticosterone depletion lead to a dramatic decrease in neuron number in the dentate gyrus of the hippocampus. Behavior deficits of ADX animals are associated with the neuron loss. In this study, the ADX model were used to determine whether Delta X can promote neurogenesis in vivo, repopulate the neuron population in the dentate gyrus, and lead to functional recovery after hippocampal damage. After one week of Delta X treatment, an increase in the number of new born neural progenitor cells (BrdU and nestin double positive cells) were observed in the ADX animals. The number of new born immature neurons (BrdU and double cortin positive cells) was also increased. At the concentration of Delta X used, the population of other cell types was not affected. Thus, Delta X’s potential for promoting endogenous neurogenesis, may have applications in repairing brain circuitry. No COI.

Painful diabetic neuropathy with streptozotocin involves plastic changes in ascending and descending pain pathways
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The number of patients with diabetes mellitus (DM) is globally increasing. Of various complications of DM, painful diabetic neuropathy (PDN) is one of the serious clinical concerns because it significantly lowers the quality of life. It is generally acknowledged that the nerve damage by hyperglycemia results in neuropathic pain. However, recent advances in the understanding of the pain-induced plasticity in the central nervous system suggest that altered central pain processing through ascending and descending pain systems would also underlie chronification and complication of pain. We challenged this by two distinct approaches using streptozotocin- (STZ-) induced PDN modes. First, we examined whether the noradrenergic system in the spinal cord is affected in STZ-treated rats because it has been shown that serotonin norepinephrine reuptake inhibitors mitigate PDN symptoms. The mechanical allodynia and thermal hyperalgesia in the PDN were significantly attenuated by duloxetine. This effect was abolished by pre-treatment with N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), which drastically eliminated nerve endings with dopamine-beta-hydroxylase in the lumbar dorsal horn. Second, we examined whether the synaptic transmission between the fibers arising from the lateral parabrachial nucleus (LPB) and the neurons in the "nociceptive" amygdala (capsular part of the central amygdala) and found that this synaptic transmission was significantly potentiated in STZ-treated mice. It is concluded that STZ and resulting hyperglycemia alters the activity of central networks regulating both ascending and descending pain pathways, which should further exacerbate the PDN-related symptoms. COI=Shionogi.
Antidepressant-like effect of bergamot oil in rats subjected to chronic restrained stress
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Depressive disorder is one of the most common psychiatric diseases and associated with a prolonged period of exposure to stressors. Bergamot essential oil (BEO) has been traditionally recommended to reduce depression however there are limited data supporting these properties. An aim was to investigate the effect of BEO, compared with fluoxetine, a well-known antidepressant, in chronic restraint rats on antidepressant-like behaviour. The hypothalamic pituitary adrenal (HPA) axis response and brain-derived neurotrophic factor (BDNF) protein levels in hippocampus were also determined. Rats were subjected to immobilization stress 15 min daily for 2 weeks. For the next 2 weeks, these rats were divided into 4 groups, control-i.p., fluoxetine-i.p., control-inhale and BEO-inhale. Fluoxetine (10 mg/kg i.p.) or saline was intraperitoneally administered daily while 2.5% BEO or saline was inhaled daily. Following the treatment, depressive-like behavior in treated rats was investigated using the forced swimming test (FST). Then, the rats were immediately decapitated and trunk blood samples were collected for the measurement of corticosterone and adrenocorticotropic hormone (ACTH) level. Hippocampus was dissected and stored in a freezer until assay for BDNF. For FST test, the immobility time was significantly reduced by both BEO and fluoxetine (p<0.05). Fluoxetine tended to decrease serum corticosterone and significantly (p<0.05) decreased serum ACTH, whereas BEO had no effect on both hormones. Moreover, either BEO or fluoxetine did not change BDNF protein levels in hippocampus. The present study indicated that BEO decrease behavior related depressive disorder similar to fluoxetine in chronic restrained stress. No COI.

Cardiovascular and single unit responses to microinjection of norepinephrine into the bed nucleus of the stria terminalis in male rat
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The bed nucleus of the stria terminalis (BNST) is a limbic structure involved in cardiovascular regulation and responses to stress. The BNST contains a high level of adrenergic fibers and receptors however its effects on the cardiovascular system and on single unit responses have not been studied yet. This study was performed to find the effects of low dose of norepinephrine (NE, 3 nmol/100 nl) by microinjection in to all parts of the BST of anesthetized rats and cardiovascular and single unit responses were recorded simultaneously. Methods: Drugs were microinjected into the BNST of urethane anesthetized male rats. The arterial pressure, heart rate and single unit responses were monitored and recorded simultaneously. Results and conclusion: Based on the stimulation site we found that NE produced two types cardiovascular responses, pressor and bradycardia at anterior part of medial division of the BNST, depressor and bradycardic at dorsal part of lateral division of the BNST. We also observed three single unit responses, consisted of short excitatory and long excitatory concomitant with depressor response and short excitatory and long excitatory correlated with pressor response. There was found an association between oscillation in blood pressure and oscillation in single unit response. Key Words: The bed nucleus of the stria terminalis (BNST), Norepinephrine, Blood pressure, heart rate and single unit recording. No COI.
Effect of low frequency stimulation on seizure-induced impairment in synaptic potentiation of hippocampal slices of kindled rats

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Synaptic plasticity is the most important physiological process in learning and memory. Disease such as epilepsy, which influences synaptic plasticity leads to impairment in learning and memory. Low-frequency stimulation (LFS) as a new therapeutic way has an inhibitory effect on kindling process. In this study we investigated if LFS can prevent the seizure induced-impairment in synaptic plasticity and memory in kindled rats. Male Wistar rats were kindled by electrical stimulation of hippocampal CA1 region. Fully kindled rats received 4 trials of LFS (each trial consisted of one train of 200 monophasic square waves of 0.1 ms pulse duration at 1 Hz) for 4 times. To test the special working memory, Y-maze test was run and to evaluate the synaptic plasticity, whole cell patch clamp was used to study the induction of long-term potentiation (LTP) in glutamatergic and GABAergic post-synaptic potentials in hippocampal slices. Kindled animals showed a significant impairment in spontaneous alternation behavior. In addition, the LTP did not induce excitatory or inhibitory synapses compared to control animals. Application of LFS in kindled animals prevented the observed impairments in spontaneous alternation behavior and LTP so that there was no significant difference between this group of animals and control. This improving effect of LFS was accompanied with a significant increase in calcineurin gene expression. Therefore, it can be postulated that LFS application can prevent seizure–induced impairment in spontaneous alternation behavior and synaptic potentiation through a mechanism involving calcineurin gene expression. No COI.
ABS0275

The physiological roles of Moesin, a cytoskeletal protein, in renal salt reabsorption
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Tubular reabsorption of electrolytes in the kidney is an essential function in regulating fluid balance in the body. In the thick ascending limb of Henle (TAL), 20–40% Na+ filtered by the glomeruli are reabsorbed by Na+-K+-2Cl– cotransporter type 2 (NKCC2). In humans, mutations in the gene coding for NKCC2 were identified in patients of Bartter syndrome type I, which is characterized by severe salt losing tubulopathy. Despite of the physiological importance of NKCC2 in NaCl homeostasis, the molecular mechanisms for its membrane trafficking have not been fully elucidated. In 2012, it was reported that moesin, which is a member of ERM (Ezrin-Radixin-Moesin) family, plays an important role in the apical membrane trafficking of NKCC2 by in vitro experiments. Here, we examined the physiological impact of moesin in the regulation of renal function in vivo by using male moesin-null (Msn-/y) mice. Fractional excretions of electrolytes were significantly increased in Msn-/y mice compared to Msn+/y mice. GFR and blood pressure were decreased in Msn-/y mice. Western blotting and immunostaining were performed to investigate the expressions and localizations of proteins in the medullary tubules. Cell surface expression level of NKCC2 was not significantly different between Msn+/y and Msn-/y mice whereas the distribution of NKCC2 in the lipid raft was decreased in Msn-/y mice. Our results suggest that moesin might play a pivotal role in the regulation of lipid raft localization of NKCC2 and in appropriate reabsorption of electrolytes in TAL. No COI.

ABS0393

Role of neuropeptide FF receptor type 2 in pain and depression
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Neuropeptide FF (NPFF) belongs to FMRF-NH2 peptide and was viewed as an opioid modulation peptide regulating nociceptive responses. Two receptor subtypes were cloned, i.e. NPFFR1 and NPFFR2. The aim of this study was to explore the physiological functions of NPFFR2 via NPFFR2 over-expressing transgenic (Tg) mice. NPFFR2 Tg mice exhibit depressive- and anxiety-like behaviors and process hyperreactivity to mechanical and thermal nociceptive stimulations as compared to WT mice. Via specific cell markers, NPFFR2 Tg mice exhibit a decrease in adult hippocampal proliferation without changes in basal neurogenesis. NPFFR2 Tg also exhibit an enhanced serum corticosterone level and decrease in GR expression in the hippocampus as compared to WT mice. In addition, after CFA or carrageenan-induced hind paw inflammation, NPFFR2 Tg mice displayed a more severe allodynia than WT mice. Consistent with these findings, levels of NPFF and NPFFR2 mRNA in the lumbar dorsal spinal cord were up-regulated after the injection of CFA or carrageenan into WT mice. Via immunohistochemical analysis, we found protein levels of CGRP were significant increased after CFA injection in the NPFFR2 Tg mice as compared to CFA-treated WT mice. Further, functional MRI with electrical stimulation was introduced to evaluate brain activity in these mice and results showed that both signal intensity and activated extents in various brain regions (sensory cortex, thalamus, PAG, etc.) were much greater in NPFFR2 Tg mice than WT mice. We conclude that NPFFR2 over-expression enhances the stress response as well as nociceptive threshold, thus displays a pain and depression comorbidity. No COI.
**ABS0415**

**Functional study of novel NaV1.8 mutations causing kidney stone disease in a northeastern Thai family**

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Kidney stone disease in Thailand is a complex multifactorial disorder with the highest prevalence in the northeastern provinces. Causes include environmental, behavioral and genetic factors. Novel mutations in the gene encoding NaV1.8, a tetrodotoxin-resistant voltage-gated sodium channel alpha subunit, have recently been identified in a northeastern Thai family of kidney stone patients. We aimed to describe the electrophysiological properties of these NaV1.8 mutations. Human embryonic kidney (HEK) cells expressing wild-type beta subunit were transfected with plasmid constructs expressing wild-type or mutant alpha subunit (NaV1.8, SCN10A) and studied by using whole-cell patch clamp technique. Results showed that cells transfected with mutant channels had significantly lower currents compared to those with wild-type subunits (wild-type vs mutant, -32.65 ± 4.525 vs -16.03 ± 2.942 pA/pF, 0 mV). However, no significant difference in the voltage dependence of activation and fast inactivation were found between wild-type and mutated channels. The reduction in mutant NaV1.8 current density may contribute to the pathogenesis of kidney stone disease in these patients. To the best of our knowledge, this is the first characterization of mutated NaV1.8 channel associated with kidney stone disease. No COI.

**ABS0482**

**Effects of morelloflavone from Gracina dulcis Kurz. on renal functions and oxidative stress in cisplatin-induced acute renal failure rats.**

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Cisplatin is widely used as a cancer therapy drug. However, its side effect is nephrotoxicity which may due to the generation of reactive oxygen species and lead to acute renal failure (ARF). The prevention of cisplatin-induced ARF with various antioxidants such as vitamin C, E and flavonoids extracted from plants have been reported. Morelloflavone, a biflavonoid from Gracina dulcis Kurz. has been proved to possess antioxidant effect both in vitro and in vivo. This study objected to investigate the protective effects of morelloflavone on ARF induced by cisplatin. Male Wistar rats weighed 250-300 g were divided into three groups including vehicle control, cisplatin and cisplatin+morelloflavone. Induction of ARF was performed using cisplatin (7.5 mg/kg, i.p.) injection. Morelloflavone (1 mg/kg i.p.) was given twice, 24 hr and 10 min before cisplatin injection. Experiments were performed in anaesthetized rats, clearance markers (0.1% inulin and 0.5% para-aminohippuric acid (PAH) dissolved in 0.9% NaCl) was infused via jugular vein. Arterial blood pressure was monitor via carotid artery. Urine samples were collected via urinary bladder. Inulin and PAH clearance were used to determine glomerular filtration rate (GFR) and renal blood flow (RBF), respectively. Osmolar clearance was used to determine electrolyte excretion. It is found that three days after cisplatin injection, renal MDA significantly increased and the administration of morelloflavone suppressed this MDA elevation. Renal functions including GFR, RBF and electrolyte excretion were impaired after cisplatin injection. It is likely that morelloflavone would restore this renal impairment via its antioxidant property. No COI.
Recognition of pathogenic components by Toll-like receptor (TLR) mediates release of cytokines. As TLR2, 3, 4 and 9 expression and function are regulated by sex steroid hormones, this study investigated the soybean isoflavone genistein (Ge) effects on the expression of these TLRs and IL6 secretion in human endometrial cell line (RL95-2). Ge effects were compared with 17β-estradiol (E2) under basal and polyinosinic-polycytidylic acid (I:C) stimulation to mimic viral infection. Cells were exposed to I:C 30 min prior to incubation with Ge (10⁻⁷, 10⁻⁶, 10⁻⁵ M) or E2 (10⁻⁹ M) for 48 h. The culture media was collected and analyzed for IL6 by ELISA and the TLR protein expression by Western blot analysis. The results revealed the differential expression of TLR2, 3, 4 and 9 proteins. I:C up-regulated TLR2 and 9 but suppressed TLR3 and 4. Ge 10⁻⁷ M increased TLR2 whereas E2 decreased TLR4 protein. All Ge treatments attenuated the I:C-induced increase in TLR2 expression. In contrast, both Ge at 10⁻⁶ M and E2 promoted the suppressive effect of I:C on TLR4 expression. In this study, the cells constitutively released IL6 which was suppressed by I:C. Ge increased IL6 secretion in correlation with the up-regulated TLR2 protein. Both Ge and E2 could reverse the inhibitory effect of I:C on IL6 secretion. These findings suggest the role of Ge on differential regulation of TLR expression and IL6 secretion. The reversibility effect of Ge on the TLR expression and IL6 secretion by viral infection provide the potential evidence for application of Ge to promote the uterine innate immunity and alleviate the inflammation following pathogen invasion. No COI.
ABS0369

Asporin localization in mouse lung alveogenesis
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Several extracellular matrix (ECM) proteins have been reported to regulate mouse alveolar formation or alveogenesis. Gene expression of asporin, one of the ECM proteins in small leucine rich proteoglycan family, was shown to be highly correlated with mouse alveogenesis. Asporin protein localization in mouse lung is still unknown and aimed to detect. C57BL/6 mouse lungs at postnatal day 14 were harvested and undergone lung inflation with constant pressure. Lung tissue was processed for sections and double-staining immunohistochemistry of asporin and several cell markers (CD31, fibroblast-specific protein 1, alpha smooth muscle actin and surfactant protein C for endothelial cells, fibroblast, myofibroblast and alveolar epithelial type 2 cells, respectively) (n = 4). To highlight asporin immunolocalization, sequential double staining was prior with staining of each cell marker followed by asporin staining. Asporin was intensely visualized at the area of airway epithelium, likely to be bronchioles identified with presence of smooth muscle and absence of cartilage in its wall. The pattern of asporin was in the cytosol and predominantly at the apical region of airway epithelial layer. The staining also showed positive signal for asporin in the connective tissue around large blood vessels, but not as strong as in the airway epithelium. The costained cell markers were manifested properly for the destined cell types. This study showed that asporin localization in mouse lung was detected in airway bronchiolar epithelium and tunica adventitia of large blood vessels. This study was supported by the Siriraj Graduate Thesis Scholarship (KC), and “Chalermphrakiat” Grant (SS). No COI.

ABS0370

Effects of CTNNAL1 on balancing regulation between epithelial and mesenchymal repair in ozone stressed HBEC
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Epithelial mesenchymal transition (EMT) and its reversible process MET is a crucial event between epithelial and mesenchymal repair, which was possible to be mechanisms for fibrosis. Our previous work showed that catenin alpha-like 1 (CTNNAL1) was participated in repair/proliferation process of human bronchial epithelial cells (HBECs). To assess the effect of CTNNAL1 on process of EMT, CTNNAL1 over-expression and silence HBEC lines were constructed by stable transfection. HBECs stressed with ozone 30 min/day for 4 days induced a decrease of epithelial markers (E-cad, CK19) and an increase of mesenchymal markers (vimentin, Fn and α-SMA), which were described by Real-time PCR, western blot and Immunocytochemical staining. Cytoskeleton reorganization (F-actin) was visualized by rhodamine-phalloidin staining. Additionally, injured HBECs reduced expression of CTNNAL1. Although the increased expression of mesenchymal markers was significant down-regulated by withdrawing ozone for 4days, reduced epithelial markers and CTNNAL1 were not reversed. Importantly, we found that CTNNAL1 up-regulated the expression of E-cadherin and CK19, while down-regulated the expression of vimentin and α-SMA. CTNNAL1 inhibited EMT marker progression and cytoskeleton reorganization. Ozone markedly enhanced the level of TGF-β1 in HBECs, whereas CTNNAL1 reduced the secretion of it. Our data, for the first time, showed an incompletely reversible EMT process in HBECs caused by ozone stress. Suppression of CTNNAL1 and increased secretion of TGF-β1 resulted in ozone may contribute to irreversible process. CTNNAL1 mediated epithelial repair by inhibiting EMT marker progression and reducing the level of TGF-β1. (This work was supported by grants #81270065 from NSFC.)
ABS0388

**Exercise capacity predicted from cardiopulmonary exercise testing in Thai adults**

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Oxygen uptake at maximal exercise (VO2max) is considered the best available index for cardiopulmonary disease prognosis and diagnosis and assessment of exercise capacity. Prediction equations for VO2max have been globally in use in cardiopulmonary exercise testing (CPET). Nonetheless, there is not adequately validated in Thais. This study aimed to create and verify equations for prediction of VO2max among Thai population and compare to those cited by previous studies. A total of 130 (48 males and 82 females) healthy Thais aged 20–78 years old underwent a CPET using a treadmill with incremental protocols until reaching symptom limitation. All subjects had normal ranges of clinical characteristics and pulmonary function. The VO2max in males was significantly higher than in females by 70% (p<0.001). The VO2max prediction equations for both genders were obtained from multiple linear regression analysis: VO2max = 2607.0 + (816.3*sex) + (-14.6*age) + (10.5*weight) + (-7.7*height) (r=0.85) (sex, male, 1; female, 0; age in years; weight in kilograms; height in centimeters). A comparison with previous studies also showed that prediction equations of VO2max derived from studies especially on Caucasian populations overestimated the actual values in Thai adults (Males: Wasserman 32%, Jones 23% and Hansen 10%; Females: Wasserman 50%, Jones 8% and Hansen 13%). The present study suggests that a prediction equation for VO2max is necessary for establishing reference values in Thai adults. Moreover, prediction equations for VO2max in Caucasians may not be readily applicable to Thai subjects. No COI.

ABS0391

**SPLUNC1 Peptide-derivative with increased efficacy and decreased renal side effects for the treatment of Cystic Fibrosis (CF) lung disease**

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CF lung disease is typified by loss of the cystic fibrosis transmembrane conductance regulator (CFTR) function and hyperactivity of the epithelial Na⁺ channel (ENaC) in the lungs, resulting in decreased airway surface liquid (ASL) volume and mucus dehydration. Short palate lung and nasal epithelial clone 1 (SPLUNC1) acts as a potent negative regulator of airway ENaC. Amiloride is another ENaC antagonist shown to improve lung function in CF patients, but cause severe renal side effects. We tested our novel SPLUNC1 derivative peptide (SPX45) for prevention of symptoms in an in vivo CF disease model, βENaC overexpressing transgenic mice. We also tested to see if SPX45 caused in vivo renal electrolyte abnormalities. βENaC-Tg mice were dosed with SPX45 or saline and survival was monitored for 14 days. In parallel, anesthetized rats were infused with amiloride, SPX45 or saline and renal parameters were recorded in real time. Conscious mice were housed in metabolic cages and tail-vein injected and then renal function was monitored for 8 hours. SPX45 treatment of βENaC-Tg mice significantly improved survival compared to saline and, unlike amiloride, the animals did not suffer stunted weight gain. Studies of renal function in rats and mice showed that IV infusion of amiloride caused increased urine flow and blunted K⁺ excretion in both, whereas treatment with SPX45 showed no significant increase in urine flow or, more importantly, they elicited no decrease in UK⁺. In conclusion, SPLUNC1-derivatives can be optimized to increase potency, which serves to (i) improve ASL re-hydration; (ii) improves survival in βENaC-Tg mice but; (iii) does not result in K⁺-retention, allowing for a viable therapeutic option for the treatment of CF lung disease. Supported by the NCBC and the NIH.
ABS0457

**Alteration in asporin expression of newborn mouse lung after hyperoxia**

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Elevated expression of asporin gene, an extracellular matrix molecule was detected in the lungs of bronchopulmonary dysplasia patients. Normally its gene expression pattern reaches the peak during mouse alveolar formation or alveogenesis. These findings imply the potential role of asporin on alveogenesis. We sought to determine the effect of high oxygen exposure on asporin expression in newborn mouse with an arrest of alveogenesis. Newborn C57BL/6 mice on postnatal day 3 were randomized for 7-day exposure of room air or FIo2 ≥ 0.95 or hyperoxia group. Mean alveolar linear intercept was measured to assess the changes in lung morphology with increased airspace size in hyperoxia group (42.33 ± 1.88 vs. 25.11 ± 1.2 μm; n = 5–11; P-value < 0.001). Lung asporin gene expression was determined by quantitative real time PCR. Asporin gene expression was significantly decreased in newborn mouse lungs after hyperoxia exposure compared with normoxia (15.91 ± 5.05 vs. 222.92 ± 14.94 [x 10^-3 arbitrary unit]; n = 5-11; P-value < 0.001). This study showed that alteration of asporin gene expression was detected in newborn mouse after hyperoxia exposure implying that asporin may have a role on alveolarization. This study was supported by the Siriraj Graduate Thesis Scholarship (NC), and “Chalermphrakiat” Grant (SS). No COI.

ABS0475

**Effects of Insulin-like growth factor-1 on the protection of hyperoxia-induced lung injury in newborn rats**

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Bronchopulmonary dysplasia is a common complication of newborn with hyperoxia and mechanical ventilation therapy. In the present study, IGF-1 was administrated to newborn rats and its effect against hyperoxia-induced lung injury was examined. Wistar newborn rats were randomly divided into room air control group, the hyperoxia group and hyperoxia + IGF-1 group. Eight rats were sacrificed in each group on day 3rd, 7th, 14th day after the treatment and the lungs were embedded. The typical pathological characteristics of acute lung injury were observed in hyperoxia group, but IGF-1 treatment group compared with the hyperoxia group was decreased significantly. TUNEL staining showed that the number of apoptotic cells of IGF-1 treatment group on the 3rd, 7th, 14th day were lower than the same days of model group and had statistical difference. Western blot was used to detect the protein, it showed that caspase-12, GRP78 and CHOP protein content of model group on the 3rd, 7th, 14th day were significantly higher than IGF-1 treatment group on the same days. These data indicate that IGF-1 may be a potential therapy in the prevention of hyperoxia-induced lung injury in neonatal rats probably via modulating the ERS pathway. This research was supported by the National Natural Science Foundation of China (81160083).
Meso-dihydroguaiaretic acid attenuates airway inflammation in an ovalbumin-induced murine asthma model

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In this study, we investigated the effect of meso-dihydroguaiaretic acid in an established mouse model of ovalbumin (OVA)-induced allergic asthma. The effects of meso-dihydroguaiaretic acid on the production of Th1 and Th2 cytokines, eotaxin, total and OVA-specific immunoglobulin E and activation of nuclear factor kappa B were measured. Mice were sensitized on days 0 and 14 with an intraperitoneal injection of 20 μg OVA emulsified in 2 mg aluminum hydroxide in 200 μL PBS buffer. On days 21, 22, and 23, mice received an airway exposure to OVA (1%, w/v, in PBS) for 1 h. Meso-dihydroguaiaretic acid was administered orally to mice at doses of 200 mg/kg per day from days 18 to 23. Intragastric administration of meso-dihydroguaiaretic acid significantly attenuated OVA-induced influx of total leukocytes and eosinophils into lungs, and decreased levels of interleukin (IL)-13 and eotaxin, in a dose-dependent manner. Meso-dihydroguaiaretic acid also significantly reduced the plasma levels of total and OVA-specific immunoglobulin (Ig)E release into the airspace. Histological studies showed that meso-dihydroguaiaretic acid inhibited OVA-induced lung tissue eosinophilia and airway mucus production. Also, MDGA treatment significantly inhibited the activation of nuclear factor kappa B (NF-κB) in OVA-challenged lungs. Collectively, these results suggest that meso-dihydroguaiaretic acid may be an effective oral treatment for allergic airway inflammation by virtue of its anti-inflammatory activity. No COI.

The effect of CTNNAL1 on the adhesion of human bronchial epithelial cells

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Adhesion molecules maintain the structural integrity of airway epithelia and may evoke inflammatory responses in airway under stress. Our previous study found that catenin alpha-like 1 (CTNNAL1) was downregulated in asthma animal model and CTNNAL1 expression in bronchial epithelial cells (BEC) was upregulated after ozone stress. To assess the possible influence of CTNNAL1 on airway epithelial cells, we examined the proliferation, adhesion and inflammatory responses of airway epithelial cells caused by different expression levels of CTNNAL1. EdU incorporation assay and cell cycle analysis showed that CTNNAL1 overexpression accelerated the DNA synthesis and promoted transformation from G1 to S phase of BEC. The adhesion between BEC and human leukocytes was observed using flow cytometry. We found that CTNNAL1 over-expression enhanced the ECM adhesion. In line with these results, over-expression of CTNNAL1 promoted the mRNA and protein expression of E-cadherin, integrin β1 and β4, while silence of CTNNAL1 decreased the expression of these molecules. In addition, we found that CTNNAL1 overexpression weakened the adhesion of BEC to leukocytes, while downregulated CTNNAL1 led to a remarkable increase of leukocyte adhesion. Furthermore, CTNNAL1 silence led to a remarkable increase of ICAM-1 expression, especially under ozone stress. Simultaneously, CTNNAL1 silence increased the basal levels of secretion of IL-1β and IL-8, and the effect was more robust under ozone stress. So we speculated that CTNNAL1 is important to maintain the structural adhesion and inflammation response of airway epithelium. (This work was supported by grants #81270065 from NSFC and #2013zztc070 from Hunan Provincial Innovation Foundation for Postgraduate.)
ABS0531

Oxidative stress induces alteration of E-cadherin expression in lungs of mice
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Oxidative stress may be involved in pathogenesis of some chronic lung diseases such as asthma. Mice exposed to ozone developed airway hyperresponsiveness (AHR) and oxidative stress impaired barrier function of bronchial epithelial cells. To further elucidate the mechanisms of airway epithelial cells injury induced by oxidative stress and its relationship to AHR, we investigated the changes of E-cadherin, an important molecule involved in maintaining airway epithelial integrity in mice with ozone exposure. Mice were exposed to ozone of 2 ppm for 30 min every day and lung tissues were analyzed on days 0, 1, 2, 4, and 8, respectively by immunohistological analysis (IHC) and western blot analysis using an anti-E-Cadherin antibody. IHC analysis showed that E-cadherin expression was up-regulated in bronchial epithelium after ozone exposure on days 1, 2 and 4 and declined on day 8. The same tendency was found in E-cadherin expression in extracts from lungs after ozone exposure by western blot. Interestingly, pretreatment of emodin (1,3,8-trihydroxy-6-methylanthraquinone, an active component present in many herbaceous plants) by intragastric administration before ozone exposure abolished the up-regulatory effect on E-cadherin expression in mice lungs induced by ozone. (This work is supported by NSFC Grants 81170024, 81270065 and 81370116, grants 14K109 and 12K003 from the open fund of Hunan College innovation platform, grants 2015JJ2147, and 2013JJ4030 from Hunan Natural Science Foundation, China).

ABS0533

Effect of some stress infectors on the intracellular chloride concentration of 16HBE14O-, HEK293T, 3T3 and RaW
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Chloride is the most abundant anion in mammalian cells, Since Cl− flux is coupled with Na+ and K+ in several processes, including cell volume control, transepithelial transport, cell multiplication and so on. We have measured the effects of some stress factors such as LPS (20 mg/ml), H2O2 (1 mM), heat (42 °C), cold (4 °C), acid (pH = 6.4), Alkali (pH = 8.4) on [Cl]i using the fluorescent chloride indicator N-(6-methoxyquinolyl) acetoxethyl ester (MQAE) with Laser confocal fluorescence microscope. 16HBE14O-, HEK293T, 3T3, RaW was selected in our experiment. Fluorescence intensity which indicate [Cl]i was measured by ImageJ software. After LPS (20 mg/ml) or H2O2 (1 mM) stimulate cells, Fluorescence intensity of cells decrease gradually. That is to say [Cl]i will increase gradually after be stimulated by LPS (20 mg/ml) or H2O2 (1 mM). However, after be stimulated by heat (42 °C), cold (4 °C), acid (pH = 6.4), alkali (pH = 8.4), [Cl]i will increase quickly and holding it for some time (about 10 seconds), then recovery to basal level. Our results showed a phenomenon that when cells be stimulated by some stress factors, [Cl]i will change. Different stress factors produce different effect. But the interesting bit was that different cells have the same effect on the same stress factor. Maybe it can give us some hints that the intracellular chloride is a potential signaling molecules like Calcium ion response to stimulation and produce some cytological effect. (This work was supported by grants #81270065, #81370116 from NSFC and grant#2013JJ4030, #2015JJ2147 from Hunan Natural Science Foundation).
Cigarette smoking is an established risk factor for cardiovascular disease. Endothelial dysfunction is a systemic disorder, which is critical element in the pathogenesis of cardiovascular disease and its complications. A noninvasive method of endothelial function assessment by ultrasound technique to evaluate brachial artery called Flow-Mediated Dilatation (FMD) has emerged as a marker of endothelial dysfunction. To study endothelial dysfunction by FMD method in smokers and non-smokers and to study the correlation of FMD with blood pressure (BP), lipid profile between smokers and non-smokers. The present study was done in young, age-matched 31 smokers and 31 non-smokers (all males). Resting state supine BP was recorded by a sphygmomanometer and brachial artery diameter (intima – intima) was recorded by ultrasonography following which BP cuff was inflated 50mmHg above systolic pressure for 5minutes and then deflated. Brachial artery diameter was again recorded for maximum dilation after deflation and FMD was calculated. Fasting lipid profile was also measured. FMD as an indicator of endothelial dysfunction was much less in smokers (7.33) compared to non-smokers (13.83). Systolic BP in smokers was 128.96 (±8) mmHg and 119.93(±7.6) mmHg in non-smokers. Dyslipidaemia was observed in smokers compared to non-smokers with a significant correlation for TG, HDL & VLDL. Significant endothelial dysfunction was observed in smokers as compared tonon-smokers. Smokers were associated with dyslipidemia, increased blood pressure all of which cause endothelial dysfunction a major trigger for cardiovascular disease. No COI.
The effect of resistance training and aquarobics on bone remodelling and obesity
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The prevalence of obesity is increased in premenopausal women. Condition of obesity provides the mechanical load on the bones and tends to cause excessive osteoporosis. Resistance training (RT) and aquarobic exercise (AE) can decrease level of obesity and increase the activity of bone remodeling in obese women. The purpose was to investigate the effect of different exercise RT and LA on the level of obesity and bone remodeling activity. Methods of research is experimental randomized pretest-posttest control group design performed in 36 obese women, aged 45-50 years, who were divided into 3 groups: RT group exercised at 75% RM, 3 sets, 12 reps, twice days for 8 weeks (n=12), AE group exercised at 75%HRmax, twice days for 8 weeks (n=12) and control group (n=12). Percentage of body fat, BMI, adiponectin levels, levels of CTX and N-MID Osteocalcin levels were examined before and after treatment. The percentage of body fat was higher in AE than the RT and control groups (p<0.05). IMT was higher in the RT group and lower in the AE group when compared to controls (p<0.05). Increased adiponectin levels were higher in the RT group than the LA group and the controls (p<0.05). Increased levels of IL-6 were higher in RT than the AE group and controls (p<0.05). Decreased level of CTX was higher in RT than AE group and controls (p<0.05). Increased levels of N-MID Osteocalcin higher in RT and LA groups than in controls (p<0.05). RT is more dominant on increasing bone remodeling activity and AE is more dominant on reduction of obesity. No COI.

Screening of Thai medicinal plants for Alzheimer’s disease
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The extracellular aggregation of amyloid-β (Aβ) protein which leads to amyloid plaque formation in the brain is a pathological hallmark of Alzheimer’s disease (AD). To develop the anti-AD agents from natural-based chemicals, nine Thai herbal plants; Caesalpinia sappan (CS), Thunbergia laurifolia (TL), Rhinacanthus nasutus (RN), Tabernaemontana divaricata (TD), Cyperus rotundus (CR), Terminalia chebula (TC), Azadirachta indica (AI), Piper retrofractum (PR), and Asparagus racemosus (AR) were selected and screened in vitro for anti-Aβ aggregation and Aβ disaggregation potency and neuronal toxicity. For the anti-Aβ aggregation, 10 µM of Aβ1-42 was co-incubated with 0.01-100 μg/mL of each herbal extract for 28 h. For the disaggregation, Aβ1-42 was first allowed to assembly into fibrils for 28 h, and the extracts were added and incubated for another 28 h. After the incubation, the Aβ aggregation and disaggregation were monitored by Thioflavin-T binding assay. Only eight plants, except AR, elicited anti-Aβ aggregation and Aβ disaggregation potency and neuronal toxicity. For the anti-Aβ aggregation, 10 µM of Aβ1-42 was co-incubated with 0.01-100 µg/mL of each herbal extract for 28 h. For the disaggregation, Aβ1-42 was first allowed to assembly into fibrils for 28 h, and the extracts were added and incubated for another 28 h. After the incubation, the Aβ aggregation and disaggregation were monitored by Thioflavin-T binding assay. Only eight plants, except AR, elicited anti-Aβ aggregation and Aβ disaggregation properties, and proceeded for the protective efficacy against Aβ-induced neuronal toxicity. For the anti-Aβ aggregation, 10 µM of Aβ1-42 was co-incubated with 0.01-100 µg/mL of each extract and 1 µM of Aβ1-42 for 48 hours and the cell viability was determined by MTT assay. Only four herbal extracts of RN (0.1 µg/mL), TD (10 µg/mL), TC (1 µg/mL) and PR (0.1 and 100 µg/mL) protected against Aβ-induced neurotoxicity, ranging by 91.74-96.87%. These effective concentrations of the four extracts were confirmed non-toxic to the cells after cytotoxicity test. From all above tests, RN, TD, TC and PR have a potential to be developed further as anti-AD agents for human use. No COI.
**Effect of Fucoidan from Sargassum sp. on joint swelling and degree of pain in osteoarthritis rat**

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Osteoarthritis (OA) is a destructive joint disease affect more than half of the world's population at the elderly and commonly manifest as chronic joint pain and edema. This study aimed to investigate the potency of fucoidan from Sargassum sp. in decreasing the degree of pain and joint swelling in osteoarthritis. A Randomized Post Test Only Controlled Group Design was performed on rat models of osteoarthritis divided into 9 groups: negative control, positive control, A, B, C, D, E, F and G. The positive control and treatment groups had been induced for osteoarthritis by injection of CFA in rats heel joint for 3 times in 2 weeks interval. Groups A, B and C were treated with fucoidan extract 20, 40 and 80 mg/kg, group D was treated with steroid treatment 10 mg/kg, groups E,F and G were treated with combination of steroid and fucoidan at dose that has been mentioned. It were given two times with two weeks interval. The degree of pain and joint swelling were evaluated every week. One Way ANOVA showed that the administration of fucoidan therapy is able to significantly reduce joint pain and edema on rat models of osteoarthritis (p <0.05). Moreover, response to the fucoidan therapy also showed better outcomes than the use of steroid injections as one commonly used in osteoarthritis. These findings suggest that administration of fucoidan can significantly improved osteoarthritis symptoms after serial intraarticular injection. Thus, it may have therapeutic value for the treatment of osteoarthritis. No COI.

**Are the reference values for different parameters of a research animal model important when conducting research?**

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Lack of availability of reference values for the Sprague-Dawley rat model led the researchers to have an additional group of rats as the control group in their experiments increasing the number of animals required for research. Hence, this project was conducted to establish a reference data base for selected physiological, haematological and biochemical parameters of Sprague-Dawley rats in the Animal House with the approval of the Ethics Review Committee of the Faculty of Medicine, Colombo. Healthy young male (n=10) and female (n=10) rats were group housed except on the days of urine collection and were fed ad libitum. Approximately 1 ml of blood was drawn from the tail vein of each rat, once a week for 15 consecutive weeks, for haematological and biochemical investigations in alternating weeks. EDTA anti-coagulated blood in haematological investigations and serum in biochemical investigations were used. Urine output, food and water intake were determined for 24 hours per week during the study period. The mean, median and ranges of the parameters were computerized separately for male and female rats. The Faculty Animal House has been supplying Sprague-Dawley rats since 1977, for various research projects through continuous breeding and the reference ranges are now established for the first time in the history. For animal models using the Sprague-Dawley rat strain, this will reduce the number of animals used in future research projects, thus applying ‘Reduction’ of the 3Rs concept of Russell and Burch. No COI.
Changes of blood pressure, blood flow and heart rate during 90° head-up tilt for 30 min in anesthetized rats
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It is well known that baroreceptor reflex (BR) is important to maintain systemic arterial pressure (BP) and blood flow (BF) toward head after transition from supine position (SP) to head-up posture. To clarify the role of BR and the changes of BP, BF and heart rate (HR) in response to transitions of 90° head-up tilt (HUT); from SP to HUT or from HUT to SP, in nerve-intact and sinoaortic denervation (SAD) rats, we measured BP, common arterial flow as BF and HR in anesthetized SD rats (urethane 1.0-1.5 g/kg, i.p.) under 15 min or 30 min HUT (approval #, H1442). After onset of HUT, BP and BF significantly decreased by -16.4% and -28.3% at 3.3 sec, respectively (mean, p<0.001: paired t-test, n=12), and then these parameters immediately increased and maintained throughout HUT; % control was -2.2% in BP or -19.9% in BF at 34.7±14.2 sec when BP was steady. After transition from 30 min, HUT, BP and BF increased by 14.6% and 44.8% at 1.6±1.1 sec (n=8), respectively, and then they decreased and increased in a short period until steady state. In the SAD rats, the changes of BP and BF during HUT showed similar pattern to nerve-intact, but each parameter was lower; % control was -7.3% in BP or -26.5% in BF at 24.1±6.0 sec (n=5). The decrease in BP after HUT in the nerve-intact rats led slightly higher level of HR, the change in HR at 34.3 sec was by 11.3±13.5 beats/min and statistically significant compared with SP control. However, this response disappeared in SAD rats. These results indicate that initial decreases or increase in BP due to the 90° transition is produced by the hydrostatic pressure gradient and the increase in HR during HUT, which is basically caused by BR, suggesting that BR is important to maintain BP and BF during long-term HUT. No COI.

Protective effect of Na-DNA on pressure ulcer and elucidation of its mechanism
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When patients are limited to change position or required keeping bedridden for a long period of time, their blood circulation becomes failure by the pressure of body weight. At this time, inflammatory response was caused by ischemia-reperfusion (IR) injury, which can induce clinical pressure ulcer. In our study, a DNA formulation prepared from sturgeon testicle, Na-DNA was used. We elucidated the mechanism of Na-DNA through investigating its therapeutic action and preventive action on pressure ulcer mouse models. Mice were anesthetized and their dorsal skin was pulled up and placed between 2 round magnetic plates with an average weight of 3.5 g and 1000 G magnetic force. We created two types of pressure ulcer mouse models. A mild IR cycle consisted of a 24-hr period of magnet placement followed by a release period of 24-hr before drug administration. A single severe IR cycle consisted of a 16-hr period of magnet placement followed by a release period of 8-hr for a 3 days period before drug administration. Na-DNA was used after diluted with agar gel. As a result, accumulation of 8-oxoguanine as a marker of DNA damage and increased expression of cyclooxygenase-2 were found in pressure ulcer mouse dorsal skin, which were inhibited by Na-DNA. We also compared the effect of Na-DNA with a pressure ulcer therapeutic agent basic fibroblast growth factor (bFGF) formulation, and Na-DNA could stand comparison to bFGF formulation. In conclusion, Na-DNA supposed to be an inexpensive and safe drug in a clinical site in future. This study is supported by Technomedservis, which provided the substance (Na-DNA) used. No COI.
ABS0155

High concentration carbohydrate mouth rinse improves endurance exercise capacity
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It has been reported that mouth rinse with a carbohydrate-containing solution can improve endurance exercise capacity. However, few studies focused on the concentration of carbohydrate (CHO) mouth rinse solution. The aim of this study was to examine the effects of different concentrations of CHO mouth rinse on endurance exercise capacity, physiological and subjective responses. Nine endurance-trained subjects participated in this study. Subjects completed cycling trials at 55% of maximum to complete exhaustion. Mouth rinse was done every 5 min for 10 seconds. CHO concentrations were set for 0%, 6% and 18%. Endurance exercise capacity (time to exhaustion), physiological indexes (rectal temperature, mean skin temperature, heart rate, cerebral oxidation) and subjective indexes (rating of perceived exertion: RPE, comfort in the mouth) were measured every 5 min. Time to exhaustion with 18% (74±18 min) was significantly longer than that with 0% (65±15 min) and 6% (69±16 min). RPE with CHO 18% was significantly lower than that with 0% and 6% at 45 and 50 min periods during exercise. Physiological indexes increased during exercise; however these are not significantly different among conditions. These results indicate that high concentration of CHO mouth rinse (18%) improves endurance exercise capacity via decrease in subjective responses without increase in physiological responses. No COI.

ABS0158

The effects of low salt and high salt intakes on insulin sensitivity in healthy subjects
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It was well known that high salt intake was associated with not only hypertension but also insulin resistance. But the association between restriction of salt intake and insulin resistance was still unclear. The present study aimed to investigate the effect of low salt and high salt intakes on insulin sensitivity in healthy subjects. This study was undertaken in 51 apparently healthy male subjects (Age 22.22 ± 3.26 years, BMI 26.51 ± 4.88 kg/m²). All participants were maintained on a low salt intake (<50 mmol/day sodium) and a high salt intake (>165 mmol/day sodium) for one week each, in random order. Fasting blood samples were collected at the end of each intervention. Fasting blood glucose was measured by glucose oxidase method. Serum insulin level was measured by ELISA kit method. Insulin sensitivity was calculated by HOMA-IR. The fasting plasma glucose level and serum insulin level in low salt and high salt intakes were 5.32 ± 0.50 mmol/l vs 5.2 ± 0.42 mmol/l and 17.32 ± 8.78 μIU/ml vs 12.68 ± 5.69 μIU/ml respectively. The fasting plasma glucose levels after two interventions were within the normal range and it was found that normal fasting plasma glucose level was maintained by compensatory hyperinsulinaemia in healthy subjects. HOMA-IR was increased during low salt intake than high salt intake (4.1 ± 2.14 vs 2.9 ± 1.32, P<0.001). Insulin sensitivity was significantly lower in low salt intake than in high salt intake in healthy male subjects. Salt loading and salt restriction can induce insulin resistance. The effects of low salt diet on insulin resistance was found to be higher than in high salt diet. No conflict of interest. Key words: Insulin sensitivity, Low salt intake, High salt intake and Homeostasis model assessment (HOMA-IR). No COI.
ABS0177

Bacopa monnieri (Brahmi) on spatial memory and long-term potentiation effects
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Bacopa monnieri (L.) Wettst. or Brahmi, the Indian medicinal plant, has been used in Ayurvedic medicine as a memory enhancer for a long time. The active ingredients of Brahmi extract are saponin glycosides which have demonstrated the anti-oxidant, anti-inflammatory, anti-anxiety properties. The administration of Brahmi extract has shown cognitive enhancing effects on both adults and aging people. Long-term potentiation (LTP), a synaptic plasticity phenomenon expressing as the strengthening of chemical synapses, played an important role in learning and memory performances. This study aimed to examine the effect of Brahmi extract on learning and memory performance and long-term potentiation magnitude in acute hippocampal slices. Forty adult male Wistar rats were divided into 4 groups and were orally fed daily with either different Brahmi extract concentrations (80, 160, and 240 mg/kg as in 3 experimental groups) or sterile water (control group) for 60 days. Morris water maze (MWM) task was performed to investigate learning and memory performances whereas the extracellular field potential recording at CA3-CA1 hippocampal synapses was used to determine LTP magnitudes. The result of chronic administration of Brahmi extract showed the significant enhancement of spatial learning and memory performance, as well as the LTP magnitudes. These data suggested that the learning and memory enhancing effect of Brahmi extract might act at least through the strengthening of CA3-CA1 hippocampal synapses. However, the detailed mechanisms of these mechanisms are still unclear, so it needs many further basic studies as well as clinical studies. No COI.

ABS0222

Fibroblast growth factor 21 (FGF21) improved cognitive impairment in obese-insulin resistant rats.
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We found that 12-week high-fat diet (HF) consumption caused insulin resistance, increased brain oxidative stress and cognitive decline. Fibroblast growth factor 21 (FGF21) is an endocrine hormone, playing an important role in the metabolic regulation and improved cognitive function in aging model. However, the effects of FGF21 on the metabolic regulation and cognition in obese-insulin resistant rats have never been investigated. We hypothesized that FGF21 can improve insulin sensitivity, reduce brain oxidative stress, and improve cognitive function in obese-insulin resistant rats. Eighteen male rats were divided into two groups to receive either normal-diet (ND) or HF for 12 weeks. At week 13, HF-fed rats were subdivided into two subgroups to receive either vehicle (HVF) or recombinant human FGF21 (0.1 mg/kg/day) for 28 days. ND-fed rats (NDV) were given vehicle for 28 days. At the end of experiment period, blood sample was collected to determine the metabolic parameters and serum malondialdehyde (MDA) level. Rats were tested with Morris Water Maze for cognition, before being sacrificed. Then, brain was removed to determine brain MDA levels. The results showed that HFV group developed obese-insulin resistance, increased serum and brain MDA levels as well as cognitive decline, when compared with NDV group. The administration of FGF21 in HF-fed rats improved insulin sensitivity and reduced oxidative stress, as indicated by decreased serum and brain MDA levels. Moreover, FGF21 improved cognitive function in HF-fed rats. These findings suggest that FGF21 improved cognitive function in obese-insulin resistant model, possibly via improved insulin sensitivity and reduced oxidative stress. No COI.
Gamma wave oscillation and synchronized neural signaling between the lateral hypothalamus and the hippocampus in response to hunger

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The lateral hypothalamus plays an important role in homeostasis. It is sensitive to negative energy balance and believed to interact with other brain regions to mediate food seeking behavior. However, no neural signaling of hunger in the lateral hypothalamus has been studied. Male ICR mice implanted with intracranial electrodes into the lateral hypothalamus and the hippocampus were randomly treated with saline for control condition, 18-20 h deprivation of food for hunger condition, and fluid for satiety condition. Therefore, local field potential (LFP) and locomotor activity of animals were simultaneously recorded. One way ANOVA with Tukey post hoc test was used for statistical analysis. Frequency analysis of LFP revealed that food deprivation significantly increased the power of gamma oscillation (65-95 Hz) in the lateral hypothalamus and the hippocampus. However, satiety did not change the oscillation in this region. Moreover, no significant difference among groups was observed for locomotor count and speed. The analysis of coherence values between neural signaling of these two brain areas also confirmed significant increase in a frequency range of 61-92 Hz for hunger. No change in coherence value was induced by satiety. In summary, this study demonstrated neural signaling of the lateral hypothalamus in response to hunger with differential power spectrum of LFP and the interplay with the hippocampus. The data may suggest critical roles of the lateral hypothalamus in detection of negative energy balance and coordination of other higher functions of food related learning or behaviors through the connectivity with the hippocampus. No COI.

Low gamma wave oscillations in mice striatum following morphine administration

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Functional role of the striatum in motor control has been widely studied. In addition, its involvement in reward function as a brain area in the dopamine system has also been mentioned. However, neural signaling in the striatum in response to consumption of emotional enhancing substances remained to be explored. This study aimed to investigate local field potential (LFP) of the striatum following morphine administration. Male ICR mice implanted with electrode into the striatum were given an intraperitoneal injection of either saline or morphine (5 or 15 mg/kg). LFP and locomotor activity of individual animals were simultaneously recorded in the recording chamber following the administration. The inspection of LFP tracings revealed the increase in fast wave induced by morphine particularly at a high dose. Statistical analyses were performed using a one way ANOVA followed by Tukey post hoc test. Frequency analysis using Fast Fourier transform also confirmed a significant elevation of low gamma (30-44.9 Hz) activity. When analyzed in time domain, significant increase in low gamma power was observed from the 15th to 65th min following 15 mg/kg morphine treatment. Moreover, morphine treatment also exhibited a stimulating effect on locomotor speed. However, regression analyses revealed no significant correlation between low gamma power and locomotor speed. In summary, this study demonstrated the increase in low gamma oscillation in the striatum and this effect was not associated with locomotor activity of animals. Thus, it is possible that low gamma oscillation induced by morphine treatment is related with the reward function. No COI.
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**Oral presentations / Young Scientist Awards (Oral)**

| O 1 | Neuroscience                                                                 |
| O 2 | Neuroscience                                                                 |
| O 3 | Exercise physiology / Cardiovascular physiology                          |
| O 4 | Teaching physiology                                                       |
| O 5 | General interest / Membrane and epithelial transport                      |
| O 6 | Cell and molecular physiology / Respiratory physiology                   |
| O 7 | YSA 1: Neuroscience                                                       |
| O 8 | YSA 2: Neuroscience / Cardiovascular physiology and microcirculations    |
| O 9 | Endocrinology and metabolism                                              |
| O 10| YSA 3: Cardiovascular physiology & microcirculation / Membrane and epithelial transport |
| O 11| Muscle physiology / Gastrointestinal physiology                           |
| O 12| Endocrinology and metabolism / Alternative and complementary medicine    |
| O 13| YSA 4: Endocrinology and metabolism / Reproductive physiology / Alternative and complementary medicine / Neuroscience |
| O 14| YSA 5: Cell and molecular physiology / Muscle physiology / Alternative and complementary medicine |
| O 15| YSA 6: Growth and development / Renal physiology / General interest       |

**Poster Presentations / Young Scientist Awards (Poster)**

| P 1 | Alternative and complementary medicine                                  |
| P 2 | Cardiovascular physiology and microcirculation                          |
| P 3 | Cell and molecular physiology                                           |
| P 4 | Endocrinology and metabolism                                            |
| P 5 | Exercise physiology                                                      |
| P 6 | Gastrointestinal physiology                                             |
| P 7 | General interests                                                        |
| P 8 | Growth and development                                                   |
| P 9 | Membrane and epithelial transport                                       |
| P 10| Muscle physiology                                                        |
| P 11| Neuroscience                                                             |
| P 12| Renal physiology                                                         |
| P 13| Reproductive physiology                                                 |
| P 14| Respiratory physiology                                                   |
| P 15| Young Scientist Awards                                                   |
The 8th Congress of the Federation of the Asian and Oceanian Physiological Societies

Keynote Lecture
Some observations on decision making in rodents

Plenary Lectures
PL 1  Short-term synaptic plasticity: inspired by biophysics
PL 2  Intracellular protein degradation: From basic mechanisms thru human diseases and on to drug targeting

Memorial Lectures
Ouay Ketusingh Lecture  Fibroblast growth factor 23 and vitamin D as feedback regulators in the bone-kidney-intestinal axis for calcium and phosphorus homeostasis
Dithi Chungcharoen Lecture  Anti-diabetic drugs, obesity, and insulin resistance: The good, the bad, and the ugly in the heart
Prasop Ratanakorn Lecture  Melatonin regulating neurogenesis and neurodegeneration
John A Young Lecture  Molecular identification and role in physiology of the volume-regulated anion channel VRAC

Special Lectures
SL 1  Myofilament dysfunction in heart failure
SL 2  Circadian rhythms, molecular clock and skeletal muscle: why muscles need to keep time
SL 3  Novel aspects of the autoregulation of blood flow: from isolated vessels to human
SL 4  Multifaceted aspects of prostaglandin E2 synthesis and degradation pathways in gastric mucosal inflammation and carcinogenesis
SL 5  Evolutionary and comparative cognitive neurobiology of human and non-human primates
SL 6  Systems biology of aquaporin-2 regulation in kidney collecting duct

Luncheon Lectures
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TPK 1  Understanding the changing learner: a critical success factor of higher education of the 21st century
TPK 2  Learning from productive failure

Teaching Physiology Lectures
TPL 1  Teaching challenges in a modern university environment
TPL 2  Creating the future: Complementary approaches to transdisciplinary learning

Teaching Physiology Symposia
TPS 1  New direction in physiology education
TPS 2  Physiology in medical education
TPS 3  Promoting inquiry in undergraduate biomedical science classes
TPS 4  Learning Physiology: The student’s voice
TPS 5  Teaching Physiology Workshop

Symposia
S 1  New insights into integral regulators of the epithelial ion transport in health and disease
S 2  The role of amino acid transport and signaling in pathophysiological conditions
S 3  The WNK and IRBIT pathways in ion transport
S 4  Zinc and zinc transporters in health and disease

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