Oral Presentations

Young Scientist Awards (Oral)

ABS0019

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.

ABS0054

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.

ABS0064

Diurnal time-of-day dependence of visual motion prediction

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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.

ABS0204

Effects of prenatal co-administration of stress and morphine and postnatal re-exposure to stress on corticosterone blood levels and pentylentetrazol-induced epileptic behaviors in rats

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The present study aimed to identify effects of co-administration of restraint stress and morphine in prenatal period and re-exposure to stress at the end of infancy on corticosterone blood levels and pentylentetrazol (PTZ)-induced epileptic behaviors in rats. Pregnant rats were allocated to six groups of control, stress, saline, morphine, stress-saline and stress-morphine. In the stressed group, rats were held immobile (2h, twice per day) for three consecutive days from day 15 of pregnancy. The rats in saline and morphine groups received saline or morphine on the same days (15-17). In the stress-saline/morphine groups, rats were exposed to stress and received either saline or morphine simultaneously. On postnatal day 22, half of the pups were re-exposed to stress, and PTZ-induced epileptic behaviors of each rat were assessed. Blood samples were collected to determine corticosterone levels. Latency of first epileptic behavior decreased significantly in stress-morphine group compared to other groups. Re-exposure to stress significantly decreased the number of clonic seizures. There was no significant difference between the experimental groups in duration of clonic attacks and mortality rate. The levels of corticosterone showed a significant increase in stressed pups and decreased in morphine group. In conclusion, these results indicated that co-administration of restraint stress and morphine during late pregnancy had profound impact on neurochemical development and might alter vulnerability to PTZ-induced epileptic behaviors. Prenatal stress is more powerful than postnatal stress on influencing neural development and seizure susceptibility in rats. 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 effetcs 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 PC12 cells. 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_2O_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_2O_2 – derived glia activation is one of free radicals that plays a role in neurovascular glia coupling process. Releasing H_2O_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_2O_2 by using kernel function of support vector machine (SVM). Kernel function is a key mapping of SVM supervised by learning algorithms. Plasma H_2O_2 was assessed by real time electrochemistry method. The training data sets were prepared from H_2O_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_2O_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 Ca^{2+} 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.

○ 3 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 +/-05 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 $Ca_v1.3$ Calcium Channel Dysregulation

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Systemic deletion of $Ca_v1.3$ Ca^{2+} channels rendered mice susceptible to atrial fibrillation (AF). This study was designed to explore the mechanisms underlying the regulation $Ca_v1.3$ involved inatrial arrhythmogenesis. A novel $Ca_v1.3$ associated protein, snapin was identified using YHT system. By co-immunoprecipitation and immunostaining assays, a physical interaction and co-localization between snapin and $Ca_v1.3$ 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 $Ca_v1.3$ were significantly impaired by snapin overexpression, causing $Ca_v1.3$ 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 $Ca_v1.3$ 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 $Ca_v1.3$ Ca^{2+} channel dysregulation. The precise molecular mechanisms contributing to snapin related regulation of $Ca_v1.3$, particularly in AF condition, should be addressed in future detailed studies. No COI.

ABS0183

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.

ABS0403

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.

ABS0013

"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.

○ 5 GENERAL INTERESTS /MEMBRANE AND EPITHELIAL TRANSPORT

ABS0164

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 life-style 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 life-style related diseases through controlling obesity, maintaining blood sugar, and preserving pancreatic beta-cells. No COI.

ABS0288

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 $\alpha 1$ (DFA) is greater in AF-stroke patient. This finding indicates remarkable complexity in physiological response associated with predominant parasympathetic drive. No COI

ABS0368

Decreasing cholesterol and fat meat using Citrus sinensis waste on Padjadjaran sheep

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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 (6^{th} 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.

ABS0360

Ambroxol activated respiratory mucociliary transport via pH_i increase and $[Cl^-]_i$ decrease in mice.

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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 ℃. ABX gradually increased CBA and CBF via pH_i increase and $[Cl^-]_i$ decrease. 1) pH_i pathway: ABX increased pH_i by activating NBC, leading to increases in CBA and CBF. 2) $[Cl^-]_i$ pathway: ABX elevated $[Ca^{2+}]_i$ by activating nifedipine-sensitive calcium channels, resulting in cell shrinkage via an increase in Ca^{2+}-dependent KCl efflux associated with a decrease in $[Cl^-]_i$ of ciliary cells. This decrease in $[Cl^-]_i$ increased CBA. In conclusion, ABX increased CBF and CBA via a pH_i increase by activating NBC and increased CBA via a $[Cl^-]_i$ decrease by causing cell shrinkage. Thus, ABX stimulated the ciliary beating coupled with transepitherial HCO_3^-/Cl^- secretion by modulating activity of ion channels/transporters. No COI.

○ 6 CELL AND MOLECULAR PHYSIOLOGY/RESPIRATORY PHYSIOLOGY

ABS0066

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.

ABS0175

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 P_i 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 integrinβ4 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-CSFtreated mice. Conclusion: The bone marrow stem cells, which are mobilized by G-CSF, have a protective effect against pulmonary fibrosis. No COI.

ABS0069 *Young Scientist Award*

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.

ABS0108 *Young Scientist Award*

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 techniquies. 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 (VO_2), 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 ℃). 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.

ABS0171 *Young Scientist Award*

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.

ABS0208 *Young Scientist Award*

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.

ABS0232 *Young Scientist Award*

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 (107 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 AUGg of OGTT ($p<0.05$; 2.78±1.0 AUGg in probiotic vs. 3.5±0.5 AUGg in vehicle). These findings suggest that probiotics improves insulin sensitivity and attenuated cognitive decline in obese-insulin resistant subjects. No COI.

ABS0103 *Young Scientist Award*

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.

ABS0141 *Young Scientist Award*

Tualang Honey Ameliorates Hydrogen Peroxide-Induced Endothelial Hyperpermeability

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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 0.5mM 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%). Immunofluorescence confirmed that TH reduced stress fiber formation and β-catenin reorganization, and significantly down-regulated intracellular calcium signaling with the highest percentage of inhibition at 0.1% (98.09%), while capable to maintain the level of cAMP when induced with H2O2. In conclusions, TH ameliorates H2O2-induced endothelial hyperpermeability via suppression of adherence junction protein re-distribution and calcium level. No COI.

ABS0193 *Young Scientist Award*

Dipeptidyl peptidase 4 inhibitor prevents left ventricular remodeling after chronic myocardial infarction in obese-insulin resistant rats

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Obesity has become a health problem world wide, and has been associated with increased risk of myocardial infarction (MI). Furthermore, adverse cardiac remodeling after MI could lead to progressive heart failure. Dipeptidyl peptidase-4 (DPP-4) inhibitor has been shown to exert cardioprotection in various animal models. However, the effects of DPP-4 inhibitor on left ventricular (LV) function and remodeling after chronic MI in obese-insulin resistant model has not been investigated. We tested the hypothesis that DPP-4 inhibitor reduces LV dysfunction and remodeling in obese-insulin resistant rats with chronic MI. Rats were fed with either high-fat diet (HFD) or normal diet (ND) for 12 weeks, followed by left anterior descending coronary artery ligation to induce MI. One week after ligation, rats in each dietary group were divided into 5 subgroups to receive vehicle, enalapril (positive control: 10 mg/kg/day), metformin (30 mg/kg/day), DPP-4 inhibitor vildagliptin (3 mg/kg/day), and combined metformin and vildagliptin for 8 weeks. Metabolic parameters, LV function, pathology and biochemical parameters of LV remodeling were determined. After MI, HFD rats had severe insulin resistance and higher mortality rate. All treatments improve insulin resistance, reduced mortality rate, preserved LV function, and attenuated LV hypertrophy and fibrosis in obese-insulin resistant rats. Interestingly, DPP-4 inhibitor effectively reduced cardiomyocyte cross-sectional area as well as ERK1/2 phoshorylation better than enalapril. However, metformin neither improved LV function nor reduced LV remodeling in ND rats with MI. In conclusion, DPP-4 inhibitor exerts better cardioprotection than enalapril by attenuating LV remodeling in obese-insulin resistant rats with MI. No COI.

ABS0194 *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 Ca^{2+} 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 Ca^{2+} via Na^{+}/Ca^{2+} 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 Ca^{2+} transient was measured with Fura-4F. The myocytes were stretched for 300 seconds. The stretch slowly increased the Ca^{2+} 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 Ca^{2+} 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.

ABS0210 *Young Scientist Award*

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.

○9 ENDOCRINOLOGY AND METABOLISM

ABS0075

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.

ABS0218

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 10uM 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.

ABS0456

Effects of phytosterols as a functional food-additive on the adrenal function in the Japanese quail

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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 resistance 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.

ABS0227 *Young Scientist Award*

Humanin exerts cardioprotection against cardiac ischemia-reperfusion injury via attenuating cardiac mitochondrial dysfunction

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Recanalization of occluded coronary arteries is a gold standard of treatment for acute myocardial infarction (AMI). However, reperfusion itself can cause myocardial damage, which known as ischemia/reperfusion (I/R) injury. Several pharmacological interventions have been extensively study 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 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.

ABS0230 *Young Scientist Award*

T-type calcium channel blocker exerts similar efficacy as deferiprone in attenuating cardiovascular dysfunction in iron-overload thalassemic mice

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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 important role in Fe^{2+} 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 bKO 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 finding 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.

ABS0231 *Young Scientist Award*

Combined therapy of iron chelator and antioxidant completely restores left ventricular dysfunction in iron-overloaded rats

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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.

ABS0153 *Young Scientist Award*

A Voltage-dependent K^+ current showing slow activation and inactivation kinetics in rat odontoblasts

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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 (E_{rev}) of the current was shifted 10–20 mV to hyperpolarizing potential (n = 6). Selected changes in $[K^+]o$ in the gluconate-based in intracellular and extracellular solution without Cl^- showed a shift in the E_{rev} 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.

○ 11 MUSCLE PHYSIOLGY / GASTROINTESTINAL PHYSIOLOGY

ABS0017

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.

ABS0293

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.

ABS0020

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 use 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.

ABS0285

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.

ABS0364

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.

ABS0464

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.

ABS0489

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.

ABS0015

The effects of meniran extracts (phyllantus nururi lyn) to COX2 mRNA expression in collitis 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.

ABS0503

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, LNCaPdcc, 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.

ABS0145 *Young Scientist Award*

High-fat high-fructose diet exacerbates hepatic steatosis under estrogen-deprived condition.

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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.

ABS0151 *Young Scientist Award*

The molecular mechanism underlying hypoxia-inhibited DNA methyltransferase 1 expression in endometriosis

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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 AUF1-mediated DNMT1 downregulation. Hypoxia-mediated DNMT1 downregulation and global DNA hypomethylation de-repressed 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.

ABS0195 *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.

ABS0147 *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.

ABS0150 *Young Scientist Award*

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 compornent 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.

ABS0165 *Young Scientist Award*

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.

ABS0144 *Young Scientist Award*

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.

ABS0126 *Young Scientist Award*

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), Ca^{2+}-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.

ABS0142 *Young Scientist Award*

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.

ABS0157 *Young Scientist Award*

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 oxegenase 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.

ABS0233 *Young Scientist Award*

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 Unclassified, 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 defection 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.

ABS0138 *Young Scientist Award*

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 $[Ca^{2+}]_i$ than that at a high $[Ca^{2+}]_i$. Moreover, in unstimulated cells, an extremely low $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ 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.

ABS0119 *Young Scientist Award*

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 mucosae. 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 fMRI 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 Ca2+ imaging under chronic pain conditions. Following peripheral nerve ligation (PSL), we observed increased Ca2+ 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.