Poster Presentations

Young Scientist Awards (Poster)

P1 ALTERNATIVE AND COMPLEMENTARY MEDICINE

ABS0106

Protective effect of vitamin E against ethanol-induced structural alteration, oxidative stress and inflammatory reaction in small intestine of rat

Alireza Shirpoor[1]*
[1]*Department of Physiology, Faculty of Medicine, Iran*
**Email : ashirpoor@yahoo.com*

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

ABS0237

Cholesterol-lowering activity of Spirogyra neglecta extract

Acharaporn Duangjai[1]*, Kanittaporn Trisat[2], Nanteetip Limpeanchob[2], Doungporn Amornlerdpison[3]
[1]*Division of Physiology, University of Phayao, Thailand* [2]*Department of Pharmacy Practice, Pharmacological Research Unit, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand* [3] *Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand*
**Email : achara.phso@gmail.com*

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

ABS0259

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

Thanakorn Ninthawat[1]*, Thamolwan Suanarunsawat[2], Oraphan Wanakhachornkrai[1]
[1]*Physiology Unit, Faculty of Science, Rangsit University, Thailand* [2]*Department of Medical Sciences, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Thailand*
**Email : tk.ninthawat@hotmail.com*

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

ABS0264

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

Komchan Chumchuen[1]*, Thamolwan Suanarunsawat[2], Oraphan Wanakhachornkrai[1]
[1]*Physiology Unit, Faculty of Science, Rangsit University, Thailand* [2] *Department of Medical Sciences, Faculty of Medicine Vajira Hospital, Navamindradhiraj University, Thailand*
**Email : kch.t8n@hotmail.com*

The present study was conducted to investigate the effect of essential oil extracts from Ocimum sanctum L. leaves (EOOS) on blood glucose, serum lipid and organ protection in rats fed with high calorie diet (HCD). The rats were divided in to three groups including normal control group, HCD group and HCD group treated with EOOS for 4 months. The results show that HCD increased blood glucose, serum insulin and HOMAR IR. It also raised serum lipid and atherogenic index (AI). Serum levels of AST, ALT and LDH were significantly increased in HCD rats. The liver and myocardial tissues were damaged as shown by histopathological examination. EOOS decreased serum lipid and AI without effect on the high levels of blood glucose, serum insulin and HOMAR IR. It also decreased the high serum levels of ALT and LDH with slightly decreased serum levels of AST and CK-MB. The liver and myocardial tissues were preserved in rats treated with EOOS. It can be concluded that HCD increases insulin resistance, resulting increased both blood glucose and serum lipid. EOOS decreased serum lipid without effect on blood glucose and insulin levels. It also protects the liver and myocardial tissues against HCD. No COI.

ABS0271

Cytotoxic effect of rice bran oil containing different concentrations of gamma-oryzanol

Papavadee Klongpityapong[1], Janpen Tangjitjareonkun[2], Roongtawan Supabphol[3]*
[1]*School of Pharmacy, Eastern Asia University, Thailand*[2]*Department of Basic Science and Physical Education, Faculty of Science at Si Racha, Kasetsart University, Si Racha Campus, Chonburi 20230, Thailand* [3]*Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Bangkok 10110, Thailand*
**Email : roongs@swu.ac.th*

Rice bran oil (RBO), is recently gaining commercial interest due to its health benefit and biological effects. Gamma-oryzanol has been suggested to be the important ingredient for health promotion, especially improving the plasma lipid profile. The present study was carried out to compare the cytotoxicity of rice bran oil containing different concentration of gamma-oryzanol. Cytotoxic activity of RBO on human prostate cancer cells, DU145, was performed by proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent. Cells were treated with RBO in a humidified atmosphere of 5% CO_2 at 37 °C for 7 days. Culture medium containing 1 mg/ml of MTT dye was added into each well, incubated for 4 h and then replaced with DMSO. The blue color of the oxidized MTT was determined by measuring the absorbance at 570 nm. Three concentrations of RBO, 5,000, 8,000 and 15,000 ppm, significantly inhibited prostate cancer cell growth in a dose- and time-dependent fashion. Half of the cells were dead at RBO concentration (IC50) of 1.06 ± 0.06, 0.36 ± 0.06 and 0.27 ± 0.04 mg/ml, respectively. These results showed the potential of RBO for anticancer activity. Its mechanism might be both direct, such as cytotoxicity, and indirect effect to the cancer cells, such as antioxidant activity as claimed about the plasma cholesterol and triglycerides in previous papers. Further study of the antioxidant gene expression is worthwhile. No COI.

ABS0338

Bathing in artificial-CO_2-hot spring between resistance training may inhibit muscle fatigue progression after the bath

Masaaki Hashimoto[1]*, Noriyuki Yamamoto[2]
[1]*Laboratory of Physiology, Department of Physical Therapy, Faculty of Medical Sciences, Teikyo University of Science, Japan* [2]*Department of Health Science, Japanese Red Cross Hokkaido College of Nursing, Japan*
**Email : mhashi@ntu.ac.jp*

Artificially made CO_2-hot spring water (CO_2-water: contains CO_2 > 1000ppm), as well as natural hot spring water, dilates skin blood vessels in the body part immersed in it, hence increases skin blood flow. Previous observations indicated that a similar vasodilation could occur in the skeletal muscle under the skin of immersed part. If muscle blood flow is improved by CO_2-water immersion, recovery of muscle fatigue also might be promoted. In the present study we investigated this hypothesis by means of water immersions of the forearms between three series of a continual measuring of grip strength. Subjects (Ss) were 11 healthy student volunteers. Ss were measured the grip strength in both hand simultaneously with one's utmost effort for 10 s and then rested for 5 s, which was repeated 25 times continuously as a set of trials. Each Ss performed 3 sets in one experiment. During 10 min of resting between two sets, Ss put both forearms in right and left separate bathtubs of two arm-bath apparatus filled with a tap water or CO_2-water in a same temperature (32~35 °C) or with a room air (24 °C). Grip strength decreased 20 to 30 % of initial level by 25 times repetition in each set. Initial levels of grip strength of each set decreased gradually as a set advances, but forearm treatment for 10 min between the sets did not affect the decreases. In the third set, attenuation of the grip strength to the end of a set was significantly smaller in the arm with water immersion than in non-immersion arm. Though a significant difference between treatments with CO_2-water and tap -water was not detected under the present conditions, CO_2-water immersion might be effective in suppressing progression of muscle fatigue. No COI.

ABS0371

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

Yoke Keong Yong[1]*, Zuraini Ahmad[2]
[1]*Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia* [2]*Department of Biomedical Science, UPM, Malaysia*
**Email : yoke_keong@upm.edu.my*

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

ABS0428

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

Dangjai Souvannakitti[1]*, Thanis Chuenmeechao[2], Ladavan Souvannakitti[2]
[1]*Phramongkutklao College of Medicine, Thailand* [2]*Baansuan Saha Clinic, Thailand*
**Email : drdangjais@gmail.com*

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

ABS0462

Vasoactive effects of semen from Psoralea Corylifolia on rat artery

Jihyun Kim[1], A Kassahun[1], TD Wijerathne[1], KP Lee[1]*
[1]*Chungnam National University, Korea*
**Email : kplee@cnu.ac.kr*

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

ABS0466

The preventive effect of Thunbergia laurifolia, Linn. on alcohol detoxification in rats

Ratirat Sangpayap[1], Watchareewan Thongsaard[2]*, Theekapun Charoenpong[3], Sunan Chainaku[4]
[1]*Faculty of Medicine, Srinakarinwirot University, Thailand* [2]*Department of Physiology, Faculty of Medicine, Srinakharinwirot University, Thailand* [3]*Department of Biomedical Engineering, Faculty of Engineering, Srinakharinwirot University, Thailand* [4]*Department of Chemistry, Faculty of Science, Srinakharinwirot University, Thailand*
**Email : watchare@gmail.com*

Thunbergia laurifolia Linn (TL) or Rangchert is a thai herbal medicine which has been used as crude extracts for various detoxification including alcohol intoxication. This study aims to evaluate the preventive effect of TL on alcohol intoxication using various behavioral models. Balance and motor coordination were determined using a narrow balance beam elevated 50 cm from the ground. The rats were placed at 30, 50, 80 and 100 cm away from the goal box and allowed to traverse the beam to the goal box and the mean number of hindlimb footslips during three trials was recorded. The effect of alcohol and TL on anxiety or sedation and exploratory behavior were also tested using elevated plus-maze and hole-board, respectively. A methanol extract of TL (200 mg/kg, orally) was administrated to Wistar rats (300–350 g) 60 minutes before 20% ethanol (2 g/kg, i.p.) administration. The behavioral tests were performed 30 minutes later started with the elevated plus maze (5 min.), hole-board (10 min) and balance beam. The results demonstrated that ethanol administration significantly increased ($P < 0.05$) the number of hindslips by 100, 250, 50 and 145% at 30, 50, 80 and 100 cm, respectively, indicating the poor motor performance when compared to control group. When TL was pretreated to the rats prior to ethanol exposure, the number of hindslips were significantly reduced ($P < 0.05$) by 20, 80, 58 and 40% at 30, 50, 80 and 100 cm distance, respectively. The results indicated that TL can reverse the effect of alcohol intoxication represented by the improvement of balance and motor behavior. However, there are no significant effect of ethanol and TL on anxiety, sedation and exploratory behavior. No COI.

P2 CARDIOVASCULAR PHYSIOLOGY & MICROCIRCULATION

ABS0050

Lumbrokinase attenuates myocardial ischemia-reperfusion injury by inhibiting TLR4 signaling

Hsiao Ting Wang[1], Shiang-Suo Huang[1]*, Jinghua Tsai Chang[1], Jiuan-Miaw Liao[1]
[1]*Chung Shan Medical University, Taiwan*
**Email : sshuang@csmu.edu.tw*

Lumbrokinase, a novel antithrombotic agent purified from the earthworm Lumbricus rubellus, has been clinically used to treat stroke and cardiovascular diseases. However, inflammatory responses in the cardioprotective effect of lumbrokinase remain unknown. In this study, the signaling pathways involved in lumbrokinase-inhibited expression of inflammation mediators were investigated in rats subjected to myocardial ischemia-reperfusion (I-R) injury. The left main coronary artery of anesthetized rats was subjected to 60 min occlusion and 3 h reperfusion. The animals were administrated with and without lumbrokinase, and the severity of I-R-induced arrhythmias and infarction were compared. Lumbrokinase inhibited I-R-induced arrhythmias and mortality. Lumbrokinase decreased the lactate dehydrogenase levels in carotid blood during the same period. Lumbrokinase also inhibited the enhancement of the I-R induced expression of cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), and matrix metalloproteinase (MMP)-9 through toll-like receptor 4 (TLR4) signaling pathway. Our results also demonstrated that stimulation with lumbrokinase decreased the phosphorylation of JNK, IÎºB, and NF-κB.These findings suggest that lumbrokinase is a potent antiarrhythmic agent with cardioprotective properties in rats with I-R injury. The cardioprotective effects of lumbrokinase may be correlated with its inhibitory effect on the I-R-induced expression of COX-2, iNOS and MMP-9 and mediated by the TLR4 signaling through the JNK and NF-κB pathways. No COI.

ABS0068

Alterations in vascular functions in experimental androgen deprivation.

Faten Diab[1]*, Mahmoud-Hani Ayobe[1], Amira Metwalli[1], Mona Ahmed[1], Mohmed Hassen[1], Ghida Hassan[1]
[1]*Faculty of Medicine, Ain Shams Univeristy, Egypt*
**Email : dtoona2000@yahoo.com*

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

ABS0081

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

Dachuan Dong[1], Liangyuan Li[1], Caihua Yuan[1], Yin Zhang[1], Xiang Gao[2], Zhao Zhang[1]*
[1]*Nanjing Normal University, China* [2]*Model Animal Research Center of Nanjing University, China*
**Email : zhangzhao@njnu.edu.cn*

We have previously observed that the mice with cardiac-targeted deletion of Ppp2ca gene (KO) developed hypertrophic cardiomyopathy at the postnatal day 11 and died around the day 13. The aim of present study was to analyze the electrical remodeling features in KO mice. As compared to control, cardiac hypertrophy was evidenced by the increases in left ventricle weight to body weight ratio and left ventricle mass, by the significant elevation of hypertrophy markers expression, by the increase in both LVED and LVSD as well as by the decrease in the FS and EF in KO mice ($P<0.05$). In addition to morphological changes observed in HE staining, notable ultrastructural alterations in KO myocytes were addressed in TEM analysis. Moreover, electrophysiological recordings indicated that the duration of APs in KO myocytes were markedly prolonged as compared with those in control. In contrast, no changes were observed in RPs and other parameters of APs. Consequently, a significant reduction in whole-cell Ito, and intermediate increase of whole-cell ICa-L were documented in KO myocytes. In consistence with changes in currents, a down-regulations of Kv4.2, Kv4.3, Kv1.4 and KChIP2 proteins co-assembling α and β subunits of Ito channel were detected by western blot analysis. Whereas, notably, not an increased expression of Cav1.2 but the increased phosphorylation of $Ca_v1.2$ Ca^{2+} channels in KO mice was demonstrated by immunohistochemistry and western blot analysis in comparison with those of control. Taken together, these findings are indicative of the fundamental role of PP2A in mouse heart and imply that deficiency of PP2A may perturb phosphorylation status of $Ca_v1.2$ and intracellular Ca^{2+} homeostasis, leading to hypertrophic cardiomyopathy along with electrical remodeling. No COI.

ABS0128

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

Fawzi Babiker[1]*, Ala'a Abwainy[1]
[1]*Kuwait University, Kuwait*
**Email : fawzi.b@hsc.edu.kw*

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

ABS0206

Combined NSAID to antidepressant treatment induces cardiovascular alterations in an animal model of depression

Petcharat Trongtorsak[1]*, Uraiwan Intamaso[1], Wanida Olankijanunt[2]
[1]*Faculty of Allied Health Sciences, Burapha University, Thailand* [2]*Faculty of Pharmaceutical Sciences, Burapha University, Thailand*
**Email : petchara@buu.ac.th*

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

ABS0249

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

Won Sun Park[1]*, Hongliang Li[1], Han Sol Kim[1], Hye Won Kim[1]
[1]*Department of Physiology, Kangwon National University School of Medicine, Korea*
**Email : parkws@kangwon.ac.kr*

We investigated the effects of the calmodulin inhibitor CGS 9343B on voltage-dependent K^+ (Kv) channels using whole-cell patch clamp technique in freshly isolated rabbit coronary arterial smooth muscle cells. CGS 9343B inhibited Kv currents in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC50) value of 0.81 μM. The decay rate of Kv channel inactivation was accelerated by CGS 9343B. The rate constants of association and dissociation for CGS 9343B were $2.77 \pm 0.04\ \mu M^{-1}s^{-1}$ and $2.55 \pm 1.50\ s^{-1}$, respectively. CGS 9343B did not affect the steady-state activation curve, but shifted the inactivation curve toward to a more negative potential. Train pulses (1 or 2 Hz) application progressively increased the CGS 9343B-induced Kv channel inhibition. In addition, the inactivation recovery time constant was increased in the presence of CGS 9343B, suggesting that CGS 9343B-induced inhibition of Kv channel was use-dependent. Another calmodulin inhibitor, W-13, did not affect Kv currents, and did not change the inhibitory effect of CGS 9343B on Kv current. Our results demonstrated that CGS 9343B inhibited Kv currents in a state-, time-, and use-dependent manner, independent of calmodulin inhibition. No COI.

ABS0250

The signaling mechanisms of cilostazol-induced vasodilation by activation of big conductance Ca^{2+}-activated K^+ channels in aortic smooth muscle

Hongliang Li[1], Won Sun Park[1]*
[1]*Department of Physiology, Kangwon National University School of Medicine, Korea*
**Email : parkws@kangwon.ac.kr*

We investigated the effect of cilostazol in phenylephrine (Phe)-induced pre-contracted aortic rings. Cilostazol induced vasorelaxation in a concentration-dependent manner. Application of the voltage-dependent K^+ (Kv) channel inhibitor 4-AP, the ATP-sensitive K^+ (KATP) channel inhibitor glibenclamide, and the inwardly rectifying K^+ (Kir) channel inhibitor Ba^{2+} did not alter the vasorelaxant effect of cilostazol; however, treatment with the big-conductance Ca^{2+}-activated K^+ (BKCa) channel inhibitor paxilline inhibited the vasorelaxant effect of cilostazol. This vasorelaxant effect of cilostazol was reduced in the presence of an adenylyl cyclase or a protein kinase A (PKA) inhibitor. Inside-out single channel recordings revealed that cilostazol induced the activation of BKCa channel activity. The vasorelaxant effect of cilostazol was not affected by removal of the endothelium. In addition, application of a nitric oxide synthase and a small-conductance Ca^{2+}-activated K^+ (SKCa) channel inhibitor did not affect cilostazol-induced vasorelaxation. We conclude that cilostazol induced vasorelaxation of the aorta through activation of BKCa channel via a PKA-dependent signaling mechanism. No COI.

ABS0251

The inhibitory effect of efonidipine, a T-type Ca^{2+} channel inhibitor, on voltage-dependent K^+ current in rabbit coronary arterial smooth muscle cells

Hye Won Kim[1], Won Sun Park[1]*
[1]*Department of Physiology, Kangwon National University School of Medicine, Korea*
**Email : parkws@kangwon.ac.kr*

The effect of efonidipine, a commercially available antihypertensive drug and Ca^{2+} channel inhibitor, on voltage-dependent K^+ (Kv) channels was studied in freshly isolated rabbit coronary arterial smooth muscle cells using the whole-cell patch clamp technique. The amplitude of Kv current was decreased by application of efonidipine in a dose-dependent manner, with IC50 of 0.26 μM and a Hill coefficient of 0.91, which suggests 1:1 binding stoichiometry. Efonidipine did not affect voltage-dependent activation of the Kv channel, but shifted the inactivation curve by –8.87 mV. The inhibitory effect of efonidipine was not significantly changed by depletion of extracellular Ca^{2+} or intracellular ATP, which indicated no involvement of the Ca^{2+} channel or intracellular protein kinase-dependent cascades. We conclude that efonidipine dose-dependently inhibits Kv current in a phosphrylation- and Ca^{2+} channel-independent manner. No COI.

ABS0252

The direct inhibition of trifluoperazine, an antipsychotic drug and calmodulin inhibitor, on voltage-dependent K^+ channels in coronary arterial smooth muscle cells

Han Sol Kim[1], Won Sun Park[1]*
[1]*Department of Physiology, Kangwon National University School of Medicine, Korea*
**Email : parkws@kangwon.ac.kr*

We investigated the effect of the calmodulin inhibitor and antipsychotic drug trifluoperazine on voltage-dependent K^+ (Kv) channels. Kv currents were recorded by whole-cell configuration of patch clamp in freshly isolated rabbit coronary arterial smooth muscle cells. The amplitudes of Kv currents were reduced by trifluoperazine in a concentration-dependent manner, with an apparent IC50 value of 1.58 ± 0.48 μM. The rate constants of association and dissociation by trifluoperazine were 3.73 ± 0.33 $\mu M^{-1}s^{-1}$ and 5.84 ± 1.41 s^{-1}, respectively. Application of trifluoperazine caused a positive shift in the activation curve but had no significant effect on the inactivation curve. Furthermore, trifluoperazine provoked use-dependent inhibition of the Kv current under train pulses (1 or 2 Hz). These findings suggest that trifluoperazine interacts with Kv current in a closed state and inhibits Kv current in the open state in a time- and use-dependent manner, regardless of its function as a calmodulin inhibitor and antipsychotic drug. No COI.

ABS0261

Optical mapping study of the circus movement of the excitatory waves in the rat isolated atrium preparation under the intracellular Ca^{2+} overloaded condition

Tetsuro Sakai[1]*
[1]*Department of Systems Physiology, University of The Ryukyus, Graduate School of Medicine, Japan*
**Email : tsakai@med.u-ryukyu.ac.jp*

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

ABS0278

Chemerin decreases contractility and coronary flow of isolated rat hearts

Özden Kutlay[1], Ziya Kaygisiz[1]*, Bilgin Kaygisiz[2]
[1]Department of Physiology, Faculty of Medicine, Eskisehir Osmangazi University, Turkey [2]Department of Pharmacology, Faculty of Medicine, Eskisehir Osmangazi University, Turkey
**Email : ziyak@ogu.edu.tr*

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

ABS0280

Has omentin a negative inotropic impact on isolated rat heart?

Özden Kutlay[1]*, Ziya Kaygisiz[1], Bilgin Kaygisiz[2]
[1]Department of Physiology, Faculty of Medicine, Eskisehir Osmangazi University, Turkey [2]Department of Pharmacology, Faculty of Medicine, Eskisehir Osmangazi University, Turkey
**Email : ozden.2007@gmail.com*

Omentin is a recently identified novel adipocytokine mainly expressed in epicardial adipose tissue. Although it has favorable effects on cardiovascular disease, impact of omentin on the hearts is still unknown. Hence we aimed to investigate the potential effects of omentin on the heart and to explore whether phosphoinositide 3-kinase (PI3K) signalling pathway mediates cardiac contractility induced by omentin. Therefore, we studied the possible effect of omentin on left ventricular developed pressure (LVDP; an index of cardiac contractility), maximal rate of pressure development of left ventricle (+dP/dtmax; another index of cardiac contractility), heart rate (HR), coronary flow (CF), monophasic action potential amplitude (MAPamp), MAP duration at 90% repolarization (MAP90). The hearts were isolated under sodium thiopental (50 mg/kg) anesthesia and perfused with modified Krebs-Henseleit solution (mK-Hs). After stabilization, 100, 200 and 400 ng/ml doses of omentin was administered to the hearts for 30 minutes. In another group, the hearts were perfused with mK-Hs containing LY294002 (10 μM), a PI3K inhibitor, for 5 minutes before 400 ng/ml omentin administration. One hundred, 200 and 400 ng/ml doses of omentin significantly decreased LVDP and +dP/dtmax. Omentin at all doses had no effect on HR, CF, MAPamp and MAP90. Our study is therefore the first one that demonstrates that an acute omentin treatment leads to a negative inotropic action on isolated rat heart. This effect may be mediated by PI3K-dependent mechanism. No COI.

ABS0292

The Study of the serum level of IL-4, TGF-β, IFN-γ and IL-6 in overweight patients with and without Diabetes and Hypertension

Gholamreza Asadikaram[1]*, Maryam Ram[1], Alireza Izadi[1]
[1]*Endocrinology and Metabolism Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran*
*Email : asadi_ka@yahoo.com, gh_asadi@kmu.ac.ir

Diabetes is one of the main reasons of mortality in the world. Overweight increases the risk of diabetes and hypertension which both expose individuals to cardiovascular diseases, renal failure and brain stroke. The cytokines are involved in the pathogenesis of these diseases. The objective of the present study is to analyze the serum levels of IL-4, TGF-β, IFN-γ, and IL-6 cytokines of overweight men with diabetes and/or hypertension so as to highlight the association of these cytokines with such diseases. Descriptive study was carried out on 164 individuals aging 20-50 years with BMI ranging 28.5-30 kg/m2 participating in Kerman coronary artery disease risk factors study (KERCADRS). In this regard, 54 men lacked diabetes and hypertension (CTL group), 36 individuals had both diabetes and hypertension (DH group), 20 individuals had diabetes without hypertension (D group), and 54 persons had hypertension without diabetes (H) all of which were selected for measurement of serum cytokines through ELISA kits. Data were analyzed using Kruskal-Wallis H test followed by Mann-Whitney U test with Bonferroni correction for pairwise comparisons. The results showed that the concentration of IFN-γ in DH group is significantly higher than the CTL group (p=0.002), IL-6 in DH and D groups is significantly lower than the CTL group (p=0.006, p=0.004, respectively). The serum level of TGF-β and IL-4 cytokines did not show any significant difference across the four groups. It seems that the proinflammatory cytokine IFN-γ has a significant role in the pathogenesis of hypertension in diabetes. No COI.

ABS0300

Cardiac progenitor cells that spontaneously develop into beating cardiomyocytes in the adult mouse heart

Yuka Nishino[1], Mariko Omatsu-Kambe[1]*, Nozomi Nozuchi[1], Hiroshi Matsuura[1]
[1]*Shiga University of Medical Science, Japan*
**Email : m_omatsu@belle.shiga-med.ac.jp*

We recently discovered a novel subpopulation of adult mouse heart cells that spontaneously develop into beating cardiomyocytes in the culture of cardiomyocyte-removed fraction cells (non-myocytes). We defined these beating cells as atypically-shaped cardiomyocytes (ACMs) based on their peculiar morphology. In cultures of the non-myocytes, some of the small cells were found to grow in size or fuse with each other to become more complexly shaped multi-nuclear beating ACMs without requiring hormones or chemicals. The ACMs then continued to beat for at least three weeks, but did not appreciably proliferate. ACMs could be identified in the neonatal heart and survive the long-term post-natal development while preserving the expression of fetal cardiac gene products, such as atrial natriuretic peptide (ANP) and T-type Ca^{2+} channel ($Ca_v3.2$). When non-myocytes and myocytes were co-cultured, the ACMs did not fuse to ventricular myocytes, but rather independently developed into beating cells. Cellular prion protein (PrP), also known as CD230, was found to serve as a marker for ACMs that enable us to identify these cells within various types of non-myocytes, such as fibroblasts, in the culture. In combination with cardiac-specific contractile protein cardiac troponin T (cTnT), PrP was demonstrated to specifically identify native ACMs in the interstitial spaces among ventricular myocytes in the heart. These data suggest that quiescent but still functionally viable cardiac progenitors, originating from the fetal stage, exist in the adult mouse heart. No COI.

ABS0335

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

Nozomi Nozuchi[1], Mariko Omatsu-Kambe[1]*, Yuka Nishino[1], Hiroshi Matsuura[1]
[1]*Department of Physiology, Shiga University of Medical Science, Japan*
**Email : m_omatsu@belle.shiga-med.ac.jp*

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

ABS0347

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

Thanaporn Kaewphaleuk[1], Wattana B. Watanapa[1]*
[1]*Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
**Email : wattana.wat@mahidol.ac.th*

Ethanol (EtOH) is known to induce coronary vasorelaxation and NO release. In other cells, it can modulate several ion channels, especially Ca^{2+}-activated potassium channel (KCa), which can influence endothelial NO release. Additionally, EtOH-induced NO release may be mediated by adenosine receptor (AdoR) in umbilical veins endothelial cells. Therefore, we hypothesized that EtOH may affect K^+ channels in human coronary artery endothelial cells (HCAECs), via AdoR, leading to NO release. We investigated the effects of acute ethanol application (1-50 mM) on HCAEC whole-cell currents in response to a voltage ramp (-110 to +100 mV in 200 ms) to compare whole-cell currents with vs. without cell exposure to EtOH. Current-voltage relation was constructed for each cell. % Control of whole-cell currents at +80 mV after acute application of 1, 3, 10, 20, 30 and 50 mM EtOH were (mean+s.e.m.) 95.31+1.87 % (n=6; not significant, ns), 102.99+4.35 % (n=7, ns), 103.48+0.90 % (n=6; $p<0.05$), 129.11+9.99 % (n=9; $p<0.05$), 124.23+6.99 % (n=9; $p<0.05$) and 141.71+10.21 % (n=6; $p<0.05$), respectively. However, after preincubation with 10 μM CGS15943, a non-selective AdoR antagonist, EtOH could no longer increase HCAEC currents significantly (n=5; ns). Our data indicate that 10-50 mM EtOH may increase whole-cell currents in HCAECs via adenosine receptor activation. No COI.

ABS0362

Bilobalide-induced SKCa current increase in human coronary artery endothelial cells is mediated by 5-HT1/2 receptor and PLC

Patcharaporn Taokum[1], Wattana B. Watanapa [1]*, Katesirin Ruamyod[1]
[1]*Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand*
**Email : wattana.wat@mahidol.ac.th*

Ginkgo biloba leaves extract and its active ingredient bilobalide have been shown to cause vasorelaxation in some vascular beds. Recently, our laboratory demonstrated in human coronary artery endothelial cells (HCAECs) that bilobalide could dose-dependently increase whole-cell currents through activation of SKCa channels, but the responsible mechanism remains to be elucidated. In this study, HCAEC currents in response to a voltage ramp (-100 to +100 mV, 200 ms) were recorded using whole-cell patch clamp technique. With external SKCa isolating solution (containing blockers of BKCa, IKCa, Kir, TRP and Cl^- channels), 100 μM bilobalide significantly enhanced SKCa currents by 63.62 ± 46.48 % compared to control (n=6, $p<0.05$), while subsequent exposure to 500 μM apamin, a specific inhibitor of SKCa channel, could significantly decrease the bilobalide-induced currents by 75.87 ± 24.86 %, (n=6, $p<0.05$). To investigate whether a 5-HT receptor was involved, HCAECs were pre-incubated with 1 μM methiothepin, a non-selective 5-HT1/2 receptor antagonist. The results showed that methiothepin pre-incubation could prevent HCAEC current increase in 100 μM bilobalide (n=5, $p>0.05$). Moreover, pre-incubation with U73122, a specific PLC blocker, also significantly decreased HCAEC currents after bilobalide exposure (n=5, $p<0.05$). Our results indicate that 100 μM bilobalide significantly increased SKCa currents in HCAECs via 5-HT1/2 receptors and possibly PLC activation. No COI.

ABS0337

The responses of pulmonary and systemic circulation and airway to allergic mediators in anesthetized BALB/c mice

Toshishige Shibamoto[1]*, Mofei Wang[1], Yuhichi Kuda[1], Mamoru Tanida[1], Yasutaka Kurata[1]
[1]*Kanazawa Medical University, Japan*
**Email : shibamo@kanazawa-med.ac.jp*

Systemic anaphylactic shock sometimes accompanies pulmonary vaso- and broncho-constriction. No systematic study in which the effects of anaphylactic substances were investigated on pulmonary vascular resistance (PVR) in in vivo mouse by directly measuring cardiac output and the inflow and outflow pressures in the pulmonary circulation. We determined the responses of pulmonary vascular resistance (PVR), total peripheral resistance (TPR) and airway pressure (AWP) to platelet-activating factor (PAF), histamine, serotonin, leukotriene (LT) C4, and prostaglandin (PG) D4 in anesthetized BALB/c mice. Pulmonary arterial pressure (PAP), left atrial pressure (LAP), and aortic blood flow were measured. PVR increased dose-dependently in response to consecutive administration of all vasoconstrictors with the order of maximal responsiveness being PAF>LTC4>serotonin>>histamine=PGD2. TPR decreased dose-dependently in response to PAF, serotonin, and histamine, while it showed an increase or no changes when LTC4 or PGD2 was injected, respectively. Serotonin, but not the other agents, increased airway pressure. In conclusion, PAF, LTC4 and serotonin cause substantial pulmonary vasoconstriction and PAF, serotonin, histamine vasodilatation. Serotonin induces airway constriction in anesthetized BALB/c mice. No COI.

ABS0392

Role of Stachytarpheta jamaicensis (L.) Vahl on lipopolysaccharides-induced inflammation in vitro

Pearl Majorie Liew[1]*, Zuraini Ahmad[1], Hasnah Bahari[1], Yoke Keong Yong[1]
[1]*Universiti Putra Malaysia, Malaysia*
**Email : pearliew920211@yahoo.com*

Inflammation is regarded as a complicated pathophysiology process that is triggered by direct activation of receptors or by the secretion of inflammatory mediators. These resulted in vasodilatation, increased vascular permeability, leakage of fluid and migration of immune cells at the site of inflammation. However, if prolonged, can lead to tissue damage as well as pathogenesis of fatal diseases. Stachytarpheta jamaicensis (SJ) or locally known as Selasih Dandi in Malaysia, belongs to the family of Verbenaceae. The plant was previously proven to have high medicinal properties, nevertheless, little is known on the anti-inflammatory benefit of SJ. Thus, this study was designed to explore the effect of SJ on inflammatory properties. The cytotoxicity of methanol extract of SJ was investigated against normal (HUVECs) and cancer cell lines (RAW 246.7) using 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. To determine the anti-inflammatory properties of SJ, nitric oxide (NO) production inhibitory activity was assessed in LPS-stimulated RAW 246.7 using Griess assay. The methanol extract of SJ was found to have no cytotoxicity against HUVECs and RAW 246.7 at concentrations ranging from 0.005 mg/ml – 0.1mg/ml. 45.28% maximum inhibition of NO in RAW 246.7 cells treated with methanol extract of SJ were observed at the dose of 0.1mg/ml. The MTT cell viability assay result revealed that the inhibitory effect of methanol extract was not due to cell damage (viability >85%). It is concluded that 0.1mg/ml of methanol extract of SJ could ameliorate LPS-induced inflammation by decreasing NO overproduction. Thus, these findings suggest that SJ might be a novel therapeutic strategy for treating vascular inflammation in the future. No COI.

ABS0399

Shear stress increase Sirtuin-1 expression to mediate shear-induced autophagy in endothelial cells

Li-Hong Wu[1], Danny Ling Wang[1]*
[1]*Institute of Medical Sciences, Tzu Chi University, Taiwan*
**Email : lingwang15@mail.tcu.edu.tw*

Blood flow-induced shear stress acted on endothelial cells (ECs) is essential for maintaining normal vascular function. Autophagy represents a homeostatic mechanism curcial for cell survival. The conversion of non-lipidated form LC3-I to autophagosome-membrane-associated lipidated form LC3-II is an indicator of autophagic activity. Sirtuins (SIRT1-7), a family of NAD+-dependent deacetylase, mediate adaptive responses to a wide spectrum of stresses. Earlier studies showed that Sirtuin 1 (SIRT1) participated in the process of autophagy. In our current study, we demonstrate that shear stress to ECs increases autophagy and SIRT1 participates in this shear stress-induced autophagy. Human Endothelial cells (ECs) were subjected to shear stress (5 or 12 dynes/cm2) in a well-defined parallel plate flow chamber system. Shear stress increased SIRT1 expression. Shear flow also enhanced NAD+/NADH ratio that is essential for Sirtuins activities. Shear stress stimulates autophagy as demonstrated by 1) an increase of LC3-II/LC3-I ratio 2) a decrease of P62 level and 3) an increase of RFP-LC3 puncta numbers in sheared ECs. Shear-induced autophagy is shown as time-dependent but shear force-independent manner. Interestingly, ECs treated with Sirtuins activator (SRT1720) also increased autophagy. Furthermore, shear stress to ECs with SIRT1 knockdown by SiRNA attenuated autophagy indicating that shear stress-induced autophagy is mediated at least partially through SIRT1. Furthermore, ECs treated with ROS scavenger (NAC) decreased shear-induced autophagy indicating ROS play a role in shear-induced autophagy. In conclusion, ECs under shear stress increase SIRT1 expression to promote autophagy and thus enhances endothelial homeostasis. No COI.

ABS0411

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

Cheng Yu Wang[1], Chun Hua Xuan[2], Xiang Dan Li[1], Dong Yuan Xu[1]*
[1]*Center of Morphological Experiment, Medical College of Yanbian University, China* [2]*Department of Cardiology, The affiliated Hospital of Yanbian University, China*
**Email : dyxu@ybu.edu.cn*

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

ABS0436

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

Yong Fen Qi[1]*
[1]*Chinese Association for Physiological Science, China*
**Email : yongfenqi@163.com*

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

ABS0471

Supraphysiological dose of testosterone induces hypertrophy of both cardiac and soleus muscles but differentially impacts on the mitochondrial functions.

Vassana Teekabut[1], Munthana Wadthaisong[1], Tepmanas Bupha-Intr[1], Jonggonnee Wattanapermpool[1]*
[1]*Department of Physiology, Faculty of Science, Mahidol University, Thailand*
**Email : jonggonnee.wat@mahidol.ac.th*

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

ABS0476

Hesperetin-induced vesorelaxation in human umbilical vein preparation

Kritsana Tipcome[1]*, Wattana Watanapa[1]
[1]*Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand*
**Email : kritsana.boom@gmail.com*

Hesperetin, a metabolized form of hesperidin found in citrus fruits, has been found to possess vasorelaxing effects in both experimental animals and human subjects, but the mechanisms involved have not been well characterized. This project aimed to study the mechanism of hesperetin-induced vasorelaxation in human umbilical vein (HUV) rings using isometric force measurement. HUV rings (3-5 mm) from normal pregnancy and delivery were suspended in modified Krebs solution (37 °C, pH 7.4, 95% O_2/5% CO_2). The effects of 10^{-3} to 10^{-5} M hesperetin were compared to corresponding DMSO concentrations, osmolality changes (using mannitol for adjustment) and time control. In endothelium-denuded HUV pre-contracted with 35 mM KCl, 10^{-4}, $3x10^{-3}$, 10^{-3} M hesperetin caused significant reduction in HUV tension by 14.43 ± 4.23%, 48.52 ± 4.38% and 71.11 ± 4.19%, respectively (n=6, $p<0.05$), compared with exposure to corresponding DMSO concentrations, osmolality and the time control. Similarly, the same hesperetin concentrations could relax endothelium-denuded HUV pre-contraction with 10 μM 5-HT (serotonin) by 15.80 ± 1.99%, 27.64 ± 0.56 and 44.07 ± 2.60%, respectively. Moreover, the hesperetin-induced vasorelaxation was comparable in both endothelium-intact and denuded HUV rings. Our data indicate that hesperetin-induced vasorelaxation was not endothelium-dependent and could be mediated by membrane potential, and possibly ion channels and/or intracellular Ca^{2+} release mechanism, in vascular smooth muscle cells. No COI.

ABS0477

Effects of morelloflavone from Garcinia dulcis on the contraction of isolated thoracic aorta of cisplatin-treated rats

Adha Sumalee[1], Wilawan Mahabusarakam[2], Pornpimol Kirirat[3], Siriphun Hiranyachattada[1]*
[1]*Department of Physiology, Faculty of Science, Prince of Songkla University, Thailand* [2]*Department of Chemistry, Faculty of Science, Prince of Songkla University, Thailand* [3]*Department of Anatomy, Faculty of Science, Prince of Songkla University, Thailand*
**Email : siriphun.h@psu.ac.th*

Cisplatin is the one of the most widely used for cancer chemotherapy, however, it has been reported to cause a variety of cardiovascular side effects such as stroke, myocardial infarction, arterial and venous thromboembolism, enhancement of carotid artery intima media thickness, coronary artery dissection and hypertension. These side effects may be due to the action of cisplatin on a reduction in endothelial nitric oxide and an increase in reactive oxygen species. Morelloflavone is a biflavonoid extracted from Garcinia dulcis (Kurz) which has been shown to possess antioxidant effects and recently shown to cause vasodilation via endothelium dependent nitric oxide release. This study was aimed to investigate the protective effect of morelloflavone on contractile function and structural changes of thoracic aorta of cisplatin-treated rats. Male Wistar rats were divided into three groups including vehicle control, cisplatin and cisplatin + morelloflavone group. DMSO and 0.9% NaCl were used as solvent for morelloflavone and cisplatin, respectively. Morelloflavone (1 mg/kg, i.p.) was given twice 1 day and 10 mins before a single dose of cisplatin (7.5 mg/kg, i.p.) injection. Seven days after cisplatin injection, the contractile responses of isolated thoracic rings were performed by cumulative addition of 10^{-10} -10^{-5} M phenylephrine from 1 g of resting tension. The relaxation responses were evaluated by cumulative addition of either acetylcholine or sodium nitroprusside (10^{-12} –10^{-5} M) after precontracted with 10^{-7} M phenylephrine. It is likely that the degree of vasoconstriction and vasorelaxation of cisplatin-treated rats were altered from control rats and morelloflavone may restore these changes as supported by histological study. No COI.

ABS0045

Analysis of the mechanism of type 2 diabetes involving ADAMTS9 /GON-1

Yong-Qi Zhao[1]*, Xue-Feng Ding[1], Yan Wu[1], Shu-Hong Liu[1], Tong Zhao[1], Li-Ying Wu[1], Xing Huang[1], Haitao Wu[1], Ling-Ling Zhu[1], Ming Fan[1]

[1]*Beijing Institute of Basic Medical Sciences, China*

**Email : yqzhaoprc@sina.com*

ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in transport from the ER to the Golgi. It has been reported that an ADAMTS9 gene variant is associated with type 2 diabetes. However, the molecular mechanisms of ADAMTS9 on the beta cell and peripheral tissues are unknown. First, we investigated how GON-1, the C. elegans homolog of ADAMTS9, is involved in the insulin signaling in C.elegans. Insulin-like proteins are secreted from neurons in the wild type background, whereas insulin-like proteins were accumulated in neurons by GON-1 depletion. To investigate the role of GON-1 in peripheral tissues, we examined the subcellular localization of DAF-16, the C. elegans homolog of FOXO. DAF-16/FOXO was present in both the nucleus and the cytoplasm in wild-type animals. DAF-16/FOXO was exclusively localized to the nucleus in peripheral tissues in the GON-1(tm3146) mutant background. Next, we investigated how ADAMTS9 is involved in the type 2 diabetes by using mammalian cell lines. Glucose-stimulated insulin secretion was gradually compromised after depletion of ADAMTS9 in the INS-1 cells, a glucose-sensitive pancreatic beta cell line. Depletion of ADAMTS9 decreased insulin-stimulated glucose uptake in differentiated 3T3-L1-derived adipocytes and differentiated C2C12-derived skeletal muscle cells. Insulin-stimulated translocation of GLUT4 to the plasma membrane was impaired by depletion of ADAMTS9 in differentiated 3T3-L1 cells. Our data suggest that ADAMTS9/GON-1 is involved in both mechanisms; insulin secretion from insulin secretory cells and insulin signaling at the peripheral tissues. No COI.

ABS0055

The role of reticulum endoplasmic stress in diabetes complication

Oski Illiandri[1]*, Achmad Rudijanto[2]

[1]*Medical Faculty of Brawijaya University, Indonesia* [2]*Department of Internal Medicine, Faculty of Medicine Brawijaya University, Indonesia*

**Email : oski_unlam@yahoo.co.id*

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

ABS0255

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

Tomoyoshi Terada[1]*, Teppei Takahashi[1], Hajime Arikawa[2], Seiichi Era[1]
[1]*Gifu University, Japan* [2]*Chubu-gakuin College, Japan*
**Email : tterada@gifu-u.ac.jp*

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

ABS0287

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

Chantarawan Saengkhae[1]*, Napaporn Uawongyart[2], Sunantha Osiri[2]
[1]*Faculty of Allied Health Sciences, Burapha University, Thailand* [2]*Faculty of Abhaibhubejhr Thai Traditional Medicine, Burapha University, Thailand*
**Email : chantars@buu.ac.th*

Ipomoea pes-caprae plant is a medicinal plant that used in folk medicine to treat jelly fish stings. The biological activities of medicinal plant are depending on solvent and method of extracts. This study is to evaluate the cytotoxic and antioxidant potential of the different solvent extracts from Ipomoea Pes Caprae. The dry plant was extracted by maceration with ethanol & water (A extracts), hexane (B extracts). Supercritical carbon dioxide (SCO2) + ethanol are used as the extracts solvent for polarity extracts (C extracts) and low polarity extracts (D extracts). The molecular cytotoxic assay was assessed by MTT, nuclear and mitochondria staining with DAPI and JC-1. The antioxidant assay was evaluated using DPPH and DCFH-DA. B and D extracts inhibited KB cells with IC50 of 200 ± 12.3 and 70 ± 4.2 μg/ml associated with the increasing of chromatin condensation. The cell population with loss of mitochondrial membrane potential (Δψm) increased significantly to 13.3% (B extracts) and 20.0% (D extracts) compared with control cells (2.0%) ($p<0.05$). Only A extracts had antioxidant activity against DPPH radical. The IC50 value of A extracts and ascorbic acid were 50 ± 4.2 and 5 ± 0.4 μg/ml, respectively. The intracellular hydrogen peroxide level was reduced to 15.1%, 9.1%, and 2.5% in KB cells following the treatment with 50, 100, and 200 μg/ml of A extract, respectively determined by DCFH-DA. In conclusion, the hexane and SCO2 low polarity extracts displayed cytotoxic activities through mitochondria-mediated pathway while the ethanol & water extracts showed antioxidant potential. No COI.

ABS0310

Homer proteins modulate RANKL-induced NFAT signaling in osteoclastogenesis

Yu-Mi Yang[1]*, Aran Son[1], Jung Yun Kang[1], Dong Min Shin[1]
[1]*Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Korea*
**Email : ymyang@yuhs.ac*

Ca^{2+} signaling and NFATc1 activation are essential for RANKL-induced osteoclastogenesis through the induction of Ca^{2+} oscillation, calcineurin activation, and translocation of NFATc1 into the nucleus. Homer proteins are scaffold proteins and have been proposed to modulate multiple Ca^{2+} signaling channels and proteins, including inositol 1,4,5-triphosphate receptors, ryanodine receptors, transient receptor potential channels, and NFAT family of transcription factors in skeletal muscle cells and T cells. However, the role of Homer proteins in Ca^{2+} signaling during osteoclast differentiation is not known. In the present work, we investigated the role of Homer proteins (Homer2 and Homer3) in RANKL-induced Ca^{2+} signaling in osteoclasts using Homer2/Homer3 (Homer2/3) double-knockout (DKO) mice. Deletion of Homer2/3 markedly decreased the bone density of the tibias, resulting in bone erosion. In contrast, Homer2/3 deletion did not affect osteoblast formation and RANKL-induced Ca^{2+} oscillation. In forty-eight hours after RANKL treatment, the Homer2/3 DKO bone marrow-derived monocytes/macrophages (BMMs) had facilitated greatly osteoclast differentiation through NFATc1 protein expression and translocation of NFATc1 into the nucleus. Notably, the interaction of Homer proteins with NFATc1 was inhibited by RANKL treatment, but restored by cyclosporine A treatment to inhibit calcineurin in wild-type osteoclasts. In addition, RANKL treatment of Homer2/3 DKO BMMs significantly increased a ~3.0-fold induction of multinucleated cells formation. These results suggest that Homer2/3 interact with NFATc1 to sequester calcineurin in the cytosol and thus modulate the NFATc1 pathway in RANKL-induced osteoclastogenesis. No COI.

ABS0311

Osmo-mechanosensitive TRP channels facilitate an increase of Ca^{2+}-mediated RANKL expression in mouse osteoblastic cells

Jung Yun Kang[1]*, Yu-Mi Yang[1], Aran Son[1], Dong Min Shin[1]
[1]*Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Korea*
**Email : hannahkang77@gmail.com*

Mechanical stress plays an important role in the regulation of bone turnover. However, the intracellular mechanisms of mechanical stress under osteoblast differentiation and proliferation are not well understood. In this study, we investigated the effects of osmo-mechanosensitive transient receptor potential (TRP) channels-induced calcium signaling in primary mouse osteoblasts and MC3T3-E1 cells. Hypotonic stress induced significant increases of RANKL mRNA expression but not OPG. In addition, hypotonic stress-induced increases of intracellular calcium concentration ($[Ca^{2+}]_i$) and RANKL express ion persisted in the presence of non-specific Ca^{2+} channel blockers or Ca^{2+}-free bath solution. Furthermore, we examined hypotonic stress-induced effects on agonists and antagonists of osmo-mechanosensitive TRP channels in order to determine the cellular mechanism of hypotonic stress-mediated increases in $[Ca^{2+}]$i and RANKL. We found that antagonists of TRPV4 and TRPM3 decreased hypotonic stress-mediated increases on $[Ca^{2+}]_i$ and protein expression levels of RANKL and NFATc1. We also identified that hypotonic stress-induced effects reduced by the genetic suppression of TRPV4 and TRPM3. Taken together, our results indicate that hypotonic stress activate the expression of RANKL and NFATc1 by $[Ca^{2+}]_i$ increases through TRPV4 and TRPM3 in osteoblasts. These effects may be important for the differentiation and proliferation of bone cells on bone remodeling that are mediated via mechanosensitive TRP channels. No COI.

ABS0313

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

Yoshiyuki Shibukawa[1]*, Maki Kimura[1], Masaki Sato[1], Yuki Kojima[1], Asuka Higashikawa[1], Miyuki Shimada[1], Kazuhiro Ogura[1], Hiroyuki Mochizuki[1], Masakazu Tazaki[1]

[1]*Tokyo Dental College, Japan*

**Email : yshihuka@tdc.ac.jp*

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

ABS0326

The $G_{i}/_{o}$ coupled muscarinic receptors form complex with the G protein gated inwardly rectifying potassium channel

Michihiro Tateyama[1]*, Yoshihiro Kubo[1]

[1]*Division of Biophysics & Neurobiology, Department of Molecular Physiology, National Institute for Physiological Sciences, Japan*

**Email : tateyama@nips.ac.jp*

The G protein gated inwardly rectifying potassium (GIRK) channel negatively regulates the excitability of neuronal and muscular cells. The GIRK channel is activated by Gβγ mostly released from Gi/o. On the other hand, Gβγ released from Gq or Gs have been reported to activate the channel when the expression level of the coupling receptors is high. Under a high surface expression condition, distance between the receptors and the channels is expected to be decreased, which may enable Gβγ release from Gq or Gs to activate the channel. To examine this possibility, the Gq coupled muscarinic receptor type1 (M1R) and the GIRK channel were connected with various lengths of glycine rich amino acid residues. The M1R activated the GIRK channel when the number of the linker residues was 100 or less, whereas it did not activate the channel when the number of the residues was 268 or more. In contrast, the Gi/o coupled muscarinic receptors, such as the M2R or the chimeric construct of the M1R and M2R (MC9), activated the GIRK channel even when the number of the linker residue was 541. Moreover, analyses of Förster resonance energy transfer (FRET) between the receptor-YFP and GIRK-CFP revealed that the MC9-YFP, but not M1R-YFP, stays in the proximity to the channel. These results suggested that the Gi/o coupled muscarinic receptors form complex with the GIRK channel for the efficient activation. No COI.

ABS0329

Voltage-gated proton channel Hv1/VSOP inhibits granule exocytosis in neutrophils and inflammation

Yoshifumi Okochi[1]*, Yasuaki Aratani[2], Hibret A. Adissu[3], Nana Miyawaki[1], Mari Sasaki[1], Kazuo Suzuki[4], Yasushi Okamura[1]

[1]Osaka University, Japan [2]Yokohama City University, Japan [3]Hospital for Sick Children, Canada [4]Teikyo University, Japan

**Email : okochyo@phys2.med.osaka-u.ac.jp*

Voltage-gated proton channel is a molecule that regulates intracellular pH and membrane potential. We discovered this molecule and have been analyzing the function of this channel at cellular and animal levels using Hv1/VSOP deficient mice. We and collaborators have reported that Hv1/VSOP helps production of reactive oxygen species (ROS, especially O_2- and H_2O_2) through the regulation of intracellular pH and membrane potential in neutrophils, where these factors are known to affect the activity of NADPH oxidase. Recently, we found that Hv1/VSOP regulates ROS production in another way: Hv1/VSOP negatively regulates HOCl production, which is made from H_2O_2 by myeloperoxidase, by inhibiting exocytosis of myeloperoxidase-containing granules (azurophilic granules) in neutrophils. Pharmacological analyses using zinc ion, an inhibitor for this channel, and valinomycin, a potassium ionophore, revealed that Hv1/VSOP on the plasma membrane regulates the granule release through in part inhibition of excess depolarization in neutrophils. Azurophilic granules contain many degradative enzymes that are known to be toxic for host itself. The release of elastase, one of the degradative enzymes, was enhanced in Hv1/VSOP deficient neutrophils during the oxidase activation. Relating to above in vitro phenotype, Hv1/VSOP deficient mice exhibited severer lung inflammation than wild-type mice after Candida infection. This result suggests that Hv1/VSOP is involved in suppressing inflammation on pathogen infection, which may be through the inhibition of azurophilic granule release in neutrophils. NO COI.

ABS0336

Overexpression of inducible nitric oxide synthase (iNOS) and increased intima/media thickness (IMT) ratio of common carotid artery with significant stenosis flow (SSF) during acute ischemic stroke

Anongnat Painupong[1], Kesorn Suwanprasert [2]*

[1]Phuket Rajabhat University, Thailand [2]Thammasat University, Thailand

**Email : kesorn.physio@gmail.com*

Vascular inflammation caused by endothelial dysfunction induced atherosclerotic plaque is evident in ischemic stroke. Recently, in significant stenosis flow (SSF) stroke, profound low level of circulating nitric oxide (NO) and increased soluble Lectin-like Oxidized LDL Receptor – 1(sLOX-1) were found and showed a significant difference from those in non SSF group. In this study, we examine an iNOS expression of macrophage from buffy coat in SSF associated with increased IMT. Five SSF- stroke aged 60.60 ± 6.78 years old and five healthy control (aged 34.20 ± 4.23 years old) were studied as follows in detail by local ethic committee (MTU-EC-PH-6-076/55). IMT ratio and 50-75% stenosis flow were measured by Doppler ultrasound at both carotid arteries. Plasma NO was measured by electrochemistry method. iNOS expression was quantitated by PCR technique. In SSF-stroke, IMT ratio was 0.61+ 0.05 mm and 0.62 + 0.03 mm compared with control 0.48 + 0.07 mm and 0.52 + 0.03 mm at right and left carotid arteries , respectively. Plasma NO levels were significantly reduced (54.72 + 0.03 nM/ml) in SSF-stroke compared with 77.12 ± 1.64 nM/L in control. Also, overexpression of mRNA iNOS was clearly evident (1.02 ± 0.01 fold of β -actin) compared with control (0.45 ± 0.01 fold of β -actin). These findings indicate activation of iNOS activity in macrophage during rapid flow through inflammatory narrow lumen of plaque vessel in ischemic stroke. Degree of iNOS activity might be vascular oxidative stress marker of plaque formation and rupture. No COI.

ABS0395

Enhancement of ciliary beating by Carbocystein via modulation of $[Cl^-]_i$ and pH_i in bronchiolar ciliary cells in mice

Takashi Nakahari[1]*, Yukiko Ikeuchi[1], Haruka Kogiso[1], Saori Tanaka[2], Shigekuni Hosogi[1], Yoshinori Marunaka[1]

[1]Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, Japan [2]Lab of Pharmacotherapy, Osaka University of Pharmaceutical Sciences, Japan

**Email : nakahari@koto.kpu-m.ac.jp*

The ciliary transport is controlled by two parameters, ciliary beat angle (CBA) and ciliary beat frequency (CBF). Carbocystein (CCys), a mucolytic, is known to activate ciliary transport. In this study, we examined the effect of CCys on bronchiolar ciliary beating, CBA and CBF, in mice. Mice were sacrificed by pentobarbital-Na (70-80 mg/kg, ip), and then bronchiolar ciliary cells were isolated by an elastase treatment. Ciliary beating was observed with a high speed camera (500Hz) at 37℃ Experiments ware performed in accordance with the Guidelines of the Animal Research Committee of Kyoto Prefectural University of Medicine. CCys respectively increased CBA and CBF by 30 % and 5% within 15 min after its application. The CCys-induced CBA increase was inhibited by Cl^- channel blockers (NPPB and CFTR (inh)-172). CCys decreased cell volume and $[Cl^-]_i$ of bronchiolar ciliary cells. Moreover, in a HCO_3^--free solution, CCys actions were mimicked by removal of Cl^- from the extracellular space, suggesting that CCys would increase CBA by decreasing $[Cl^-]_i$ associated with cell shrinkage caused by activation of Cl^- channel (CFTR). On the other hand, the CCys-induced increase in CBF was not observed in a HCO_3^--free solution or in the presence of DIDS. Moreover, in a HCO_3^- containing Cl^--free solution, CCys still increased CBF. This suggests that CCys would increase CBF by elevating pH_i caused by activation of NBC. In conclusion, CCys increases CBA by a decrease in $[Cl^-]_i$ via CFTR activation, and increases CBF by a pH_i elevation via NBC activation. No COI.

ABS0426

VRAC channel composition determines its substrate specificity and resistance to the induction of apoptosis

Rosa M Planells-Cases[1]*, Darius Lutter[2], Florian Ullrich[2], Deborah Elger[2], Felizia Voss[2], Momsen Reincke[2], Tobias Stauber[2], Thomas Jentsch[2]

[1]FMP (Leibniz-Institut fuer Molekulare Pharmakologie) and MDC (Max-Delbrueck-Centrum fuer Molekulare , Germany [2]Leibniz-Institut für Molekulare Pharmakologie (FMP) and Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany

**Email : Rosa.Planells-Cases@mdc-berlin.de*

One of the first events observed during apoptosis is a decrease in cell volume due to efflux of K^+, Cl^- and H_20, a phenomenon known as apoptosis volume decrease (AVD), that later is followed by caspase activation and DNA fragmentation. Volume-regulated anion channels (VRAC) and volume sensitive organic anion channels (VSOAC) have been show to play an important role in drug-induced AVD and apoptosis by physiological and pharmacological characterization. Thus, cell death induced by staurosporine and the anticancer drug cisplatin could be reduced by non-specific inhibitors of the volume-regulated anion channel VRAC, now known to be a LRRC8 heteromer. Disruption of the obligatory subunit LRRC8A and another subunit indeed reduced cisplatin- or staurosporine-induced caspase activation, while both drugs activated VRAC. Further analysis showed that the LRRC8 subunit composition determines VRACs selectivity for several substrates, providing evidence that LRRC8 heteromers directly form the pore of VRAC. No COI.

ABS0446

Comparative study on the redox state of HSA products for laboratory use and healthy young subjects

Teppei Takahashi[1]*, Tomoyoshi Terada[1], Takeshi Minami[2], Hajime Arikawa[3], Hajime Imai[1], Seiichi Era[1]
[1]*Gifu University, Japan* [2]*Gifu University of Medical Science, Japan* [3]*Chubu Gakuin College, Japan*
**Email : md13teppei@gmail.com*

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

ABS0480

The physiological role of endothelin in periodontitis

So Yun Lee[1]*, Ga-Yeon Son[1], Sue Young Oh[1], Inik Chang[1], Dong Min Shin[1]
[1]*Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Korea*
**Email : my1000id@naver.com*

Periodontitis is a very common oral inflammatory disease and results in the destruction of supporting connective and osseous tissues of tooth. Although the etiology is still unclear, Gram-negative Porphyromonas gingivalis in subgingival pockets has been thought as one of the major etiologic agent. It has been known that endothelin is involved in the occurrence and progress of various inflammatory process and diseases. However, functional roles of endothelin in periodontitis are still unclear. In this study, we explored cellular and molecular mechanisms of ET-1 actions in periodontitis using human gingival epithelial cells (hGECs) and human gingival fibroblasts (hGFs). ET-1 and ETA, but not ETB were abundantly expressed in both hGECs and hGFs. Stimulation of hGECs with *P. gingivalis* LPS increased the expression of ET-1 and ETA suggesting the activation of endothelin signaling pathway. Production of pro-inflammatory cytokines, IL-1 β, IL-6, and IL-8 was significantly enhanced by exogenous ET-1 treatment in both hGECs and hGFs. Moreover, ET-1 augmented the number of multinucleated osteoclasts implicating the acceleration of alveolar bone loss. Together, our study showed that activation of ET-1/ETA signaling pathway by *P. gingivalis* may exacerbate periodontitis by stimulating production of pro-inflammatory cytokines in hGECs and hGFs and provoking the alveolar bone loss through the increment of multinucleated osteoclasts at the same time. To directly examine the endothelin antagonism as a potential therapeutic approach for periodontitis, the inhibitors for ET receptors will be applied to the animal periodontitis model. Infiltration of immune cells, production of pro-inflammatory cytokines, and alveolar bone loss will be evaluated. No COI.

ABS0545

Quinacrine reduces the microwave caused neuronal damage by stabilizing cell membrane

Yong-Qi Zhao[1]*, Xue-Feng Ding[1], Yan Wu[1], Shu-Hong Liu[1], Tong Zhao[1], Li-Ying Wu[1], Xing Huang[1], Haitao Wu[1], Ling-Ling Zhu[1], Ming Fan[1]

[1]*Beijing Institute of Basic Medical Sciences, China*

**Email : yqzhaoprc@sina.com*

Quinacrine (QA) once was widely used in treatment of parasitic diseases such as malaria and giardiasis, and autoimmune diseases. Although QA has been substituted with more effective drugs, research related to this old drug has never been stopped. Microwave (MW) has been widely used in various industrial, communications, medical, and domestic applications, though its adverse effect remain unclear. However, the thermal effect is the most important characteristic of microwave. Recently, we found that QA administration could decrease the neuronal damage of hippocampus caused by MW. However, the detailed molecular mechanism remains unknown. For this purpose, here, PC12 cells were successfully induced into neuronal cells with RA. Next, the induced cells were pretreated before receiving microwave. The results showed that microwave exposure (3h and 6h group) significantly increased the neuronal apoptosis and necrosis compared with control, but QA pretreatment (low and high dose) can dramatically decrease the MW induced cell apoptosis and necrosis. Analysis based on Atomic force microscope (AFM) showed that QA pretreated cells displayed much less cell membrane damage. Further investigations demonstrated that QA may be involved in MW caused neuronal injury by increasing Hsp70 expression level. This work may further facilitate the research on thermal effect of MW, and also to discuss the new use of QA. No COI.

ABS0063

Optogenetic approach to control neuronal activity in rat vasopressin neuron in in vitro preparation

Yoichi Ueta[1]*, Jun-ichi Ohkubo[1], Toru Yokoyama[2], Kanako Shoguchi[1], Mitsuhiro Yoshimura[1], Takashi Maruyama[1], Hirofumi Hashimoto[1]
[1]*Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Japan*
[2]*Department of Anesthesiology and Critical Care Medicine, Jichi Medical University, Japan*
**Email : yoichi@med.uoeh-u.ac.jp*

Neurohypophysial hormone, arginine vasopressin (AVP) is synthesized in the magnocellular neurosecretory cells (MNCs) of the supraoptic (SON) and the paraventricular nuclei (PVN) of the hypothalamus. AVP release from the posterior pituitary (PP) into the systemic circulation is mainly regulated by neuronal activity of MNCs that project their axon terminals to the PP. In the present study, we have generated a transgenic rat that expresses the AVP and channelrhodopsin 2 (ChR2)-eGFP fusion gene to regulate the neuronal activities of AVP MNCs by blue light-activated ion channel (ChR2). The eGFP that indicates the expression of the ChR2 gene was observed in the SON, the magnocellular divisions of the PVN that are known to localize AVP MNCs. Confocal laser scanning microscopic observation revealed that ChR2-eGFP was mainly localized in the membrane of MNCs. The intensities of eGFP in the SON and the PVN were markedly increased after chronic salt loading. Using whole cell patch-clamp recordings in in vitro preparations such as a single cell isolated from the SON and brain slice including the SON from the transgenic rats, it was found that repeated blue light evoked action potentials repetitively in a current clamp mode, and caused inward currents in a voltage clamp mode. Thus, optogenetic approach is a powerful tool to regulate neuronal activity of AVP MNCs. No COI.

ABS0077

Age and sex differences in monosodium glutamate obesity

Faten Diab[1]*, Magda Youssef[2], Nermine Saleh[1], Abd El-Hamid Mohamed [1], Doaa Abou-Bakr[1]
[1]*Physiology Department, Faculty of Medicine, Ain Shams University, Egypt* [2]*Physiology department, Faculty of Medicine, Taibah University Al Madinah Al Monawarrah, Saudi Arabia*
**Email : dtoona2000@yahoo.com*

Aim: This study aimed to assess the impact of age and sex differences on the effects of monosodium glutamate (MSG) induced obesity as regard body mass index (BMI), lee Index (LI), body weight gain, and percentage gain of body weight, fasting plasma glucose and glucose uptake by diaphragm, plasma insulin, corticosterone, testosterone and estradiol in rats. Methods: Adult and old Wistar rats of both sexes were divided into: Control groups, receiving control diet; Monosodium glutamate (MSG) obese groups, receiving control diet and MSG. At the end of the experiment, BMI, LI, body weight gain and, percentage gain of body weight were assessed. Blood samples were collected for plasma glucose and hormonal assays. The diaphragms were dissected for determination of glucose uptake by the muscles. Results: MSG obese rats showed increased values of BMI, and LI. The percentage gain of body weight was more enhanced in the adult groups compared with the old groups. MSG obese groups showed significant decrease in energy expenditure, which was more apparent in the old obese male group, with significantly decreased food intake. Fasting plasma glucose was significantly increased, while plasma insulin and glucose uptake by diaphragm were significantly decreased in the MSG obese groups. Plasma testosterone level was significantly decreased in MSG male obese groups, but insignificant changes in plasma estradiol and corticosterone were observed. Conclusion: MSG had been shown to have adverse effects on body weight, energy expenditure, glucose homeostasis of the rats, as well as, on plasma insulin and testosterone levels. Age and sex influenced some of these effects. No COI.

ABS0120

Rosiglitazone improved hepatic circadian-clock gene expression in the insulin resistance and diabetes of mice

Huey-Ling Tseng[1]*, Kun-Ruey Shieh[2], Julie Y.H. Chan[1]
[1]*Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital, Taiwan*
[2]*Department of Physiology, School of Medicine, Tzu Chi University, Taiwan*
**Email : 95351111@gms.tcu.edu.tw*

Circadian rhythms are exhibited in the physiological and behavioral processes of all mammals, and are generated by a set of circadian-clock genes which includes Bmal1 (brain and muscle Arnt-like protein-1), Clock (circadian locomotor output cycles kaput), Per1 (period 1), Per2, Per3, Cry1 (cryptochrome 1) and Cry2. Previous evidence suggests a strong link between circadian rhythms and energy metabolism; however, the underlying mechanisms remain uncertain. In the present study, neonatal streptozotocin (STZ)-treated mice were used to model the molecular and physiological progress from insulin resistance to diabetes. Two-day-old male C57BL/6 mice received a single injection of STZ to induce the non-obese, hyperglycemic and hyperinsulinemic conditions in the early stage, insulin resistance in the middle stage, and diabetes in the late stage. Levels of the hepatic circadian-clock gene expression were examined by real-time quantitative PCR. Most components of the hepatic circadian-clock gene expression, such as the mRNAs of Bmal1, Per2 and Cry1, were elevated, and circadian patterns were retained in the early and middle stages of insulin-resistant conditions. Rosiglitazone, an insulin sensitizer, returns the physiological and molecular changes associated with the diabetic phenotype to normal levels through peroxisome proliferator-activated receptor γ (PPARγ) rather than PPARα. Early and chronic treatment with rosiglitazone has been shown to be effective to counter the diabetic condition. Thus, these results support an essential role for the hepatic circadian-clock system in the coordinated regulation and/or response of metabolic pathways. No COI.

ABS0159

Nocturnal dipping behaviour of blood pressure and diurnal urinary sodium excretion in normotensive adolescents in response to changes in salt intake

Aung Khaing[1]*, Ohnmar[2], Mya-Thanda Sein[3], Mya-Mya Thwin[3], Tint-Swe Latt[4]
[1]*University of Medicine 2,* [2]*Department of Physiology, University of Medicine 1* [3]*Department of Physiology, University of Medicine 2, Yangon, Myanmar* [4]*Department of Physiology, University of Medicine 2,Yangon, Myanmar (retired)*
**Email : aungkhaing76@gmail.com*

Nocturnal nondipping of blood pressure (BP) is associated with salt intake especially in hypertensive individuals but nocturnal dipping behaviour of BP in normotensive adolescents gain less attention. The aim of the study was to determine the effect of salt intake on nocturnal dipping behaviour of BP and diurnal urinary sodium excretion in normotensive adolescents. Subjects (n = 36) were maintained on high salt intake (200.8 ± 56.3 mmol/day) for one week followed by one week washout and then salt reduction (86.2 ± 22.3 mmol/day) for another one week. During high salt intake, 19 subjects (52.8%) were classified as non-dippers and 17 subjects (47.2%) as dippers. During salt reduction, 13 out of 19 previously non-dippers (68.4%) changed to dippers. A significant increase in nighttime urinary sodium excretion rate was observed in non-dippers during high salt intake ($p<0.05$) and salt reduction ($p<0.01$). These results demonstrated that high salt intake would induce attenuated nocturnal dipping of BP with concomitant changes in enhanced nighttime natriuresis, and modest salt reduction would restore normal dipping pattern of BP and normal pattern of urinary sodium excretion. It can be concluded that nocturnal dipping behaviour of BP could change as a consequence of salt intake even in healthy normotensive adolescents. No COI.

ABS0186

Tyrosine hydroxylase expression in CD4+ T cells is associated with joint inflammatory alleviation in collagen type II-induced arthritis

Yi-Hua Qiu[1]*
[1]*Nantong University, China*
**Email : yppeng@ntu.edu.cn*

We have recently reported that CD4+ T cells synthesize and secrete catecholamines that facilitate a shift of T helper 1 (Th1)/Th2 balance toward Th2 polarization. In this study, we used an animal model of human rheumatoid arthritis (RA), and collagen type II-induced arthritis (CIA), to explore relationship between catecholamine production in CD4+ T cells and Th1/Th2-mediated joint inflammation. Histopathological observation of ankle joints of CIA mice displayed an evident inflammatory change on day 35 and a major damage to bones on day 55 post-immunization. Expressions of Th1 specific transcription factor, T-bet, and cytokines, IL-2 and IFN-γ, and Th2 specific transcription factor, GATA-3, and cytokines, IL-4 and IL-10, were all upregulated on days 35 and 55 post-immunization, but the elevated Th1 response tended to decrease and the enhanced Th2 response tended to increase with the CIA progression. Expression of tyrosine hydroxylase (TH), a rate-limiting enzyme for synthesis of catecholamines, dramatically increased in ankle joints of CIA mice, although this increase was reduced on day 55 relative to day 35 post-immunization. In synovial tissue of CIA ankle joints but not normal joints, CD4-, T-bet-, GATA-3- and TH-immunoreactive cells were found. Importantly, co-expressed cells with CD4 and TH, T-bet and TH, and GATA-3 and TH were observed in synovial tissue of CIA ankle joints. These results suggest that an increase in catecholamine production occurs in inflamed joints of CIA. The catecholamines are, at least in part, from Th1 and Th2 cells, and they may be related to joint inflammatory alleviation in CIA progression. No COI.

ABS0203

Hyperhomocysteinemia activates Aryl hydrocarbon receptor-CD36 pathway to promote hepatic steatosis in mice liver.

Liu Yao[1]*, Yi Zhu[2]
[1]*Tianjin Medical University, China* [2]*Tanjin Medical University, China*
**Email : yl_yaoliu@163.com*

Hyperhomocysteinemia (HHcy) is associated with liver diseases, such as fatty liver and hepatic fibrosis. However, the underlying mechanism is largely unknown. The current study aims to explore the signaling pathway involved in HHcy-induced hepatic steatosis. First, we performed a meta-analysis of studies involving 2,057 subjects and found that plasma Hcy levels was associated with an increased risk of nonalcoholic fatty liver disease (WMD = 2.56, 95% CI: 1.17, 3.38, P = 0.011). Next, C57Bl/6 mice were fed a high-methionine diet (HMD) (2%, wt/wt) for 8 weeks to establish a HHcy mouse model with hepatic steatosis and elevated CD36 gene expression in liver. The increased CD36 expression was associated with activation of aryl hydrocarbon receptor (AHR). Furthermore, mass spectrometry analysis showed that hepatic content of lipoxin 4A, a well-known ligand of AHR, was significantly elevated in HHcy mice. In primary hepatocytes, the Hcy-induced CD36 expression and subsequent lipid uptake were significantly attenuated by AHR siRNA. Transient transfection assays showed that the activity of AHR response element was dramatically increased in a ligand-dependent manner by Hcy. In addition, Hcy treatment promoted the binding of AHR to CD36 promoter. Finally, AHR antagonist CH223191 reversed lipid accumulation caused by HHcy by inhibiting AhR-CD36 pathway. In conclusion, HHcy activated AHR-CD36 pathway by increasing hepatic lipoxin 4A content, which resulted in hepatic steatosis. No COI.

ABS0302

20-hydroxyecdyzone alleviates hypertension and improves glucose tolerance in a rat model of metabolic syndrome

Jariya Buniam[1], Yupaporn Rattanavichit[1], Natsasi Chukijrungroat[1], Vitoon Saengsirisuwan[1]*
[1]*Department of Physiology, Faculty of Science, Mahidol University, Thailand*
**Email : vitoon.sae@mahidol.ac.th*

Ecdysteroids are polyhydroxylated steroids present in plants and invertebrates. In mammals, anabolic effects on skeletal muscle cells and metabolic effects on glucose metabolism of hepatic cells of ecdysteroids have been reported. Although the favorable metabolic effects including hypoglycemic and cholesterol-lowering properties have been shown in obese rodents, no study thus far has evaluated the effects of ecdysteroids on metabolic syndrome. Thus, this study was designed to investigate how chronic treatment with a phytoecdysteroid 20-hydroxyecdysone (20HE) would affect the phenotypic characteristics of metabolic syndrome in rat model induced by high caloric diet. Female Sprague–Dawley rats were ovariectomized and fed with high-fat high-fructose diet (OVX+HFFD), and received daily oral administration of either vehicle or 20HE (5 mg, 10 mg, or 20 mg/kg body weight) for 8 weeks. Body weight, visceral fat weight, blood pressure, serum triglyceride, glucose tolerance test, and insulin action on skeletal muscle glucose transport activity were determined. 20HE treatment tended to decrease body weight and significantly reduced visceral fat content and blood pressure by 20% and 12-15%, respectively. Whole-body insulin sensitivity was increased 38% ($P < 0.05$) in the 20HE-treated groups. However, no significant improvements were observed for insulin-stimulated glucose transport activity in the soleus muscle of 20HE-treated animals. These data indicate that 20HE can alleviate the development of hypertension in OVX+HFFD rats and the improvement in whole-body insulin sensitivity in 20HE-treated rats occur independently of modulation of the insulin action on skeletal muscle glucose transport activity. No COI.

ABS0402

Protective effect of chronic intermittent hypobaric hypoxia on Diabetes Mellitus rats

Yanming Tian[1], Huijie Ma[1], Yi Zhang[1]*
[1]*Hebei Medical University, China*
**Email : zhyhenryphy@163.com*

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

ABS0424

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

Hiroshi Nagano[1]*, Hayato Matsuyama[1], Shoichiro Saito[2], Hiroki Sakai[1], Wess Jurgen[3], Seiichi Komori[1], Toshihiro Unno[1]

[1]Department of Pathogenetic Veterinary Science [2]Department of Basic Veterinary Science, The United Graduate School of Veterinary Sciences, Gifu University, Japan [3]Laboratory of Bioorganic Chemistry, NIDDKD, United States

**Email : t5110015@edu.gifu-u.ac.jp*

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

ABS0434

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

Akira Kohsaka[1]*, Tomomi Nakao[1], Tsuyoshi Otsuka[1], Hue Le[1], Masanobu Maeda[1]

[1]Department of Physiology, Wakayama Medical University, Japan

**Email : kohsaka@wakayama-med.ac.jp*

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

ABS0449

Anti apoptotic and anti oxidative effects of salvianolic acid B in multiple low-dose streptozotocin-induced diabetes

Safoura Raoufi[1]*, Javad Giahshenas[2], Turandokht Turandokht[3], Mehrdad Roghani[4], Jalal Hasanshahi[3]
[1]Hamadan University of Medical Sciences, Department of Physiology, Faculty of Medicine, Iran [2]Islamic Azad University of Hamedan, Iran [3]Department of Physiology, Faculty of Medicine, Iran University of Medical Sciences, Iran [4]Department of Physiology, Faculty of Medicine, Shahed University, Tehran, Iran
**Email : safoura.raoufi@gmail.com*

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

ABS0327

The effect of exercise and its stability on interleukin-17, melatonin and cortisol concentrations in serum and lymphocytes /whole blood culture in non-athletic and healthy subjects

Mohammad Zarei[1]*, Ghodrat Farokhi Fard[1], Iraj Salehi[2], Alireza Zamani[3], Abdolrahman Sarihi[1], Mohammad Mahdi Zarei[4]

[1]Department of Physiology, Faculty of Medicine, [2]Department of Anesthesia, Faculty of Paramedical Sciences, Hamadan University of Medical Sciences, [3]Department of Immunology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, [4]Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Alborz, Iran

**Email : zarei@umsha.ac.ir*

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

ABS0345

Sustained vocalizations during *kendo* exercises suppress expiration of carbon dioxide

Hajime Arikawa[1]*, Tomoyoshi Terada[2], Teppei Takahashi[2], Hajime Imai[2], Seiichi Era[2]

[1]Chubu Gakuin College, Japan [2]Gifu University, Japan

**Email : arikawa@chubu-gu.ac.jp*

One distinctive trait of kendo, the traditional Japanese martial art of fencing, is the execution of sustained, high-effort vocalizations during actions. The purpose of this study was to determine the effect of these vocalizations on breathing patterns and respiratory functions. Respiratory indicators of eight university kendo athletes were analyzed using a portable breath gas analyzer during the most intensive kendo exercise, kakari-keiko, with and without vocalization. Sustained vocalization was found to prolong expiratory time, and suppress breathing frequency and expired minute ventilation. Analysis of exhaled gases revealed no effect of sustained vocalization on oxygen uptake, but did reveal reduced carbon dioxide output (VCO_2) and increased fraction of end-tidal carbon dioxide ($FetCO_2$) during exercise session and enhanced VCO_2 in recovery periods. Thus, we conclude that the sustained high-effort vocalizations greatly affect expiration breathing patterns in kendo. Moreover, repetition of kakari-keiko caused a reduction in VCO2 and an increase in CO_2 storage and $FetCO_2$. We hypothesize that these vocalizations of kendo may increase cerebral blood flow in athletes. No COI.

ABS0354

The possibility of increase in both the local oxygen consumption and blood flow of the skeletal muscle by the forearm immersion to artificial CO_2 water

Noriyuki Yamamoto[1]*, Masaaki Hashimoto[2]
[1]*Japanese Red Cross Hokkaido College of Nursing, Japan* [2]*Laboratory of Physiology, Department of Physical Therapy, Faculty of Medical Sciences, Teikyo University, Japan*
**Email : yama@rchokkaido-cn.ac.jp*

Clinical observations of CO_2-hot spring (CO_2 ~1000 ppm) immersion revealed the effects, e.g. an immersed part reddening, skin blood flow improvements, etc. In this study, whether O_2 consumption (MO_2) and blood flow (BFmuscle) in the local muscle of forearm were influenced by immersion of the forearm into water containing high concentration-CO_2 (CO_2-water) was investigated by using near-infrared spectroscopy (NIRS). Six healthy female volunteers (21-22 years) seated in upright position were instrumented on the right forearm with a laser Doppler flowmetry probe for recording skin blood flow (BFskin), and a NIRS-probe for recording muscle hemoglobin (Hb) contents (oxy-Hb, total-Hb). Blood flow of the forearm was restricted by comprising the inflatable cuff on the upper arm to perform three consecutive venous occlusions (<50 mmHg for 20 s) followed by an arterial occlusion (<280 mmHg, 50 s). MO_2 was determined by evaluating the slope of linear regression line of oxy-Hb decreases during arterial occlusion, and BFmuscle was determined by the rate of increase of total-Hb during venous occlusion. Bath water (30 °C) was exchanged for another, i.e. tap water (CO2<20 ppm) for CO_2 water (860-990 ppm) or vice versa, every about 20 min. All subjects reported that CO_2-water was warmer than the tap water. The BFskin was significantly 3.6 times larger during CO_2-water immersion than tap water immersion. MO_2 and BFmuscle during CO_2-water immersion was respectively 15 % and 32 % larger than during tap-water immersion. Results suggest that the bathing with artificial CO_2-water may stimulate the muscle metabolism hence increased muscle-blood flow, as well as skin blood flow. No COI.

ABS0355

The role of brain serotonin levels and its related gene expressions in regulating amount of daily spontaneous physical activity

Shinya Yanagita[1]*, Natsuko Kubota[1], Yurika Takano[1], Masakazu Umezawa[1], Ken Takeda[1]
[1]*Tokyo University of Science, Japan*
**Email : yanashin@rs.noda.tus.ac.jp*

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

ABS0378

Negative rebound in hippocampal neurogenesis following exercise cessation

Takeshi Nishijima[1]*, Yoshika Kamidozono[1], Atsushi Ishiizumi[1], Ichiro Kita[1]
[1]*Tokyo Metropolitan University, Japan*
**Email : t-nishijima@tmu.ac.jp*

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

ABS0445

Lactate threshold during exercise in a cool environment is decreased by whole body surface cooling prior to exercise with an enhanced sympathetic nerve activity

Daiki Imai[1]*, Kazunobu Okazaki[1], Ryosuke Takeda[1], Yoshihiro Yamashina[1], Nooshin Naghavi[1], Akemi Ota[1], Yoshikazu Hirasawa[1], Hisayo Yokoyama[1], Toshiaki Miyagawa[1]
[1]*Osaka City University, Japan*
**Email : dimai@sports.osaka-cu.ac.jp*

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

ABS0473

Functional capacity and maximal oxygen consumption in obese, overweight and normal weight young adults and the effects of arm swing exercise training: A preliminary study

Piyapong Prasertsri[1]*, Naruemon Leelayuwat[2]

[1]Faculty of Allied Health Sciences, Burapha University, Thailand [2]Department of Physiology, Faculty of Medicine, Khon Kaen University, Thailand

**Email : piyapong@buu.ac.th*

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

ABS0516

Effects of gender and intensity of exercise on immunity during exercise in patients with diabetes type 2

Naruemon Leelayuwat[1]*, Nantaya Krasuaythong[2], Chongchira Boonthongkaew[3], Yupaporn Kanpetta[2], Terdthai Tong-un[1]

[1]Department of Physiology, Thailand [2]Exercise and Sport Sciences Program, Thailand [3]Biomedical Sciences Program, Graduated School, Khon Kaen University, Thailand

**Email : naruemon@kku.ac.th*

The effects of gender and intensity of exercise on immunity during exercise in patients with type 2 diabetes mellitus (T2DM) are unclear. This study aimed to determine the effects of gender and intensity of exercise on immunity during exercise in patients with T2DM. Six women and 6 men with T2DM (aged between 30 and 60 years) cycled on ergometer at a target workload equal to 25 (low), 65 (moderate) and 85 (high) % of peak oxygen consumption for 10, 10 and 5 minutes, respectively with at least 7 days apart. Blood samples were taken to count their immune cells (white blood cells (WBCs), neutrophils (NE), lymphocytes (LY) and monocytes (MO)) before and immediately after the exercise. Peak oxygen consumption of male and female subjects were 25.6±6.4 and 19.0±7.5 ml/kg body mass/min. Men had significantly increased WBCs and LY after the exercise at high intensity, whereas women had significantly increased WBCs and LY after the exercise at moderate and high intensity. In addition, men had significantly increased in NE after the exercise at moderate and high intensity. However, women had significantly increased NE immediately after the exercise at every intensity. At moderate intensity, women had significantly higher NE and LY after the exercise than men. MO was not significantly affected by the intensity and gender. The present study suggests that there was the effect of intensity of exercise on WBCs, NE and LY. No COI.

ABS0248

Effects of ezrin knockdown on the architecture of gastric glandular epithelia

Saori Yoshida[1], Hiroto Yamamoto[2], Ryo Hatano[1], Ken-ichi Mukaisho[2], Takanori Hattori[2], Hiroyuki Sugihara[2], Shinji Asano[1]*

[1]*Department of Molecular Physiology, Ritsumeikan University, Japan* [2]*Department of Pathology, Shiga University of Medical Sciences, Japan*

*Email : ashinji@ph.ritsumei.ac.jp

Ezrin, an adaptor protein that cross-links plasma membrane-associated proteins with actin cytoskeleton, is concentrated on apical surface of epithelial cells especially in microvilli of small intestines and stomachs. In stomachs, ezrin is predominantly expressed on apical canalicular membrane of parietal cells. It is involved in remodeling of apical surface membrane and in gastric acid secretion. The ezrin knockdown (Vil2kd/kd) mice showed achlorhydria due to the impairment of membrane fusion between tubulovesicles and apical membrane. However, the effects of knockdown of ezrin expression on the architecture of gastric epithelia have not been studied yet. Here, we studied the architecture of gastric epithelia of theVil2kd/kd mice by immunohistochemistry, and newly found that the mice showed hypergastrinemia and foveolar hyperplasia in the gastric fundic region. Dilation of fundic glands was observed with the percentage of parietal and chief cells being decreased, and that of mucous-secreting cells being increased. The parietal cells of the Vil2kd/kd mice contained dilated tubulovesicles and abnormal mitochondria, and subsets of these cells contained abnormal vacuoles and multilamellar structures. Therefore, ablation of ezrin causes not only achlorhydria and hypergastrinemia, but also changes in the architecture of gastric gland with severe perturbations in the secretory membranes of the parietal cells. No COI.

ABS0312

Effects of dietary zinc supplementation on the intestinal epithelial histomorphology in Bama miniature pig

Xin Zhou[1], Yansen Li[1], Yun Cao[1], Fei Wang[1], ChunMei Li[1]*

[1]*Nanjing Agricultural University, China*

**Email : chunmeili@njau.edu.cn*

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

ABS0333

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

Atcharaporn Ontawong[1], Acharaporn Duangjai[2], Chatchai Muanprasat[3], Anchalee Pongchaidecha[1], Chutima Srimareong[1]*

[1]*Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand* [2]*Divison of Physiology, School of Medical Sciences, University of Phayao, Phayao, Thailand* [3]*Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand*

**Email : chutima.srimaroeng@cmu.ac.th*

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

ABS0343

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

Nurul Ain Abu Bakar[1]*

[1]*Universiti Putra Malaysia, Malaysia*

**Email : nurulain37@gmail.com*

Introduction: Bitter gourd or Momordica charantia L. (MC), a cucurbitaceae family plant is a plant native to the semi-tropical climate of Thailand, India, Asia and Africa and has been traditionally used as a folk remedy and best known for its anti-diabetic, anti-inflammatory, anti-microbial, anti-ulcer and antihelmintic properties. Objective: This study was conducted to evaluate the anti-ulcerogenic effect of MC essential oil on HCl/ethanol-induced gastric ulcer in rats. Method: Five groups of Spraque Dawley rats (n = 6) were given treatment orally for 7 consecutive days. These group consist of I=negative control, II = positive control, III = 10 mg/kg MC, IV = 50 mg/kg MC and V = 100mg/kg MC. On the 7^{th} day, after 1 hour of treatment administration, the rats were induced with HCl/ethanol to produce ulcers and were kept for 6 hours. Thereafter, the rats were sacrificed and their stomachs were removed to measure the ulcer index and percentage of inhibition Results: At the dosage of 10, 50 and 100 mg/kg, MCEO significantly ($p < 0.05$) reduced the total area of gastric lesion (mm^2) from 59.7 ± 7.4 to 18 ± 4.0, 7.8 ± 3.4 and 5.0 ± 1.9 respectively. Ranitidine reduced the total area of gastric lesion from 59.7 ± 7.4 to 40.5 ± 3.2. Inhibition was well shown in the 100 mg/kg MC (91.6%) concentration followed by 50 mg/kg (86.9%) and 10 mg/kg (69.8%) after induced by HCl/ethanol. All inhibiting effects obtained were compared with ranitidine, 100 mg/kg (32.1%). Conclusion: Based on the results obtained, MC essential oil has the properties to reduce the severity of gastric ulcer in rats. No COI.

ABS0349

Effects of Xenin on spontaneous circular muscle contraction in rat distal colon

Atsukazu Kuwahara[1]*, Yuko Kuwahara[2], Shin-Ichiro Karaki[1], Yuka Tomizawa[1], Ikuo Kato[3]
[1]University of Shizuoka, Japan [2]Aichi Medical University, Japan [3]Kobe Pharmaceutical University, Japan
**Email : kuwahara@u-shizuoka-ken.ac.jp*

In search of a mammalian counterpart for the octapeptide xenopsin of amphibian skin was detected in the mucosa of the upper gastrointestinal tract of humans and various mammals. This peptide was named Xenin25 (Xen) which is a 25 amino acid neurotensin-related peptide produced by GIP-producing K cells in the small intestine and peptide sequence is highly conserved during evolution. We have shown that Xen functions as a mediator on the ion transport in the rat colon [J. Physiol. Sci., 65 (Suppl.1):S195, 2015]. Previous morphological and functional studies support the concept that xenin represents a new member of regulatory peptide. To support the concept, in the present study we have evaluated the effect of Xen on smooth muscle activity in the colon. Xen was synthesized by a solid-phase methodology with Fmoc-strategy using an automated peptide synthesizer (Model Pioneer; Life Technologies, CA, USA). For the mechanical experiments, full-thickness circular muscle strips (approximately 2 mm wide and 8~10 mm long) were attached to isometric transducer under a constant load of 3~8 mN in 15 mL oxygenated (95%O_2 and 5% CO_2) Krebs solution maintained at 37 °C. An amplifier (Quad Bridge Amp.) and a PowerLab system (ML846: ADInstruments, Bella Vista, NSW, Australia) were used to record circular muscle activity. The tissues were allowed to equilibrate for at least 2 h to develop spontaneous contractions of varying amplitude. Xen concentration-dependently inhibited spontaneous circular muscle contractions in rat distal colon. These results indicate that Xen may contribute to an inhibitory modulator in colonic circular smooth muscle. No COI.

ABS0377

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

Shin-Ichiro Karaki[1]*, Junji Ishikawa[2], Yuka Tomizawa[1], Atsukazu Kuwahara[1]
[1]Department of Environmental and Life Sciences, University of Shizuoka, Japan [2]FANCL Research Institute, Japan
**Email : karaki@u-shizuoka-ken.ac.jp*

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

ABS0382

Agonists and antagonists for the free fatty acid receptor 3, FFA3 (GPR41) – Short-chain fatty acid-evoked anion secretion in the mice and rat intestinal mucosa

Yuka Tomizawa[1], Shin-Ichiro Karaki[1]*, Ikuo Kimura[2], Atsukazu Kuwahara[1]
[1]*Department of Environmental and Life Sciences, University of Shizuoka, Japan* [2]*Department of Applied Biological Science, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Japan*
**Email : karaki@u-shizuoka-ken.ac.jp*

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

ABS0429

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

Hongfang Li[1]*, Qiankun Liang[1], Lanfang Mao[2], Yunxia Li[1], Xiaojuan Du[2], Junhong Liu[2], Longde Wang[2]
[1]*Department of Physiology, College of Basic Medicine, Lanzhou University, China* [2]*Affiliated Hospital of Traditional Chinese Medicine College, China*
**Email : lihf@lzu.edu.cn*

Functional dyspepsia (FD), a functional gastrointestinal disorder and a typical psychosomatic disease, is characterized by the presence of one or more chronic dyspepsia symptoms in the absence of any organic disease. The present study was to establish an adult rat model of liver stagnation and spleen deficiency syndrome with functional dyspepsia and to investigate the changes of gastrointestinal motility and brain-gut peptide levels in rat serum and gastrointestinal tissue, which aimed to elucidate the mechanisms of gastrointestinal motility disorder. Male Wistar rats were divided into control group (Con) and FD model group. FD model was established by stimulating semi-starvation rats via tail damping, provocation and forced exercise fatigue until gastrointestinal motility disorder appeared, and then the levels of motilin, leptin, cholecystokinin (CCK), vasoactive intestinal peptide (VIP) and P substance were detected in serum by Elisa and in gastrointestinal tissues by RT-PCR, Western blotting and Immunohistochemistry. The results showed the rates of gastric emptying and intestinal propulsion slowed down markedly as compared to Con（$P<0.05$）and gastrointestinal myoelectric activity attenuated, interdigestive myoelectric activity complex (IDMEC) interrupted and instead of irregular myoelectric activity. The level of leptin and VIP markedly increased as compared to Con（$P<0.05$ in serum. The expression of leptin, P substance, VIP increased, motilin and CCK decreased as compared to Con（$P<0.05$）in gastrointestinal tissues. The data suggest parasecretion of brain-gut peptides is the main reason to cause gastrointestinal motility disorder in FD rats. No COI.

ABS0433

Colokinetic effect of serotonin is mediated by activation of lumbosacral 5-HT2 and 5-HT3 receptors in rats.

Hiroyuki Nakamori[1]*, Kiyotada Naitou[1], Yuuki Sano[1], Takahiko Shiina[1], Yasutake Shimizu[1]
[1]*Department of Basic Veterinary Science, Laboratory of Physiology, The United Graduate School of Veterinary Sciences, Gifu University, Japan*
**Email : nakahiro.handball@gmail.com*

The monoamine neurotransmitter serotonin is released from spinal terminals of nucleus raphe magnus (NRM) neurons. One of major serotonergic effects in the spinal cord is to depress ascending nociceptive transmission. Since pattern of release from NRM neurons uses volume transmission, we hypothesized that serotonin diffuses and influences the defecation center in the lumbosacral spinal cord. In the present study, therefore, we examined effects of 5-HT receptor stimulation in the lumbosacral defecation center on colorectal motility in rats. Rats were anesthetized with α-chloralose and ketamine, and colorectal intraluminal pressure and expelled liquid volume were recorded in vivo. Intrathecal administration of serotonin into the L6-S1 spinal cord caused propulsive contractions of the colorectum. This serotonergic colokinetic effect remained unaffected even after disconnecting from supraspinal regions by severing the T10 spinal cord. On the other hands, transection of the pelvic nerves prevented the serotonin-induced enhancement of colorectal motility. Pharmacological experiments revealed that the effect of serotonin is mediated by 5-HT2 and 5-HT3 receptors. In conclusion, lumbosacral serotonin acting on 5-HT2 and 5-HT3 receptors promotes propulsive colorectal motility via the pelvic nerves. Considering that visceral pain activates descending inhibitory pain modulation pathways including NRM neurons, our results provide a rationale for the concurrence of abdominal pain and colonic motility disorder such as irritable bowel syndrome. No COI.

ABS0443

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

Saravut Satitsri[1], Pawin Pongkorpsakol[1], Potjanee Srimanote[2], Varanuj Chatsudthipong[1], Chatchai Muanprasat[1]*
[1]*Department of Physiology, Faculty of Science, Mahidol University, Thailand* [2]*Graduate Study, Faculty of Allied Health Sciences, Thammasat University, Thailand*
**Email : chatchai.mua@mahidol.ac.th*

Vibrio cholerae is known to cause non-inflammatory diarrhea. However, inflammatory responses were detected in the intestinal mucosa of cholera patients, suggesting that intestinal inflammation may play roles in pathogenesis of cholera, especially by enhancing severity of cholera diarrheas as a result of intestinal barrier disruption. In this study, the effect of a current circulating strain of V.cholerae known to cause severe diarrheas, V.cholerae O1 El Tor variant, on intestinal barrier function was investigated in comparison with the O1 classical strain of V.cholerae. The two strains of V.cholerae were inoculated into the mouse closed ileal loops and, 12 hours later, intestinal barrier integrity was evaluated by determining trans-intestinal flux of 4-kDa dextran tagged with fluorescein thiocyanate (FITC). V. cholerae O1 classical strain did not induce intestinal barrier leakage. In contrast, V.cholerae O1 El Tor variant significantly promoted intestinal barrier leakage compared with control. Interestingly, NF-κB activity and mRNA levels of proinflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8 were dramatically increased in V.cholerae O1 El Tor variant-infected group compared with control and the classical strain. Indeed, inhibition of NF-κB activation by NF-κB inhibitor, BAY 11-7082, diminished intestinal barrier leakage and expression of proinflammatory cytokines in the V.cholerae O1 El Tor variant-infected group. The present study reveals that the circulating strain of V.cholerae O1 El Tor variant induces intestinal barrier disruption through NF-κB-mediated inflammatory responses. This mechanism may be accountable for the more severe diarrheas observed in patients infected with the current circulating strain of V.cholerae. No COI.

ABS0458

Alteration of gastric emptying in diabetic rats

Nipaporn Muangchan[1], Supatra Lohsiriwat[1], Suwattanee Kooptiwut[1], Sompol Tapechum[1], Pravit Akarasereenont[1], Reawika Chaikomin[2]*
[1]Department of Physiology, Faculty of Medicine Siriraj Hospital, Thailand [2]Department of Pharmacology and Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
**Email : reawika.cha@mahidol.ac.th*

Gastroparesis, a delay in gastric emptying rate, is a complication found more common in diabetes. However, accelerated gastric emptying has also been reported in both type 1 and type 2 diabetes. There are few studies about gastric emptying changes during the progression of diabetes in the same animal. Moreover, gastric emptying study in human is very expensive and exposed to radioactive substance. We aimed to determine the alteration of gastric emptying rate during the progression of diabetes in rats. The rats were divided into control group (CON) and diabetic group (DM; streptozotocin (STZ), i.p. 60 mg/kg BW). Fasting blood glucose (FBG) levels were measured weekly in blood from tail vein. The [13C] acetic acid breath test was used to evaluate the gastric emptying changes at the early diabetic state (12 weeks of diabetes) and long-term diabetes (20 and 28 weeks of diabetes). The results showed that the FBG of DM was significantly increased as compared to CON ($P<0.05$) whereas the body weight of DM was gradually decreased as compared to CON ($P<0.05$). Interestingly, the gastric emptying rate was accelerated at 12 weeks of diabetes and returned to normal at 20 and 28 weeks of diabetes. These data suggest that in STZ-induced diabetic rats, the gastric emptying was accelerated in early state of diabetes and then declined to normal. The delayed gastric emptying was not found up to 28 week of diabetes. The effect of long-standing diabetes (> 28 weeks) needs to be further investigated. No COI.

ABS0472

Effect of Tiliacora triandra (Colebr.) diels on cholesterol synthesis and absorption in vitro.

Tipthida Pasachan[1], Atcharaporn Ontawong[1], Acharaporn Duangjai[2], Anchalee Pongchaidecha[1], Chatchai Muanprasat[3], Chutima Srimareong[1]*
[1]Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand [2]Division of Physiology, School of Medical Sciences, University of Phayao, Phayao, Thailand [3]Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand
**Email : chutima.srimaroeng@cmu.ac.th*

Tiliacora triandra (Colebr.) Diels (TT) or Yanang, in Thai is the native plant of Southeast Asia and widely used in northeastern Thai cuisines. In vivo and in vitro studies, TT demonstrated anti-pyretic, anti-inflammatory, anticancer, antioxidant, and anti-dyslipidemia effects. However, the nutraceutical effects of TT on cholesterol synthesis and absorption are limited. Therefore, this study aimed to investigate the direct effects of TT aqueous extract (TTE) on cholesterol synthesis and absorption using human intestinal Caco-2 cells. Cholesterol synthesis was examined using the HMG-CoA reductase (HMGCR) assay and cholesterol absorption, represented by the physicochemical property of cholesterol micelles size and solubility formations, was also investigated. Moreover, the effect of TTE on [3H]-cholesterol transport into human intestinal Caco-2 cells was determined. The results showed that TTE inhibited the activity of HMGCR, a key enzyme for liver cholesterol synthesis, in a concentration-dependent manner. In addition, marked decreases in micellar solubility of cholesterol and significant increases in micelle size by TTE were observed in a dose-dependent. Consistently, TTE significantly reduced [3H]-cholesterol uptake into human intestinal Caco-2 cells compared to that of control. Thus, these findings suggest that TTE exhibits lipid lowering action by decreasing both liver synthesis and intestinal absorption of cholesterol. Further in vivo studies are required to elucidate the mechanisms involvement of anti-dyslipidemia effects by TTE. This study is supported by the Research and Researcher for Industry (RRI) by Thailand Research Fund (TRF) and Ampol Food Processing Co., Ltd (Bangkok, Thailand).

ABS0481

The effects of furosemide on ileal motility in male mice.

Kannaree Kaewsaro[1]*, Sara Bumrungsri[1], Pissared Khuituan[2]
[1]*Department of Biology, Faculty of Science, Prince of Songkla University, Thailand* [2]*Department of Physiology, Faculty of Science, Prince of Songkla University, Thailand*
**Email : kannaree24@gmail.com*

Furosemide is a loop diuretic that act on the Na^+-K^+-$2Cl^-$ co-transporter in the thick ascending of Henle's loop to inhibit Na^+ and Cl^- reabsorption resulting in increased urine production. Furosemide also has non-diuretic effect on gastrointestinal tract. Associate with the side effects of patients taking furosemide have diarrhea or constipation probably resulting from gastrointestinal motility disorder. Therefore, the aim of this study was to investigate the effect of furosemide on the longitudinal smooth muscle contractions in male ICR mice ileum by using in vitro organ bath study. After 20 minutes equilibration, the isolated ileal tissue is exposed to DMSO (vehicle control), 10, 50, 100, 500 μM or 1 mM furosemide added directly into the tissue chamber containing 37ºC oxygenated Krebs solution. The amplitude and frequency of smooth muscle contraction were recorded every 2 minutes throughout the 20 minutes experiment. It was found that at 50, 100, 500 μM and 1 mM furosemide, the contractile amplitude was significantly decreased ($P<0.001$, $P<0.01$, $P<0.001$ and $P<0.05$), but had no impact on the frequency. Time-course studies showed that optimal period for furosemide inducing intestinal smooth muscle contractile inhibition was 18 minutes post-incubation. These studies showed that furosemide inhibited ileal smooth muscle motility, which might be possible to base on pharmacological considerations for applying to severe diarrhea patients. Further studies should demonstrate the mechanisms of furosemide on gastrointestinal motility. No COI.

ABS0483

Effect of morelloflavone on duodenal contraction of cisplatin-treated rats

Nongyao Kitjaroennirut[1]*, Suphap Nuanplab[1], Wilawan Mahabusarakam[2], Siriphun Hiranyachattada[1]
[1]*Department of Physiology* [2]*Department of Chemistry, Faculty of Science, Prince of Songkla University, Thailand*
**Email : nongyao.k@psu.ac.th*

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

ABS0505

Effect of genistein attenuates NSAIDs-induced gastropathy in rats

Duangporn Werawatganon[1]*, Kanjana Somanawat[1], Sarocha Vivatvakin[1], Sakolwan Suchartlikitwong[1], Kasiphak Kaikaew[1], Naruemon Klaikeaw[1], Prasong Siriviriyakul[1]
[1]*Chulalongkorn University, Thailand*
**Email : dr.duangporn@gmail.com*

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed worldwide and known to induce gastric injury from multiple mechanisms. Genistein, one of the flavonoid compounds, isoflavone, is found mostly in soybean. Previous studies show that flavonoid compounds have good outcomes with gastritis, ulcer, and cancer. The aim of this study was to investigate the anti-inflammation effect of genistein on NSAIDs-induced gastropathy. Male Sprague-Dawley rats were randomly divided into three groups (n = 6, each). Group 1 (control) was fed with distilled water. Group 2 (NSAIDs) was fed with 150 mg/kg indomethacin dissolved in 5% $NaHCO_3$- at time 0th and 4th h. Group 3 (NSAIDs + Gen) was fed with 150 mg/kg indomethacin as previously described and 100 mg/kg genistein dissolved in 0.1% DMSO at time 0th and 4th h. Stomach was removed to study histopathology at 8 h after treatment. Serum was collected to determine TNF-α and PGE2 using ELISA technique. In NSAIDS group, serum TNF-α was significantly increased and PGE2 was significantly decreased when compared to control group (210.28 ± 0.10 vs 126.40 ± 0.13 pg/mL and 152.83 ± 0.01 vs 303.33 ± 2.16 pg/mL, P = 0.000, respectively). These significantly attenuated in NSAIDs + Gen group when compared to NSAIDs group (156.59 ± 0.10 vs 210.28 ± 0.10 pg/mL and 247.65 ± 0.01 vs 152.83 ± 0.01 pg/mL, P = 0.000, respectively). In NSAIDs group, rats developed moderate to severe gastric inflammation, erosion, and ulcer. In the NSAIDs + Gen group, histopathology was improved when compared to the NSAIDs group. Genistein attenuated NSAIDs-induced gastropathy by increased PGE2, reduced inflammatory cytokines, and improved histopathology. No COI.

ABS0517

Protective effects of manassantin A against ethanol-induced gastric ulcer in rats

Ji-Won Song[1], Young-Soek Kim[1], Charith ub Wijerathne[1], Hwa-Young Son[1], Hyo-Jung Kwon[1]*
[1]*Department of Veterinary Pathology, College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea, Korea*
**Email : hyojung@cnu.ac.kr*

We examined the protective effect of manassantin A on the ethanol-induced gastric ulcer in rats. Gastric injury was induced by intragastric administration of 5 mL/kg body weight of absolute ethanol to each rat. The positive control group and the manassantin A group were given oral doses of omeprazole (50 mg/kg) or manassantin A (200 mg/kg), respectively, 2 h prior to the administration of absolute ethanol. The stomach of each animal was excised and examined for gastric mucosal lesions. To confirm the protective effects of manassantin A, we evaluated the degree of lipid peroxidation, the level of reduced glutathione (GSH), and the activities of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD). In addition, Prostaglandin E2 (PGE2) and iNOS level were measured. Furthermore, we conducted an acute toxicity study to evaluate the safety of manassantin A according to OECD guideline. Manassantin A reduced ethanol-induced hemorrhage, hyperemia, and loss of epithelial cell in the gastric mucosa. Manassantin A reduced the increased lipid peroxidation associated with ethanol-induced acute gastric lesions, and increased the mucosal GSH content and the activities of antioxidant enzymes. In addition, manassantin A pretreatment prevented the ethanol-induced decrease of Prostaglandin E2 and decreased iNOS level in gastric mucosa. Furthermore, manassantin A did not cause any adverse effects. These results indicate that manassantin A protects the gastric mucosa against ethanol-induced gastric injury by increasing the antioxidant status. We suggest that manassantin A could be developed as an effective drug for the treatment of gastric injury caused by alcohol intake. No COI.

ABS0146

Role of KCC2 down-regulation on motor functional recovery after sciatic nerve injury

Takuya Toda[1], Hiroaki Wake[2], Junichi Nabekura[2]*
[1]*Sokendai, Japan* [2]*National Institute for Physiological sciences, Japan*
**Email : nabekura@nips.ac.jp*

The K^+-Cl^- co-transporter (KCC2) is a major Cl^- efflux pathway in neurons necessary for hyperpolarizing GABAA and Glycine-receptor mediated inhibitory responses. Down-regulation of KCC2 expression occurs in several pathological conditions and results in a conversion of GABA responses from the inhibitory to the excitatory. Excitatory GABA signaling at post-injury has been proposed to facilitate the recovery of neuronal circuits and appropriate firing patterns. To directly test this hypothesis we manipulated KCC2 expression using a tetracycline-inducible transgenic mouse and examined motor function recovery following peripheral nerve injury. In wild type control mice, KCC2 mRNA expression in the ventral horn ipsilateral to the nerve injury was significantly decreased 3 days after injury, as compared to the contralateral side, and recovered to control levels by 42 days after injury. Motor function, assessed by the rotor rod test, was markedly decreased in WT mice following injury and recovered to control levels by 28 days. In contrast, in mice with overexpression of KCC2 motor function recovery was delayed, and was still incomplete by 42 days post injury. Furthermore the expression of the GABA synthesizing enzymes GAD65 and GAD67 were decreased at 42 days post-injury in WT mice but not in KCC2 overexpressing mice. Hence injury induced loss of KCC2 and subsequent excitatory GABA and reduced GABA-ergic signaling is beneficial for neuronal recovery after injury. Down-regulation of GABA inhibitory signaling may enhance excitability of neural circuits and contribute to promoting recovery of motor function. No COI.

ABS0174

P2Y6 purinoceptor mediates the effect of H5N1 avian influenza virus on cytokine production in human respiratory epithelium

Nawiya Huipao[1]*, Suparerk Borwornpinyo[2], Siriphun Hiranyachattada[1], Arunee Thitithanyanont[3], Chumpol Pholpramool[4], David Cook[5], Anuwat Dinudom[5]
[1]*Department of Physiology, Faculty of Science, Prince of Songkla University, Thailand* [2]*Department of Biotechnology* [3]*Department of Microbiology* [4]*Department of Physiology, Faculty of Science, Mahidol University, Thailand* [5]*Discipline of Physiology, The Bosch Institute, School of Medical Sciences, The University of Sydney, Australi*a
**Email : nawiya.h@psu.ac.th*

Important pathogenesis of H5N1 infection is the cytokine storm and severe respiratory distress. Recent reports suggested that H5N1 may trigger cytokine production via unknown infection-independent mechanisms. We investigated the mechanism underlying the effect of β-propiolactone (BPL)-treated, non-replicated, H5N1 on cytokine production in 16HBE14o- human respiratory epithelial cells. We found that BPL-H5N1 increased mRNA expression of IL-6 and IL-8, which was inhibited by suramin, MRS2578, and apyrase. The effect of H5N1 was attenuated by α-2,3 sialidase, suggesting that an interaction between H5 hemagglutinin and α-2,3 sialic acid receptors of the host cells is essential for inducing the effect. In addition, an inhibitory effect of apyrase suggests that the effect of H5N1 is mediated by nucleotides released from the epithelium. Furthermore, PLC, PKC, intracellular Ca^{2+}, NF-κB, ERK1/2 and p38 MAPK were found to be involved in the H5N1-mediated effect. Taken together, our findings suggest that the infection-independent effect of H5N1 is mediated by one of the viral structural proteins that triggers nucleotide releases from the respiratory cells. Consequently, activation of P2Y6R signaling leads to increasing transcription of IL-6 and IL-8 genes. Our data also suggest that pharmacological inhibitors of PLC, PKC and MAPK may be useful therapeutic tools for delaying an onset of hypercytokinemia known to be the major cause of fatality in H5N1 infected patients. No COI.

ABS0242

Inhibition of the extrinsic aging-related ion channels TRPV1 and ORAI1 by constituents of Cyperus rotundus rhizome

Joohyun Nam[1]*, Dong-Ung Lee[1]
[1]*Korean Physiological Society, Korea*
**Email : jhnam@dongguk.ac.kr*

Exposure to solar ultraviolet (UV) radiation is the major cause of extrinsic skin aging (photoaging), which is clinically characterized by deep skin wrinkling and pigmentation. These phenomena are caused by an increase in metalloproteinase-1 (MMP-1) expression in keratinocytes and tyrosinase activation in melanocytes. In a recent study, it was reported that two Ca^{2+} channels, transient receptor potential vanilloid type-1 (TRPV1) and calcium release-activated calcium channel protein 1 (ORAI1), are respectively involved in MMP-1 expression and tyrosinase activity induced by exposure to UV radiation. In the present study, we evaluated whether an extract of Cyperus rotundus rhizomes has inhibitory effects on TRPV1 and ORAI1 by using the whole-cell patch-clamp technique and measuring intracellular Ca^{2+} levels. In our electrophysiological study, the hexane fraction of the Cyperus rotundus extract was found to strongly block capsaicin-induced TRPV1 and ORAI1 currents in HEK293T cells overexpressing TRPV1 or a combination of ORAI1 and STIM1. Furthermore, of the 5 compounds isolated from the hexane fractions, valencene had inhibitory effects on both ORAI1 (95 ± 5% at 90 μM) and TRPV1 (69 ± 15% at 90 μM) activation. Our findings suggest that the C. rotundus extract and its constituents provide a novel approach for treating and preventing UV-induced skin aging. No COI.

ABS0262

Enhanced expression of epithelial sodium channel (ENaC)-α, β and γ in kidneys of orchidectomized rats by testosterone

Su Yi Loh[1], Nelli Giribabu[1], Naguib Salleh[1]*
[1]*University of Malaya, Malaysia*
**Email : naguib.salleh@yahoo.com.my*

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

ABS0301

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

Ayako Katagi[1]*, Li Sui[1], Kazuyo Kamitori[1], Toshisada Suzuki[1], Takeshi Katayama[1], Akram Hossain[1], Chisato Noguchi[1], Youyi Dong[1], Fuminori Yamaguchi[1], Masaaki Tokuda[1]

[1]*Kagawa University, Faculty of Medicine, Japan*

**Email : s12d733@stu.med.kagawa-u-ac.jp*

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

ABS0361

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

Kazushi Yamaguchi[1]*, Ryoji Kitamura[1], Ryosuke Kawakami[2], Tomomi Nemoto[2]

[1]*Graduate School of Information Science and Technology, Hokkaido University, Japan* [2]*Research Institute for Electronic Science, Hokkaido University, Japan*

**Email : kazushi0320@eis.hokudai.ac.jp*

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

ABS0413

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

Ryosuke Takeda[1]*, Daiki Imai[1], Akemi Ota[1], Nooshin Naghavi[1], Yoshihiro Yamashina[1], Yoshikazu Hirasawa[1], Akina Suzuki[1], Hisayo Yokoyama[1], Toshiaki Miyagawa[1], Kazunobu Okazaki[1]

[1]*Department of Environmental Physiology for Exercise, Graduate School of Medicine, Osaka City University, Japan*

**Email : m2065903@med.osaka-cu.ac.jp*

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

ABS0454

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

Chau-Zen Wang[1], Tzu- Lin[2], Mei-Ling Ho[1], Je-Ken Chang[3]*

[1]*Department of Physiology* [2]*Orthopaedic Research Center* [3]*Department of Orthopedics, College of Medicine, Kaohsiung Medical University, Taiwan*

**Email : jkchang@cc.kmu.edu.tw*

Injectable thermo-responsive hydrogels are advantageous cell delivery-biomaterials for applications in the minimally invasive tissue engineering. We previously demonstrated that a hyaluronic acid (HA)-enriched microenvironment enhances the chondrogenesis of adipose-derived stem cells (ADSCs). In this study, we investigated the chondrogenic potential of two newly developed HA-modified thermo-responsive poly(N-isopropylacrylamide) (PNIPAAm) hydrogels in vitro and in vivo in neo-cartilage formation combined with rabbit ADSCs (rADSCs) for articular cartilage tissue engineering. The thermo-responsive HA-mixed PNIPAAm (HA-PNIPAAm-CP) and HA-cross-linked PNIPAAm (HA-PNIPAAm-CL) were fabricated using physical interaction and chemical cross-linking methods, respectively. The in vitro results showed that, compared to unmodified PNIPAAm, both HA-modified hydrogels significantly increased the cell viability, expression of chondrogenic marker genes (collagen type II and aggrecan) and formation of sulfated glycosaminoglycan (sGAG) in embedded rADSCs. The chondrogenic effects of HA-modified hydrogels on rADSC were confirmed in vivo by intra-articular injection of rADSC/hydrogels constructs into rabbit joint cavities for 3 weeks, in which embedded-rADSCs in hydrogels were traced using CM-Dil labeling. The in vivo results showed that implanted rADSC/HA-PNIPAAm-CL constructs had markedly more neo-cartilage formation than that of rADSC/HA-PNIPAAm-CP and rADSC/PNIPAAm constructs. These results indicated that the HA-PNIPAAm-CL hydrogel provides a suitable microenvironment to enhance ADSC chondrogenesis for cartilage tissue engineering applications. No COI.

ABS0532

Apelin-13 impaired acquisition but not consolidation or expression of contextual fear in rats

Li Han[1], Huaiqing Luo[2], Shaowen Tian [3], Yang Xiang[1], Chi Liu[1], Xiangping Qu[1], Huijun Liu[1], Xiaoqun Qin[1]*
[1]Central South University, China [2]Changsha Medical University, China [3]University of South China, China
**Email : Qinxiaoqun@csu.edu.cn*

Recently apelin-13 was demonstrated to be involved in the processes that contribute to learning and memory. While contextual fear conditioning is a form of associative learning that has been used to elucidate mechanisms that are involved in aversive memory processes. At present study, we tested the effects of exogenous apelin-13 (1.0, 2.0 and 4.0 μg/rat) on contextual fear conditioning (experiment 1), fear consolidation (experiment 2) and fear expression (experiment 3) in rats. Behavior procedure involved three training phases: habituation, fear conditioning and test, each separated by 24 h. Apelin-13 was injected i.c.v. 10 min before conditioning, immediately after conditioning and 10 min before testing. Percent freezing time on each time block during which no footshock was presented was scored. We found that apelin-13 administrations had no effect on freezing in experiment 2 and 3 but produced a decrease in experiment 1[$F(3,28) = 3.494$, $P < 0.05$]. Apelin-13 (2.0 μg/rat) group in experiment 1 presented a significant lower freezing during both fear conditioning (time block 4, $P < 0.05$; 5 and 6, $P < 0.01$) and test phases ($P < 0.01$). Further results indicated that the decreased freezing in experiment 1 was not attributed to nonspecific changes of locomotor activity. These results showed that apelin-13 impaired fear acquisition but not fear consolidation or expression, which extend previous research on the apelin-13 effects on learning and memory. (This work was supported by grants #81270065, #81370116 from NSFC, grant#2013JJ4030, #2015JJ2147from Hunan Natural Science Foundation, and grants #14C0128 from Scientific Research Fund of Hunan Provincial Education Department). No COI.

ABS0209

Comparison between MRI findings and motor development in the cases with neonatal HIE

Sayaka Okuno[1]*, Natsuko Tabata[1], Makoto Nabetani[2], Takashi Morita[1], Tamaki Wada[1], Natsuko Shiomi[1], Nanae Yutaka[1], Masato Nishihara[1], Hiroyuki Sano[1]

[1]Osaka, Japan [2]Osaka , Japan

**Email : sayakazaki@yahoo.co.jp*

In the cases with moderate to severe neonatal hypoxic-ischemic-encephalopathy (HIE), it is well known that those MRI findings are one of the most sensitive predictor of neurological prognosis of these patients. However, there are some cases show differences between MRI findings and their development. We investigated correlative of 43 cases with moderate to severe HIE, comparing MRI findings at six to twelve months old with neurological prognosis at one and a half year old. No COI.

P9 MEMBRANE AND EPITHELIAL TRANSPORT

ABS0047

Microcirculation and paracellular fluid secretion in the perfused submandibular gland

Masataka Murakami[1]*, Fei Wei[1], Takanori Narita[2], Miwako Fukushima[3], Sadamitsu Hashimoto[4], Yoshiyuki Shibukawa[4], Masaki Sato[4]

[1]National Institute for Physiological Sciences, NINS, Japan [2]Nihon University College of Bioresource Scis, Japan [3]Showa University School of Dentistry, Japan [4]Tokyo Dental College, Japan

**Email : masataka@nips.ac.jp*

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

ABS0056

Role of TRPM6 and TRPM7 in maternal-fetal calcium transport

Yoshiro Suzuki[1]*, Masaki Watanabe[1], Claire Saito[1], Makoto Tominaga[1]

[1]Okazaki Institute for Integrative Bioscience (National Institute for Physiological Sciences), Japan

**Email : yoshiro@nips.ac.jp*

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

ABS0330

In vitro study of organic cation drug transport mediated by human organic cation transporter 1 (SLC22A1) and 3 (SLC22A3) in hepatocellular carcinoma (HepG2) cells

Metee Jinakote[1], Sunhapas Soodvilai[2], Atcharaporn Ontawong[1], Chutima Srimaroeng[1]*
[1]*Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand* [2]*Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand*
**Email : chutima.srimaroeng@cmu.ac.th*

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

ABS0334

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

Oranit Boonphang[1], Atcharaporn Ontawong[1], Anchalee Pongchidecha[1], Tipthida Pasachan[1], Acharaporn Duangjai[2], Chutima Srimaroeng[1]*
[1]*Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand* [2]*Division of Physiology, School of Medical Sciences, University of Phayao, Phayao, Thailand*
**Email : chutima.srimaroeng@cmu.ac.th*

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

ABS0375

Properties of volume-sensitive anion channels in butyrate-triggered apoptosis of murine colonic epithelial cells

Takahiro Shimizu[1]*, Hironao Ohtake[1], Takuto Fujii[1], Yoshiaki Tabuchi[2], Hideki Sakai[1]
[1]*Department of Pharmaceutical Physiology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Japan* [2]*Life Science Research Center, University of Toyama, Japan*
**Email : takshimi@pha.u-toyama.ac.jp*

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

ABS0405

Human organic anion transporter 10 (OAT10) transports urate and Ketone bodies

Promsuk Jutabha[1]*, Naohiko Anzai[1]
[1]*Dokkyo Medical University School of Medicine/ Department Pharmacology & Toxicology, Japan*
**Email : pjutabha@dokkyomed.ac.jp*

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

ABS0431

Evidence for a modulatory role of annexin A2 in the maxi-anion channel activity

Md. Rafiqul Islam[1]*, Toshiaki Okada[2], Petr Merzlyak[2], Ravshan Z. Sabirov[2], Yasunobu Okada[3]
[1]*National Institute for Physiological Sciences, Japan* [2]*National Institute for Physiological Sciences, Japan*
[3]*Sokendai (The Graduate University for Advanced Studies), Japan*
**Email : rislam@nips.ac.jp*

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

ABS0518

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

Joohyun Nam[1]*, Woo Kyung Kim[2]
[1]*Department of Physiology, Dongguk University College of Medicine, Korea* [2]*Department of Internal Medicine, Dongguk University College of Medicine, Korea*
**Email : jhnam@dongguk.ac.kr*

Intracellular Ca^{2+} signaling via various calcium channels has been shown to directly modulate skin inflammation. Ca^{2+} influx through these channels eventually generates intracellular Ca^{2+} signaling that results in different outcomes dependent on the individual Ca^{2+} channel type, for example, immune cell modulation through Orai1, epidermal barrier formation and keratinocyte differentiation through TRPA1, and keratinocyte cornification through TRPV3. Therefore, a specific agonist/antagonist for each calcium channel is required for maintaining skin barrier homeostasis and for the treatment of dermatological diseases. We prepared 70% MeOH extracts of 30 medicinal herbs, performed bioassay-guided fractionation of the active extracts, and then isolated and identified the bioactive constituents. By performing the combination of automated and conventional whole-cell patch clamp studies, we found eight medicinal herb fractions for Orai1, four for TRPV1, two for TRPA1, and one for TRPV3 that showed >50% inhibition rates at 30 μg/mL. We also found three fractions with TRPA1 agonist activity. Further, we also identified chemical constituents that inhibit Orai1 (compound V: 95 ± 5% inhibition at 90 μM) and TRPV1 (compound M: 93.9 ± 2.45% inhibition at 90 μM). Considering that most regional plants have not been investigated chemically or pharmaceutically, they remain as untapped potential sources of topical agents for drugs and other application. We found major active components and chemical constituents of plant extracts for the modulation of various calcium ion channels, which may have potential clinical applications for abnormal skin barrier functions such as atopic dermatitis. No COI.

ABS0309

Selenium-induced autophagy is associated with decreased mitochondrial marker and contributes to regulation of oxidative fiber proportion in L6 myoblasts

Ronny Lesmana[1]*, Shu Yun Chong[2], Putri Teesa Radhiyanthi[3], Hanna Goenawan[3], Vita Muniarti Tarawan[3], Reni Farenia[3], Winifred Yau Wing Yiu[2], Rohit Sinha[2], Paul Yen[4]

[1]Universitas Padjadjaran/DUKE-NUS, Singapore [2]DUKE NUS, Singapore [3]UNPAD, Indonesia [4]Duke NUS, Singapore

**Email : cloencool@gmail.com*

Selenium is a trace element that is naturally present in many foods. It appears to be necessary for normal muscle function and homeostasis. Hypo- or hyperselenium is associated with several muscular diseases affecting both cardiac and skeletal muscles in both cattle and humans, and has led to the identification of a new clinical syndrome called nutritional muscular dystrophy. Indeed, excess selenium intake has been reported to be associated with muscle weakness and peripheral neuropathy, whereas selenium deficiency caused dilated cardiomyopathy (keshan disease). Unfortunately, there is limited information about how selenium maintains muscle function. Therefore, we studied the effects of selenium on mitochondria content and fiber type arrangement and autophagy induction in a L6-rat myoblast derived cell line (L6). L6 cells were seeded into a 6/12-well plate with cell density of 4×10^6 cells/well and cultured for 3 days until 80-90% confluency. 24h before cell harvesting, the myoblasts were treated with different concentrations of Selenium (1, 10, 100 μM). We observed that 10 μM selenium significantly decreased LC3II (autophagosome marker) and p62 protein levels indicative of increased autophagic flux. Furthermore, 10 μM selenium increased phosphorylation of AMPK and ULK1 (S555), and decreased mTOR signaling, two key pathways that regulate autophagy. Interestingly, we also observed that PPAR1A (PGC1α), COX IV and HSP60 protein levels were decreased by selenium treatment. Moreover, selenium also reduced significantly the expression of the oxidative muscle fiber marker, βMHC. Taken together, selenium induced autophagy and decreased mitochondrial content to reduce oxidative fiber ratio and potentially may lead to suboptimal muscle performance. No COI.

ABS0522

Expression level of importin and exportin of mouse skeletal muscle in response to unloading with or without reloading

Katsumasa Goto[1]*, Takao Sugiura[2], Yoshinobu Ohira[3], Toshitada Yoshioka[4]

[1]Graduate School of Health Sciences, Toyohashi SOZO University, Japan [2]Yamaguchi University, Japan [3]Graduate School of Sport and Health Science, Doshisha University, Japan [4]Hirosaki Gakuin University, Japan

**Email : gotok@sepia.ocn.ne.jp*

Loading is a regulatory factor for skeletal muscle mass as well as function. However, the molecular mechanisms of loading-associated gene expressions in skeletal muscle remain unclear. Recently, it has been suggested that an accumulation of proteins, such as muscleblind-like 1 (MBNL1), in nuclei plays an important role in skeletal muscle atrophy in myotonic dystrophy (DM1). In the present study, therefore, we investigated the response of the nuclear transport proteins, importin b and exportin, to unloading in mouse skeletal muscle. Mice (C57BL/6J) were randomly divided into two groups, untreated control and hindlimb suspension (HS) groups. The mice of HS group were subjected to continuous hindlimb suspension for 2 weeks with or without 2-week ambulatory recovery. The medial gastrocnemius (MGAS) and plantaris muscles of mice in both groups were dissected bilaterally immediately after the suspension and/or 2 weeks of recovery. Significant reduction of muscle mass was observed in MGAS, but not plantaris. Although the expression level of importin was not changed in both muscles, the expression level of exportin in plantaris was upregulated by unloading, but not in MGAS. Therefore, the accumulation level of proteins in nuclei might be a loading-sensitive regulatory factor of skeletal muscle. No COI.

ABS0016

Activation of cannabinoid system in nucleus accumbens affects cost-benefit decision making

Zahra Fatahi Vanani[1]*, Abbas Haghparast[1], Bahman Sadeghi[1], Abbas Khani[1], Marziyeh Moradi[1]
[1]*Neuroscience Research Center, Shahid Beheshti University of Medical Science, Iran*
**Email : biofatahi2001@yahoo.com*

Ability to choose goals based on usefulness or the time of reaching goals are the important aspects of decision-making. Previous evidence showed that decision making altered in cannabis abusers but, the role of the cannabinoid system in decision-making circuits has not been investigated exactly. In the current study, we examined the effects of cannabinoid modulation during cost-benefit decision making in the nucleus accumbens (NAc). We trained different groups of rats in a delay-based and an effort-based form of cost-benefit T-maze decision-making task. During test days, the rats received local injections of either vehicle or win 55,212-2, a cannabinoid receptor agonist, (0.125, 0.25, 0.5, 1 and 2 mM/0.5 µl DMSO) in the NAc bilaterally. We measured spontaneous locomotor activity following the same treatments. Then, AM251, a cannabinoid antagonist, (250 µM/0.5 µl DMSO) was injected in the NAc, 5 min before the administration of the most effective dose of win 55,212-2 (2 mM). The results showed that activation of cannabinoid receptor in the NAc impaired decision making such that rats were less willing to invest physical effort to gain high reward. Similarly, cannabinoid receptor activation in this area induced impulsive pattern of choice such that rats preferred small immediate rewards to large delayed rewards. Control tasks ensured that the effects were specific for differential cost-benefit tasks. These finding revealed that the cannabinoid system in the NAc plays a critical role in regulating cost-benefit decision making. No COI.

ABS0037

Wnt signaling regulates blood pressure by downregulating a GSK-3b–mediated pathway to enhance insulin signaling in the central nervous system

Ching-Jiunn Tseng[1]*, Luo-Ping Ger[1], Pei-Wen Cheng[1]
[1]*Kaohsiung Veterans General Hospital, Taiwan*
**Email : cjtseng@vghks.gov.tw*

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

ABS0043

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

Yung-Mei Chao[1]*, Julie Y.H. Chan[2]

[1]*Center for Translational Research in Biomedical Science, Kaohsiung Chang Gung Memorial Hospital, Taiwan*[2]*Center for Translational Research in Biomedical Science, Kaohsiung Chang Gung Memorial Hospital, Taiwan*

**Email : ymchao47@gmail.com*

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

ABS0051

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

Suguru Kobayashi[1]*

[1]*Tokushima Bunri University, Japan*

**Email : kobayashis@kph.bunri-u.ac.jp*

Synchronous oscillatory activity in a laminar structure is common in the olfactory nervous system of both vertebrates and invertebrates. In the terrestrial slugs, periodic oscillation is recorded from the surface of the laminar structure of procerebrum (PC) and its frequency changes are suggested to encode the olfactory information and memory. We recently found that oscillatory neuronal network was formed from dispersed cell culture of PC neurons. Increases in neurite arborization, neurite connection and cell aggregation were observed with time in culture. Calcium imaging for each PC neurons showed that acetylcholinesterase inhibitor or nicotine increased the number of spontaneous calcium transients and induced synchronous oscillatory activity. On the other hand, histamine increased the number of calcium transients without synchronous oscillatory activity in a smaller number of PC neurons. These results suggest that acetylcholine can function as an excitatory modulator on the synchronous oscillatory activity of the PC neuron network via nicotinic acetylcholine receptors activation. No COI.

ABS0052

Spinal IL-33/ST2 signaling contributes to neuropathic pain via neuronal CaMKII-CREB and astroglial JAK2-STAT3 cascades in mice

Yanqing Wang [1]*, Shenbin Liu[2], Wenli Mi[3]

[1]Fudan University, China [2]Department of Integrative Medicine and Neurobiology, Shanghai Medical College

[3]Department of Integrative Medicine and Neurobiology, Shanghai Medical College, Fudan University, China

**Email : wangyanqing@shmu.edu.cn*

Nerve damage-initiated neuroinflammation and immune response contribute to the development of neuropathic pain. This study investigates the role of spinal interleukin (IL)-33 and its receptor ST2 in the spared nerve injury (SNI)-induced neuropathic pain. Intrathecal administration of ST2-neutralising antibody or ST2 gene knockout (ST2-/-) significantly attenuated the SNI-induced mechanical and cold allodynia. The expression of spinal IL-33 and ST2 were substantially elevated by 255.8±27.3% and 266.4±83.5% (mean±SD) on the 7th day following SNI, respectively. Mechanistic studies showed that the increased expression of spinal NR1 subunit of N-methyl-D-aspartate (NMDA) receptor after SNI was reduced by ST2 antibody administration or ST2-/-. The induction of nociceptive behaviours in naïve mice by rIL-33 was reversed by the non-competitive NMDA receptor antagonist MK-801. ST2 antibody administration or ST2-/- markedly inhibited the increased activation of the astroglial janus kinase 2 (JAK2)-signal transducer and activator of transcription 3 (STAT3) cascade and the neuronal calcium-calmodulin dependent kinase II (CaMKII)-cAMP response element-binding protein (CREB) cascade after SNI. Moreover, intrathecal pretreatment with the CaMKII inhibitor KN-93 or the JAK2-STAT3 cascade inhibitor AG490 attenuated the rIL-33 induced nociceptive behaviours and NR1 up-regulation in naïve mice. The results demonstrate that spinal IL-33/ST2 signaling contributes to neuropathic pain by activating astroglial JAK2-STAT3 cascade and neuronal CaMKII-CREB cascade. No COI.

ABS0070

Anxious behavioural responses in male Formosan wood mice

Kun-Ruey Shieh[1], Ching-Lu Chen[2], Shu-Chuan Yang[3]*

[1]Department of Physiology, Tzu Chi University, Taiwan [2]Institute of Radiological Sciences, Tzu Chi College of Technology, Taiwan [3]General Education Center, Tzu Chi College of Technology, Taiwan

**Email : scyang291@gmail.com*

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

ABS0071

Behavioural responses in depression and performance of learning and memory in male Formosan wood mouse

Shu-Chuan Yang[1]*, Ching-Lu Chen[2], Kun-Ruey Shieh[3]
[1]General Education Center, Tzu Chi College of Technology, Taiwan [2]Institute of Radiological Sciences, Tzu Chi College of Technology, Taiwan [3]Department of Physiology, Tzu Chi University, Taiwan
**Email : scyang291@gmail.com*

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

ABS0072

The effect of estrogen on iron metabolism in astrocytes and neurons

Hong Jiang[1]*
[1]Qingdao University, China
**Email : jhkyk@163.com*

Estrogen affects iron metabolism in peripheral tissues, and what is the role of estrogen on iron metabolism in neurons and astroctyes in midbrain? In this study, we investigated the effect of estrogen on the iron transport proteins as well as its mechanisms. The results were as follows: Iron exporter ferroportin1 (FPN1) and iron importer divalent metal transporter 1 (DMT1) was up-regulated after estrogen was treated for 12h in primary cultured astrocytes. Hypoxia inducible factor-1alpha (HIF-1α) was up-regulated, but hypoxia inducible factor 2 alpha (HIF-2α) remained unchanged after estrogen was treated for 12 h in primary cultured astrocytes. In neurons, DMT1 was decreased but FPN1 was up-regulated after estrogen was treated for 12h in primary cultured neurons. IRP1 was down-regulated while HIF-1α and HIF-2α remained unchanged after estrogen was treated in primary cultured neurons. The results suggest that the regulations of estrogen on astroctyes and neurons are different. Estrogen can increase FPN1 and DMT1 expressions by elevating HIF-1α in astrocytes. However, the decreased expression of IRP1 may account for the decreased DMT1 and increased FPN1expressions in neurons. No COI.

ABS0073

Rosmarinic acid protects SK-N-SH cells by inhibiting iron induced α-synuclein aggregation

Junxia Xie[1]*
[1]*Qingdao University, China*
**Email : jxiaxie@public.qd.sd.cn*

Rosmarinic acid (RA) is a naturally occurring polyphenolic compound, and is composed of caffeic acid and danshensu. Our previous studies have confirmed RA could protect against 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl pyridine cation (MPP+) induced cell injury. Improving evidence showed iron-induced α-synuclein aggregation played important roles in the etiology of Parkinson's disease (PD). However, whether RA could protect dopaminergic neurons through inhibiting the aggregation of α-synuclein in PD is unclear and the regulation mechanisms underlying this inhibition were not elucidated. Therefore, the experiment proposed to explore the effects of rosmarinic acid against iron-induced α-synuclein aggregation in dopaminergic cells and elucidate the possible mechanisms in the SK-N-SH cells. Results showed that iron could reduce the mitochondrial transmembrane potential (ΔΨm) and induce α-synuclein aggregation in the SK-N-SH cells. In accordance with iron responsive element/iron regulatory protein (IRE/IRP) system, iron could increase the mRNA levels of α-synuclein. Results also showed that RA pretreatment could restore the ΔΨm reduction induced by iron and alleviate iron induced α-synuclein aggregation. Further results showed that RA pretreatment could inhibit iron induced α-synuclein aggregation by up-regulating hemeoxygenase-1 (HO-1). In addition, RA pretreatment could decrease the mRNA levels of α-synuclein via decreasing the protein levels of IRP1. These results provide new findings and new strategies for the prevention and treatment of PD. No COI.

ABS0074

Interleukin 6 regulates iron related proteins through c-Jun N-terminal kinase activation in BV2 microglial cell lines

Jun Wang[1]*
[1]*Qingdao University, China*
**Email : junwangqdu@163.com*

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

ABS0087

The neuronal and glial reorganization in the intact hemisphere contributes functional remodeling after focal stroke.

Yusuke Takatsuru[1]*, Junichi Nabekura[2], Noriyuki Koibuchi[1]
[1]*Gunma University Graduate School of Medicine, Japan* [2]*NIPS, Japan*
**Email : takatsur@gunma-u.ac.jp*

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

ABS0179

Long-lasting sound-evoked afterdischarge in the auditory midbrain

Munenori Ono[1]*, Deborah Bishop[2], Douglas Oliver[2]
[1]*Kanazawa Medical University, Japan* [2]*UConn Health Center, United States*
**Email : onomn@kanazawa-med.ac.jp*

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

ABS0260

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

Wachirapong Saleeon[1], Ukkrit Jansri[2], Anan Srikiatkhachorn[1], Saknan Bongsebandhu-Phubhakdi[1]*
[1]*Department of Physiology, Faculty of Medicine* [2]*Research Affairs, Faculty of Medicine, Chulalongkorn University, Thailand*
**Email : saknan@live.jp*

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

ABS0263

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

Sungjun Jung[1]*, Sanghoon Lee[1], Seongah Kim[2], Seogbae Oh[2]
[1]*Hanyang University, Korea* [2]*Seoul National University, Korea*
**Email : eurijj@naver.com*

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

ABS0265

The effect of N-acetyl-L-cysteine on β-amyloid induced Alzheimer disease model in rat: A behavioral and electrophysiological study

Siamak Shahidi[1]*, Somayeh Zargooshnia [1], Sara Soleimani Asl [1], Abdolrahman Sarihi [1], Alireza Komaki[1]
[1]*Neurophysiology Research Center, Hamadan University of Medical Sciences, Hamadan, Iran*
**Email : siamakshahidi@yahoo.com*

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

ABS0273

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

Mohammad Khaksari Haddad[1]*, Mojdeh Hajmohammadi[1], Nader Shahrokhi Sardoo[1]
[1]*Department of Physiology, Kerman Medical Faculty, Kerman University of Medical Sciences, Iran*
**Email : khaksar38@yahoo.co.uk*

The neuroprotective effect of alone administration of estrogen and candesartan (angiotensin receptor blocker) has reported in traumatic brain injury (TBI).In this study we evaluated the effect of combined administration of estrogen and candesartan (AT1R blocker)on intracranial pressure afterTBI. Material and Methods: The ovarectomized (OVX) female rats were divided to 7 group as follows: 1- sham, 2-TBI, 3-TBI+ estrogen, 4-TBI+OIL, 5-TBI+estrogen+low dose of candesartan, 6-TBI+estrogen+high dose of candesartan, 7-TBI+OIL+VEH.The diffuse TBI was induced by Marmarou method. Intracranial pressure (ICP) was evaluated in -1, 1,4,24 hours after TBI. Results: The ICP was higher in group2 compared to group 1 in all times. Estrogen decreased ICP compared to TBI in all times. Although the combined administration of estrogen and high dose of candesartan wasn't effective on ICP in the first hour after TBI, but the effect of estrogen on ICP was inversed by this group at 4 hour after TBI. Conclusion: The results of this study indicated that the combined administration of estrogen and high dose of candesartan after TBI eliminated the neuroprotective effect of estrogen on ICP. No COI.

ABS0274

Epigenetic regulation of Nrf2 transcription in oxidative stress-associated hypertension induced by angiotensin II

Pei-Chia Tsai[1], Julie Y.H. Chan[1]*
[1]*Kaohsiung Chang Gung Memorial Hospital, Taiwan*
**Email : jchan@adm.cgmh.org.tw*

The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a principal player in cellular antioxidant defense mechanisms via regulation of transcription of antioxidants bearing the antioxidant response element (AREs) in their promoter regions. Downregulation of many Nrf2-regulated antioxidant enzymes is known to be involved in the pathogenesis of redox-associated cardiovascular disease, including hypertension. The Nrf2 is subjected to epigenome control. The present study investigated the significance of epigenetic regulation of Nrf2 in angiotensin II (Ang II)-induced hypertension. Exposure with Ang II (0.01, 0.1 or 1 uM) to the N2a cells resulted in a decrease in Nrf2 expression, decreased expressions of the ARE-regulated antioxidants, including peroxisome proliferator-activated receptor gamma (PPARr), heme oxygenase-1 (HO-1), superoxide dismutase 1 (SOD1), SOD2 and Kelch-like ECH-associated protein 1 (Keap1), increased protein expression of histone deacetylases (HADC) 1-5, an upregulation in trimethyl-histone H3-Lysine 9 and a downregulation of acetylated-histone H3-Lysine 9 expression. Chromatin immunoprecipitation results further showed a reduction in biding affinity of H3K9-Ac to promoter region in the Nrf2 gene. Pyrosequencing for the evaluation of methylation of the CpG islands in promoter regions of Nrf2 gene, however, showed no significant change following Ang II treatment. In normotensive WKY rats, Ang II-induced pressor response was augmented in rats subjected to treatment with Nrf2 siRNA. In contrast, the gene transfer of lentivirus encoding Nrf2 to SHR resulted in a moderate decrease in arterial pressure. Together our results indicate that Ang II-dependent epigenetic downregulation of Nrf2 expression may be involved in the pressor response to Ang II. No COI.

ABS0304

Nrf2 mediated mitochondrial biogenesis deficit in neuron of rostral ventrolateral medulla led to systemic inflammation-associated hypertension

Kay LH Wu[1]*
[1]*Center for Translational Research in Biomedical Sciences, Chang Gung Memorial Hospital, Taiwan*
**Email : wlh0701@yahoo.com.tw*

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

ABS0314

Antinociceptive effects of Rhus coriaria L. extract in male rats

Saeed Mohammadi[1], Mohammad Zarei[2]*, Mohammad Mahdi Zarei[3]
[1]Department of Biology, Faculty of Basic Sciences, Islamic Azad University of Hamadan, [2]Department of Physiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan [3]Faculty of Veterinary Medicine, Karaj Branch, Islamic Azad University, Karaj, Alborz, Iran
**Email : mmmzarei@yahoo.com*

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

ABS0318

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

Wan-Yun Meng[1], Yen-Ting Chang[1], Ging-Ting Tseng[2], Yu-Li Chen[3], Mei-Shiuan Shen[1], Shih-Han Chang[1], Yi-Ru Chen[1], Ying-Jui Ho[1]*
[1]Chung Shan Medical University, Department of Psychology, Taiwan [2]School of Medical Laboratory and Biotechnology, Taiwan [3]Department of Biomedical Sciences, Taiwan
**Email : yjho@csmu.edu.tw*

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

ABS0319

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

Tomio Hayama[1]*

[1]*Department Morphological and Physiological Sciences, Faculty Life Science, Kumamoto University, Kumamoto, Japan*

**Email : to_hayama@yahoo.co.jp*

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

ABS0322

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

Tin Tin Win Shwe[1]*, Chaw Kyi-Tha-Thu[2], Yadanar Moe[2], Yuiji Fujitani[1], Shinji Tsukahara[2], Hidehiro Watanabe[1], Seishiro Hirano[1]

[1]*National Institute for Environmnetal Studies, Japan* [2]*Division of Life Science, Graduate School of Science and Engineering, Saitama University, Japan*

**Email : tin.tin.win.shwe@nies.go.jp*

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

ABS0324

Effect of developmental exposure to acetamiprid on behavioral profiles in mice

Kazuhiro Sano[1], Jiaxin Yang[1], Tin Tin Win Shwe[1], Keiko Nohara[1], Fumihiko Maekawa[1]*
[1]*Center for Environmental Health Sciences, National Institute for Environmental Studies, Japan*
**Email : fmaekawa@nies.go.jp*

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

ABS0339

Role of parabrachial monosynaptic inputs in the central amygdala nucleus network

Yae K Sugimura[1], Yukari Takahashi[1], Ayako M Watabe[1], Fusao Kato[1]*
[1]*The Jikei University School of Medicine, Japan*
**Email : fusao@jikei.ac.jp*

The central nucleus of the amygdala (CeA) is a key structure underlying nociception-induced emotional responses. Nociceptive information is conveyed from the superficial layer of the dorsal horn to the capsular part of the CeA (CeC) via the lateral parabrachial nucleus (LPB). It has been demonstrated that LPB-CeC synaptic transmission is enhanced in various pain models by using electrical stimulation of the fibers arising from the LPB in brain slices. However, it is difficult to selectively stimulate monosynaptically projecting fibers without activating local neurons and passing fibers. To overcome this limitation and elucidate the role of LPB inputs in CeA network activities, we transfected AAV vector for channelrodopsin (ChR2) expression to the LPB in rats and prepared brain slices containing the amygdala 5-7 weeks after transfection. We found that blue light illumination on the CeC, but not the surrounding areas, resulted in monosynaptic EPSCs with very small latency fluctuation in CeC neurons regardless of the firing pattern type. These EPSCs were followed by large polysynaptic IPSCs. This feedforward inhibition rapidly brought the membrane potential back to the resting state level after depolarization. Moreover, intraplanter formalin injection made 24 hours before the slice preparation resulted in a significantly larger EPSC amplitude than those with saline injection only in the CeC neurons showing late-firing pattern. These results indicate that direct monosynaptic inputs from the LPB not only excite the CeC neurons but also regulate the CeA network excitability through robust feedforward inhibition, which is under plastic modulation in response to persistent inflammatory pain. YKS is a JSPS Research Fellow. No COI.

ABS0341

Area MT neurons at the population level represent binocular disparity in a manner between correlation-based and match-based representation

Toshihide Yoshioka[1], Takahiro Doi[2], Mohammad Abdolrahmani[1], Ichiro Fujita[1]*
[1]*Graduate School of Frontier Biosciences, Osaka University, Japan* [2]*Department Neurosciences, University of Pennsylvania, United States*
**Email : fujita@fbs.osaka-u.ac.jp*

Binocular disparity is a cue for stereopsis. Psychophysical evidence suggests that the visual system represents binocular disparity based on both binocular-correlation and binocular-match signals. We previously showed that area V4 employs a match-based representation of disparity in responses pooled across neurons. As a step towards identifying the brain areas that represent disparity with binocular-correlation signals, we examined the disparity representation of MT, a counterpart of V4 in the dorsal visual pathway. We recorded single-unit activity of MT neurons from a monkey performing a fixation task and analyzed the pooled-average of the resulting tuning curves. We then manipulated the level of binocular correlation in random-dot stereograms (RDSs) by reversing the luminance contrast of a varying proportion of dots in one eye (graded anti-correlation). The amplitude of pooled disparity-tuning curve gradually decreased as the level of correlation was decreased from 100% (normal RDSs) to 0% (RDSs with half of the dots contrast-reversed). At 0% correlation, the tuning curve became completely flat. With further decrease from 0% to −100% (anti-correlated RDSs), response modulation by disparity reappeared, gradually grew, and exhibited an inverted tuning-curve shape. The tuning amplitude at −100% correlation was approximately 40% of the amplitude at 100% correlation. These changes in disparity tuning functions fall between correlation-based and match-based representation of binocular disparity. MT thus represents disparity in a more correlation-based manner than V4, whose representation is completely match-based at the population level. No COI.

ABS0344

Evaluation of spatial resolution in “in-vivo” two-photon microscopy by fluorescent microbeads injected into living mouse brain cortex

Ryoji Kitamura[1]*, Kazushi Yamaguchi[1], Ryosuke Kawakami[2], Tomomi Nemoto[2]
[1]*Graduate School of IST, Hokkaido University, Japan* [2]*RIES, Hokkaido University, Japan*
**Email : ryoji2k@gmail.com*

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

ABS0346

Non-selective NSAIDs do not affect long term synaptic plasticity but induce memory impairment

Narges Hosseinmardi[1]*, Jafar Doost Mohammadpour[2], Mahyar Janahmadi[1]
[1]*Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Iran*
[2]*Neurophysiology Research Centre, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Iran*
**Email : nargeshosseinmardi@gmail.com*

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

ABS0348

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

Yoshimasa Komatsuzaki[1], Kouya Kato[1], Minoru Saito[1]*, Ken Lukowiak[2]
[1]*Nihon University, Japan* [2]*University of Calgary, Canada*
**Email : msaito625_chaos@hotmail.com*

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

ABS0356

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

Natsuko Kubota[1]*, Shinya Yanagita[1], Yurika Takano[1], Ken Takeda[1]
[1]*Tokyo University of Science, Japan*
**Email : nkubota@rs.tus.ac.jp*

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

ABS0358

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

Chaw Kyi-Tha-Thu[1]*, Kota Okoshi[1], Hiroto Ito[1], Ken-Ichi Matsuda[2], Mitsuhiro Kawata[2], Shinji Tsukahara[2]
[1]*Division of Life Science, Graduate School of Science and Engineering, Saitama University, Japan* [2]*Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Japan*
**Email : s12db051@mail.saitama-u.ac.jp*

Estradiol, which originates from testicular testosterone and binds to estrogen receptor-α (ERα) during the perinatal period, is necessary for organization of the male-type brain in mice. In this study, we examined transgenic mice, in which ERα-expressing cells of the brain can be visualized by green fluorescence protein (GFP) under the control of the ERα promoter, to determine whether the transgenic mice are useful for the study of the sexual differentiation of the brain. Fluorescence microscopy of the brain sections obtained from adult transgenic mice showed the existence of many GFP-expressing cells in the medial preoptic area, medial preoptic nucleus, bed nucleus of the stria terminalis (BNST), striohypothalamic nucleus (StHy), and anterior hypothalamic area in both sexes. Most GFP-expressing cells in the hypothalamic brain expressed neuronal nuclear antigen, a neuronal marker. Analysis of GFP-expression area in adult transgenic mice revealed that females had a larger area of GFP-expressing cells in a region including the caudal portion of BNST and StHy when compared to males. Such female-biased sex difference of GFP-expression area was also observed in transgenic pups on postnatal day 5 and 8. Moreover, the GFP-expression area of adult female transgenic mice was decreased by postnatal treatment with testosterone or estradiol. These findings indicate that a sex difference in ERα-expressing neurons of the hypothalamus can be visualized by GFP. The transgenic mice may be useful for the analysis of the sexual differentiation of the brain. No COI.

ABS0363

Effect of Rosa damacena mill hydroalcoholic extract on passive avoidance learning and memory in high fat diet adult rats

Iraj Salehi[1]*, Arezoo Rezvani Kamran [2], Siamak Shahidi[2], Alireza Komaki[2], Shirin Moradkhani[3], Mohammad Zarei[2]

[1]Hamadan University of Medical Sciences [2]Department of Physiology and Neurophysiology Research Center, Hamadan University of Medical Sciences [3]Department of Pharmacognosy and Pharmaceutical Biotechnology, Faculty of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

**Email : mfathi685@gmail.com*

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

ABS0366

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

Kei Eto[1]*, Hitoshi Ishibashi[1], Junichi Nabekura[1]

[1]Division of Homeostatic Development, Department of Developmental Physiology, National Institute for Physiological Sciences, Japan

**Email : keieto@nips.ac.jp*

Neurons in the primary somatosensory cortex (S1) receive nociceptive information from peripheral nerve and code location, intensity, and duration of pain. Under chronic pain conditions, brain activities of S1 increase and recent study demonstrates that S1 has critical roles in chronic pain. Under chronic pain conditions, S1 excitatory neuronal activities are enhanced and these hyperactivities contribute to chronic pain. Inhibitory GABAergic neurons in the S1 project to S1 excitatory neurons and can attenuate excessive excitation of excitatory neurons. However, little is known about how inhibitory neurons in the S1 modulate excitatory neuronal activities and pain behavior under chronic pain conditions. Using two-photon calcium imaging and electrophysiological methods, we found that inhibitory neuronal activities increased in S1 in inflammatory chronic pain. Local application of a GABAA receptor blocker further enhanced excitatory neuronal activities in S1 and pain behavior in chronic pain. This suggests that enhanced inhibitory neuronal activities in S1 contribute to reduce exaggerated cortical excitatory neuronal activities and pain behavior. However, reduction of K-Cl cotransporter expression in S1 excitatory neurons resulted in inhibition being less efficacious. Thus, although there is a net increase in inhibition within S1 cortical circuit, it is not enough to balance the enhanced excitatory neuronal activities and prevent chronic pain behavior. No COI.

ABS0383

Male-biased sexually dimorphic nuclei found in the brain of Suncus murinicus

Yadanar Moe[1], Tomoko Tanaka[2], Masahiro Morishita[2], Chihiro Nakahara[2], Ichiro Sakata[2], Takafumi Sakai[2], Shinji Tsukahara[2]*

[1]Saitama University, Japan [2]Division of Life Science, Graduate School of Science and Engineering, Saitama University, 255 Shimo-Okubo, Sakuraku, Saitama City, Saitama 338-8570, Japan, Japan

**Email : stsuka@mail.saitama-u.ac.jp*

The brain of mammals contains sexually dimorphic nuclei (SDNs) that underlie sex-biased physiological functions. In mice (Mus musclus) belong to Rodentia, the calbindin-sexually dimorphic nucleus (Calb-SDN) of the preoptic area and the principal nucleus of the bed nucleus of the stria terminalis (BNSTp) are SDNs that abundantly express calbindin, a calcium-binding protein. Both the SDNs exhibit male-biased sex differences in the volume and neuron number. Suncus (Suncus murinicus), which belongs to Insectivore, is the useful model for physiological study. However, the existence of SDNs in suncus is largely unknown. This study was aimed to determine the existence of SDNs in suncus. Coronal brain sections obtained from adult suncus of both sexes were subjected to Nissl-staining and calbindin-immunohistochemistry. In the results of histological analysis, we found a cluster of calbindin-immunoreactive cells in the preoptic area. This cluster of males was larger and contained more calbindin-immunoreactive cells compared to females, suggesting that this is a homologue of the Calb-SDN. The BNSTp of suncus showed male-biased sex difference in the volume and Nissl-stained neurons, although few calbindin-immunoreactive cells were in the BNSTp of suncus. These results indicate that male-biased SDNs that may be homologues of the Calb-SDN and BNSTp of mice exist in suncus, although there may be a species difference in the BNSTp. No COI.

ABS0384

Analgesic effects of Bee venom derived phospholipase A2 in a mouse model of oxaliplatin-induced neuropathic pain

Dongxing Li[1], Woojin Kim[1], Ji Hwan Lee[1], Hyunsu Bae[1], Sun Kwang Kim[1]*

[1]Kyung Hee University, Korea

**Email : skkim77@khu.ac.kr*

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

ABS0386

CEF recovers neuronal density and activity changes in an MPTP-induced Parkinson's disease rat model: an MEMRI study

Yen Ting Chang[1]*
[1]*Chung Shan Medical University, Taiwan*
**Email : siphinie@gmail.com*

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

ABS0404

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

Kei Shinohara[1], Yukari Takahashi[1], Tomokazu Tsurugizawa[2], Ryo Ikeda[3], Keishi Marumo[3], Fusao Kato[1]*
[1]*Department Neuroscience, Jikei University School Medicine, Japan* [2]*Neurospin, CEA, France* [3]*Department Orthopedic, Jikei University School Medicine, Japan*
**Email : fusao@jikei.ac.jp*

Persistent activation of brain regions involved in emotion and self-consciousness is the signature of the chronic pain in human patients (Apkarian et al., 2013). To understand the neuronal process for development of chronic pain, we employed manganese-enhanced MRI (MEMRI), a brain imaging technique based on visualization of activity-dependent accumulation of Mn^{2+} (Aoki et al., 2004) to follow the changes in MEMRI during the chronification process after formalin-induced inflammation. $MnCl_2$ was injected intravenously to mice and formalin solution or saline was administered into the left hind paw at 2, 6 or 24 h before MRI acquisition with ultra-high field scanner under anesthesia at 23-24 h after $MnCl_2$ injection. Two distinct types of methods, region of interest- (ROI-) and voxel-based analyses were performed to evaluate changes after formalin injection. The ROI-based analysis revealed an early activation of the right CeA (6 h) and latent activation of the bilateral CeA, left BLA and right dentate gyrus (24 h). The voxel-based analysis revealed wide-spread brain activities in addition to the amygdala in 6- and 24-h inflammation groups. MEMRI combined with ultra-high field scanner is powerful and useful in identifying wide-spread brain areas spontaneously activated in the course of pain chronification in animal model of inflammatory pain. Identification of neural networks and molecular basis underlying sequential activation of these brain areas would provide new targets of pain neuroscience. No COI.

ABS0410

Basal ganglia and cerebellar control of thalamocortical activity

Satomi Chiken[1]*, Hiromi Sano[1], Yasuo Kawaguchi[1], Minoru Kimura[2], Atsushi Nambu[1]
[1]*National Institute for Physiological Sciences, Japan* [2]*Tamagawa University, Japan*
**Email : chiken@nips.ac.jp*

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

ABS0412

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

Atsushi Nambu[1]*, Daisuke Koketsu[1], Satomi Chiken[1], Tatsuhiro Hisatsune[2], Shigehiro Miyachi[3]
[1]*National Institute for Physiological Sciences, Japan* [2]*University of Tokyo, Japan* [3]*Kyoto University, Japan*
**Email : nambu@nips.ac.jp*

The subthalamic nucleus (STN) receives direct cortical inputs and projects to the substantia nigra pars reticulata (SNr), the output station of the basal ganglia (BG), and thus forms the cortico-STN-SNr hyperdirect pathway. We observed BG activity and motor behaviors in mice before and after the selective elimination of the cortico-STN projection by a photodynamic technique. We injected retrogradely transportable microspheres conjugated with chlorin e6 into the STN. Then, we irradiated the motor cortex with a near-infrared laser to activate chlorin e6 and selectively induce apoptosis in the labeled cortico-STN neurons. We recorded the activity of globus pallidus (GP) and SNr neurons to stimulation of the forelimb motor cortex in awake mice by constructing peri-stimulus time histograms. Before the elimination, a triphasic response composed of early excitation, followed by inhibition and late excitation was the most common. After the elimination, early excitation diminished in both GP and SNr neurons. On the other hand, no significant changes of firing rates and patterns were observed. We also examined locomotor activity of mice before and after the bilateral cortico-STN elimination. The locomotor activity gradually increased after one week from the laser irradiation and became significantly higher than that of control mice in 2–4 weeks. These results suggest that the hyperdirect pathway conveys fast excitatory signals from the motor cortex to the SNr, inhibits the thalamic and cortical activity and suppresses motor behaviors. No COI.

ABS0416

Angiotensin II facilitates GABAergic neurotransmission at postsynaptic sites in rat central amygdala neurons

Bo Hu[1], Yi Lu[2], Hu Qiao[1], Bo Sun[1], Bo Lu[1], Ru Jia[1], Yuanyuan Fan[1], Nan Wang[1], Jianqun Yan[1]*
[1]*Department of Physiology and Pathophysiology, Xi'an Jiaotong University Health Science Center, China*
[2]*Department of Prosthodontics, Xi'an Jiaotong University College of stomatology, China*
**Email : jqyan@mail.xjtu.edu.cn*

The central nucleus of the amygdala (CeA) in the forebrain is a critical brain site for the regulation of sodium appetite. Angiotensin II type-1 (AT1) receptors and angiotensin II (ANG II) terminals are present in the CeA. However, there is no clear evidence for the role of ANG II in the CeA. In this study, we determined the effect of ANG II on the excitatory and inhibitory synaptic inputs to the CeA neurons. Whole-cell patch-clamp recordings were performed on CeA neurons in brain slices. Application of 0.1–5 μM ANG II significantly potentiated the amplitude of spontaneous GABAergic inhibitory postsynaptic currents (IPSCs) in a concentration-dependent manner. Also, 2 μM ANG II increased the amplitude of miniature IPSCs from 22.86 ± 2.54 to 29.45 ± 3.32 pA ($P < 0.05$, n=11), without affecting the frequency. The effect of ANG II on miniature IPSCs was blocked by the AT1 receptor antagonist losartan, but not by the AT2 receptor antagonist PD123319. Furthermore, inclusion of a G protein inhibitor, GDP-β-s, in the pipette internal solution attenuated the facilitatory effect of ANG II on GABAergic transmission. By contrast, ANG II had no effect on the spontaneous glutamatergic excitatory postsynaptic currents (EPSCs) and did not alter the frequency and amplitude of miniature EPSCs at concentrations that facilitated IPSCs. Thus, this study provides substantial new evidence that ANG II facilitates GABAergic synaptic inputs through activation of postsynaptic AT1 receptors in the CeA. No COI.

ABS0420

Effect of valproate sodium on electrical activity of Helix aspersa F1 neuron in a pentylenetetrazol-induced epileptic model using intracellular recording system

Mahin Ganjkhani[1]*, Marzieh Marahem[2]
[1]*Zanjan University of Medical Sciences, Iran* [2] *Zanjan Univ. of Medical Sciences, Iran*
**Email : ghanjkhani@zums.ac.ir*

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

ABS0423

Complexity of autonomic modulation and neurovascular oxidative stress during hypertensive response in acute stroke

Phumdecha Chanbenjapipu[1]*, Kesorn Suwanprasert[2]
[1]*Thammasart University, Thailand* [2]*Faculty of Medicine, Thammasart University, Thailand*
**Email : taewakan@gmail.com*

Stroke is closely related with vascular oxidative stress in atherosclerosis. During acute large artery ischemic stroke, variations of high blood pressure and heart rate have been reported. In this study, we investigated autonomic control and neurovascular oxidative stress during hypertensive response in large artery ischemic stroke. Sixty five patients were studied as follows local ethic committee (MTU-EC-IM-018154). Three groups of systolic blood pressure (SBP) were classified as follows: Gr I, SBP< 139 mmHg (n, 23 aged 59.45 ± 2.34); Gr II, SBP =140-159 mmHg (n, 21 aged 63.09 ± 2.79); and Gr III, SBP > 160 mmHg (n, 21 aged 62.38 ± 2.86). Plasma nitric oxide (NO) and hydrogen peroxide (H_2O_2), neurovascular oxidative stress marker, were recorded by electrochemistry technique. Autonomic control was assessed by Lead II ECG - short term heart rate variability (HRV) using Kubios Program. Carotid stenosis by means of flow and intima/media thickness (IMT) ratio was assessed using Doppler ultrasound. Gr III had greater IMT ratio than in Gr I and Gr II and differed significantly. Profound lower NO and higher (H_2O_2) were also presented. In all groups by SD2, SD1 Poincare plot, sympathetic drive gradually increased whereas parasympathetic activity decreased dramatically. Significant correlation of SBP and SD2/SD1 was evident (r = 0.81). Surprisingly, less scatter plot and fractal scaling exponent by α1/α2 were shown in Gr III. These findings indicate that reorganized complexity of autonomic modulation plays a vital role in hypertensive response. No COI.

ABS0432

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

Rie Fujiyama[1]*, Yukio Okada[1], Kazuo Toda[1]
[1]*Integrative Sensory Physiology, Graduate School of Biomedical Sciences, Nagasaki University, Japan*
**Email : rierika@nagasaki-u.ac.jp*

The alteration in taste (dysgeusia) is a common and severe side effect in approximately 60% of cancer patients undergoing chemotherapy. However, its symptoms remain unclear. Dysgeusia causes malnutrition in a majority of the patients and affects their quality of life. However, research on this problem is lacking. The aim of the present experiments was to investigate the effects of paclitaxel (Taxol®) on voluntary NaCl intake using twenty-eight rats and a two bottle choice. For this purpose, the rats were presented with a NaCl solution (0.1 M) and water, and their consumption was measured daily. The rats received two cycles of paclitaxel chemotherapy. The cycles were repeated at 14-day intervals. Paclitaxel was administered for five consecutive days in rats. We did not observe any effects of paclitaxel on NaCl intake and preference such as the effect on sucrose. We have reported that paclitaxel decreased sucrose intake and preference in a few days after its administration and sucrose preference improved within several days after we stopped administering it. These results suggest that paclitaxel alters sucrose, but not NaCl, preference and there are different side effects of paclitaxel on the preference of NaCl and sucrose in rats. These findings indicate that paclitaxel modifies the specific taste function. No COI.

ABS0439

Opt-fMRI imaging of somatosensory cortical activity using transgenic rat which expresses channelrhodopsin-2 in the peripheral mechanoreceptive neurons

Yukinobu Yokoyama[1]*, Akira Sumiyoshi[2], Ryuta Kawashima[2], Hiromu Yawo[1]
[1]*Tohoku University Graduate School Life Science, Japan* [2]*Tohoku University IDAC, Japan*
**Email : y-yokoyama@dc.tohoku.ac.jp*

The rodent whisker-barrel cortical system has been a model to reveal somatosensory representation in the brain. Optogenetics would facilitate this with high spatiotemporal resolutions. Recently, we have identified the expression of ChR2 in the mechanoreceptive neurons in the trigeminal ganglion (TG) in one of thy1.2-channelrhodopsin 2 (ChR2)-Venus transgenic rat lines, W-TChR2V4 (Honjoh et al., 2014). Each whisker follicles were also richly innervated by the ChR2-positive nerve endings. The whiskers of a ChR2-expressing rat were attached with plastic optic fibers in array of 4x4 in the awake state. Another end of the optic fiber was connected to blue LED, individual of which was turned on and off by a computer-generated pulse. Therefore, the whiskers could be stimulated with various spatiotemporal patterns (optogenetic tactile pattern, OTP). The functional magnetic resonance imaging (fMRI) responses of barrel cortex were investigated using OTP under 7T-MRI system. The whisker irradiation induced a change of blood oxygenation level-dependent (BOLD) responses in the barrel field of contralateral somatosensory cortex in a manner dependent on time. The response to a single whisker irradiation was more regional than the simultaneous 16-whiskers irradiation. It is suggested that the OTP was accompanied with specific spatiotemporal changes of BOLD response. Our OTP, in combination with fMRI, would facilitate to study how the spatiotemporal pattern of the whisker mechanoreception would be represented in the cortex. All animal procedures were conducted in accordance with the guiding principles of Physiological Society of Japan and NIH. No COI.

ABS0440

Aldosterone induces rapid sodium intake by a nongenomic mechanism in the nucleus tractus solitaries

Hu Qiao[1], Bo Hu[1], Jianqun Yan[1]*
[1]*Xi'an Jiaotong University, China*
**Email : jqyan@mail.xjtu.edu.cn*

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

ABS0452

Blockade of Smad4 SUMOylation impairs spatial learning and memory in rats

Hsiao-Yuan Lee[1]*, Wei-Lun Hsu[1], Yun-Li Ma[1]
[1]*Institute of Biomedical Sciences, Academia Sinica, Taiwan*
**Email : eminy@gate.sinica.edu.tw*

We have previously found that the protein inhibitor of activated STAT1 (pias1) gene is differentially expressed between fast learners and slow learners from the water maze learning task with fast learners showing a higher expression level of pias1 in hippocampal neurons. Spatial training also increases PIAS1 expression in rat CA1 area. Further, overexpression of PIAS1 in CA1 neurons enhances whereas knockdown of PIAS1 impairs spatial learning and memory performance in rats. Because PIAS1 is a transcriptional regulator that possesses small ubiquitin-like modifier (SUMO) E3 ligase activity, in this study, we aimed to examine the mechanism underlying PIAS1-mediated learning and memory facilitation. Smad4 is a downstream target of transforming growth factor-beta (TGF-beta) signaling. Smad4 has been well studied in the immune system, but its role in the brain has been rarely examined. In this study we examined whether Smad4 could be SUMO-modified by PIAS1 in the brain and whether Smad4 SUMOylation plays a role in spatial learning and memory formation. Our results showed that Smad4 could be SUMO-modified by PIAS1 at Lys-113 and Lys-159 both in cell lines and in rat hippocampus in vivo. Further, water maze training increased the level of Smad4 SUMOylation in CA1 neurons compared with the swim controls. Smad4 SUMOylation is also induced by neuronal activation. On the other hand, transduction of the lenti-Smad4WT vector did not apparently affect spatial learning and memory, but transduction of the lenti-Smad4 sumo-mutant vector (Smad4K113RK159R) significantly impaired spatial learning and memory performance. In future studies, we will identify the downstream genes that are regulated by Smad4 SUMOylation and are involved in spatial learning and memory formation. No COI.

ABS0459

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

Kanyaratana Bamrungsuk[1], Thanaporn Rungruang[2], Sorachai Srisuma[1], Sompol Tapechum[1], Kanokwan Tilokskulchai[1], Supin Chompoopong[2]*
[1]*Department of Physiology* [2]*Department of Anatomy, Faculty of Medicine Siriraj hospital, Thailand*
**Email : supin.cho@mahidol.ac.th*

Recently, many experimental devices have been designed to construct standardized animal spinal cord injury (SCI) models, because electromagnetic SCI devices are expensive. To evaluate adopted weight drop device inducing contusive SCI, the fixed weight was used and dropped down from varied heights, then followed by behavioral and histopathological studies. Fifteen adult male Sprague Dawley rats were divided into laminectomy (L), moderate injury (MI) and severe injury (SI) groups. The C5 hemicontusion injury was performed and resulted in the right side hemiplegia and forepaw deficits. Both MI and SI rats showed the clubbing forepaw at 24 h after injury. The skilled locomotion using the horizontal ladder test was analyzed. The SI showed a significant increase in error scores, percentage of total rungs used and decrease in percentage of correct placement when compared to L group, $p<0.05$. The normal recovered placement (type II) was shown at day 7 after injury but higher numbers in MI than SI group. The somatosensory function using sticker removal test was also analyzed. The SI group showed a significant somotosensory deficit at day 3, 7 when compared to L group, $p<0.05$. Behavioral deficits were related to histopathological study using H&E counterstained with luxol fast blue staining, the higher degree of injury, the larger area of lesion. The lesion was mostly in lateral funiculus related to rubrospinal and lateral corticospinal tract involving skilled movement of forepaw. Results indicate that this more suitable rat SCI model should be simple, reliable and induced as moderate injury for allowing significant recovery of function. No. COI.

ABS0468

Delta X, a new synthetic compound that promotes adult neurogenesis

Pei-Jung Wu[1], Guey-Jen Lai[1]*
[1]*National Chengchi University, Taiwan*
**Email : jen.lai@mail2.nccu.tw*

There are currently two known pools of cells in the adult brain that can continuously generate new neurons. One of these pools of cells is located in the subgranular zone (SGZ) of the hippocampus. Here we report a new synthetic compound "Delta X" that is capable of promoting proliferation of neural progenitor cells (NPC) from SGZ of the hippocampus in vivo. Previously, we have demonstrated that Delta X can promote the proliferation of adult rat hippocampus derived neural progenitor cells in primary culture. The average number of neurospheres in the Delta X treated group was 2.3 fold higher than that in the control group. The observed effect of Delta X treatment on cultured cells is dose-dependent and this information was used to identify a working concentration for in vivo treatment. In a rat model, adrenalectomy (ADX, removal of adrenal glands) and the resulting corticosterone depletion lead to a dramatic decrease in neuron number in the dentate gyrus of the hippocampus. Behavior deficits of ADX animals are associated with the neuron loss. In this study, the ADX model were used to determine whether Delta X can promote neurogenesis in vivo, repopulate the neuron population in the dentate gyrus, and lead to functional recovery after hippocampal damage. After one week of Delta X treatment, an increase in the number of new born neural progenitor cells (BrdU and nestin double positive cells) were observed in the ADX animals. The number of new born immature neurons (BrdU and double cortin positive cells) was also increased. At the concentration of Delta X used, the population of other cell types was not affected. Thus, Delta X's potential for promoting endogenous neurogenesis, may have applications in repairing brain circuitry. No COI.

ABS0485

Painful diabetic neuropathy with streptozotocin involves plastic changes in ascending and descending pain pathways

Yukari Takahashi[1], Toshitaka Ochiai[2], Jun Kinoshita[1], Ayako M Watabe[1], Junzo Kamei[2], Fusao Kato[1]*
[1]*Jikei University School of Medicine, Japan* [2]*Hoshi University, Japan*
**Email : fusao@jikei.ac.jp*

The number of patients with diabetes mellitus (DM) is globally increasing. Of various complications of DM, painful diabetic neuropathy (PDN) is one of the serious clinical concerns because it significantly lowers the quality of life. It is generally acknowledged that the nerve damage by hyperglycemia results in neuropathic pain. However, recent advances in the understanding of the pain-induced plasticity in the central nervous system suggest that altered central pain processing through ascending and descending pain systems would also underlie chronification and complication of pain. We challenged this by two distinct approaches using streptozotocin- (STZ-) induced PDN modes. First, we examined whether the noradrenergic system in the spinal cord is affected in STZ-treated rats because it has been shown that serotonin norepinephrine reuptake inhibitors mitigate PDN symptoms. The mechanical allodynia and thermal hyperalgesia in the PDN were significantly attenuated by duloxetine. This effect was abolished by pre-treatment with N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), which drastically eliminated nerve endings with dopamine-beta-hydroxylase in the lumber dorsal horn. Second, we examined whether the synaptic transmission between the fibers arising from the lateral parabrachial nucleus (LPB) and the neurons in the "nociceptive" amygdala (capsular part of the central amygdala) and found that this synaptic transmission was significantly potentiated in STZ-treated mice. It is concluded that STZ and resulting hyperglycemia alters the activity of central networks regulating both ascending and descending pain pathways, which should further exacerbate the PDN-related symptoms. COI=Shionogi.

ABS0499

Antidepressant-like effect of bergamot oil in rats subjected to chronic restrained stress

Somrudee Saiyudthong[1]*, Chantana Mekseepralard[2]

[1]Department of Physiology, Srinakrarinwirot University, Thailand [2]Department of Micrology, Srinakrarinwirot University, Thailand

**Email : somrudee@swu.ac.th*

Depressive disorder is one of the most common psychiatric diseases and associated with a prolonged period of exposure to stressors. Bergamot essential oil (BEO) has been traditionally recommended to reduce depression however there are limited data supporting these properties. An aim was to investigate the effect of BEO, compared with fluoxetine, a well-known antidepressant, in chronic restraint rats on antidepressant-like behaviour. The hypothalamic pituitary adrenal (HPA) axis response and brain-derived neurotrophic factor (BDNF) protein levels in hippocampus were also determined. Rats were subjected to immobilization stress 15 min daily for 2 weeks. For the next 2 weeks, these rats were divided into 4 groups, control-i.p., fluoxetine-i.p., control-inhale and BEO-inhale. Fluoxetine (10 mg/kg i.p.) or saline was intraperitoneally administered daily while 2.5% BEO or saline was inhaled daily. Following the treatment, depressive-like behavior in treated rats was investigated using the force swimming test (FST). Then, the rats were immediately decapitated and trunk blood samples were collected for the measurement of corticosterone and adrenocorticotropic hormone (ACTH) level. Hippocampus was dissected and stored in a freezer until assay for BDNF. For FST test, the immobility time was significantly reduced by both BEO and fluoxetine ($p<0.05$). Fluoxetine tended to decrease serum corticosterone and significantly ($p<0.05$) decreased serum ACTH, whereas BEO had no effect on both hormones. Moreover, either BEO or fluoxetine did not change BDNF protein levels in hippocampus. The present study indicated that BEO decrease behavior related depressive disorder similar to fluoxetine in chronic restrained stress. No COI.

ABS0510

Cardiovascular and single unit responses to microinjection of norepinephrine into the bed nucleus of the stria terminalis in male rat

Masoumeh Hatam[1]*, Ali Nasimi [2]

[1]Shiraz University of Medical Sciences, Iran [2]Isfahan University of Medical Sciences, Iran

**Email : hatam_m@yahoo.com*

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

ABS0546

Effect of low frequency stimulation on seizure-induced impairment in synaptic potentiation of hippocampal slices of kindled rats

Samireh Ghafouri[1], Saeed Semnanian[1], Amir Shojaei[1], Javad Mirnajafi-Zadeh[1]*
[1]*Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*
**E-mail: mirnajaf@modares.ac.ir*

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

ABS0275

The physiological roles of Moesin, a cytoskeletal protein, in renal salt reabsorption

Kotoku Kawaguchi[1], Ryo Hatano[1], Shinji Asano[1]*
[1]*Ritsumeikan University, Japan*
**Email : ashinji@ph.ritsumei.ac.jp*

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

ABS0393

Role of neuropeptide FF receptor type 2 in pain and depression

Jin-Chung Chen[1]*, Ya-Ting Lin[1], Tse-Yu Liu[1], Ching-Yao Yang[1], Yuan-Ji Dey[2]
[1]*Chang-Gung University, Taiwan* [2]*Chang-Gung Memorial Hospital, Taiwan*
**Email : jinchen@mail.cgu.edu.tw*

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

ABS0415

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

Katesirin Ruamyod[1]*, Wattana B. Watanapa[1], Oranud Praditsap[1], Choochai Nettuwakul[2], Sittideth Sangnual[2], Nunyawan Rungroj[2], Nunghathai Sawasdee[2], Pa-thai Yenchitsomanus[2]

[1]Department of Physiology [2]Division of Molecular Medicine, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

**Email : katesirin.ry@gmail.com*

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

ABS0482

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

Tippawan Damrongongtrakool[1], Wilawan Mahabusarakam[2], Sara Bumrungsri[1], Siriphun Hiranyachattada[3]*

[1]Department of Biology [2]Department of Chemistry [3]Department of Physiology, Faculty of Science, Prince of Songkla University, Thailand

**Email : siriphun.h@psu.ac.th*

Cisplatin is widely used as a cancer therapy drug. However, its side effect is nephrotoxicity which may due to the generation of reactive oxygen species and lead to acute renal failure (ARF). The prevention of cisplatin-induced ARF with various antioxidants such as vitamin C, E and flavonoids extracted from plants have been reported. Morelloflavone, a biflavoniod from Gracinia dulcis Kurz. has been proved to possess antioxidant effect both in vitro and in vivo. This study objected to investigate the protective effects of morelloflavone on ARF induced by cisplatin. Male Wistar rats weighed 250-300 g were divided into three groups including vehicle control, cisplatin and cisplatin+morelloflavone. Induction of ARF was performed using cisplatin (7.5 mg/kg, i.p.) injection. Morelloflavone (1 mg/kg i.p.) was given twice, 24 hr and 10 min before cisplatin injection. Experiments were performed in anaesthetized rats, clearance markers (0.1% inulin and 0.5% para-aminohippuric acid (PAH) dissolved in 0.9% NaCl) was infused via jugular vein. Arterial blood pressure was monitor via carotid artery. Urine samples were collected via urinary bladder. Inulin and PAH clearance were used to determine glomerular filtration rate (GFR) and renal blood flow (RBF), respectively. Osmolar clearance was used to determine electrolyte excretion. It is found that three days after cisplatin injection, renal MDA significantly increased and the administration of morelloflavone suppressed this MDA elevation. Renal functions including GFR, RBF and electrolyte excretion were impaired after cisplatin injection. It is likely that morelloflavone would restore this renal impairment via its antioxidant property. No COI.

ABS0419

Isoflavone genistein modulates the expression and function of Toll-like receptors in human endometrial cells

Chatsri Deachapunya[1]*, Norathee Buathong[2], Sutthasinee Poonyachoti[3]
[1]*Srinakharinwirot University* [2]*Department of Physiology, Faculty of Medicine, Srinakharinwirot University* [3]*Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand*
**Email : chatsri@swu.ac.th*

Recognition of pathogenic components by Toll-like receptor (TLR) mediates release of cytokines. As TLR2, 3, 4 and 9 expression and function are regulated by sex steroid hormones, this study investigated the soybean isoflavone genistein (Ge) effects on the expression of these TLRs and IL6 secretion in human endometrial cell line (RL95-2). Ge effects were compared with 17β-estradiol (E2) under basal and polyinosinic-polycytidylic acid (I:C) stimulation to mimic viral infection. Cells were exposed to I:C 30 min prior to incubation with Ge (10^{-7}, 10^{-6}, 10^{-5} M) or E2 (10^{-9} M) for 48 h. The culture media was collected and analyzed for IL6 by ELISA and the TLR protein expression by Western blot analysis. The results revealed the differential expression of TLR2, 3, 4 and 9 proteins. I:C up-regulated TLR2 and 9 but suppressed TLR3 and 4. Ge 10^{-7} M increased TLR2 whereas E2 decreased TLR4 protein. All Ge treatments attenuated the I:C-induced increase in TLR2 expression. In contrast, both Ge at 10^{-6} M and E2 promoted the suppressive effect of I:C on TLR4 expression. In this study, the cells constitutively released IL6 which was suppressed by I:C. Ge increased IL6 secretion in correlation with the up-regulated TLR2 protein. Both Ge and E2 could reverse the inhibitory effect of I:C on IL6 secretion. These findings suggest the role of Ge on differential regulation of TLR expression and IL6 secretion. The reversibility effect of Ge on the TLR expression and IL6 secretion by viral infection provide the potential evidence for application of Ge to promote the uterine innate immunity and alleviate the inflammation following pathogen invasion. No COI.

ABS0369

Asporin localization in mouse lung alveogenesis

Khemapa Chusamer[1]*, Lakkana Wongkhat[2], Julalux Thongam[1], Sorachai Srisuma[1]
[1]Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University [2]Department of Biopharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Burapha University, Thailand
**Email : khemapa@gmail.com*

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

ABS0370

Effects of CTNNAL1 on balancing regulation between epithelial and mesenchymal repair in ozone stressed HBEC

Meiling Tan[1], Xiaoqun Qin[1], Xiangping Qu[1], Huijun Liu[1], Chi Liu[1], Caixia Liu[1], Wei Xie[1], Yang Xiang[1]*
[1]Central South University, China
**Email : xiangyang@csu.edu.cn*

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

ABS0388

Exercise capacity predicted from cardiopulmonary exercise testing in Thai adults

Tichanon Promsrisuk[1], Wilaiwan Khrisanapant[1]*, Orapin Pasurivong[1], Watchara Boonsawat[2], Boonsong Patjanasoontorn[2], Burabha Pussadhamma[2], Paitoon Benjapornlert[3]

[1]Department of Physiology [2]Department of Medicine [3]Department of Rehabilitation Medicine, Faculty of Medicine, Khon Kaen University, Thailand

**Email : wilkhr@kku.ac.th*

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

ABS0391

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

Robert Fellner[1]*, Shawn Terryah[2], Jessica Hairr[2], John Taylor[2], Dale Christensen[2], Robert Tarran[1]

[1]Cystic Fibrosis Center at University of North Carolina at Chapel Hill, United States

[2]Spyryx Biosciences, United States

**Email : rfellner@unc.edu*

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

ABS0457

Alteration in asporin expression of newborn mouse lung after hyperoxia

Nattawat Chaiyawong[1], Supitsara Thorsuwan[1], Julalux Thongam[1], Sorachai Srisuma[1]*
[1]*Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand*
**Email : sorachai.sri@mahidol.ac.th*

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

ABS0475

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

Xiang Dan Li[1], You Chen Zhang[2], Chun Hua Xu[2], Hui Wen Li[2], Zheng Yong Jin[2]*
[1]*Center of Morphological Experiment, Medical College of Yanbian University, China* [2]*Department of Neonatology, The affiliated Hospital of Yanbian University, China*
**Email : jinzhengyong2003@163.com*

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

ABS0513

Meso-dihydroguaiaretic acid attenuates airway inflammation in an ovalbumin-induced murine asthma model

Ji-Won Song[1], Young-Soek Kim[1], Charith ub Wijerathne[1], Hyo-Jung Kwon[1], Hwa-Young Son[1]*
[1]*Department of Veterinary Pathology, College of Veterinary Medicine, Chungnam National University, Korea*
**Email : hyson@cnu.ac.kr*

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

ABS0530

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

Cai Xia Liu[1], Xiaoqun Qin[1], Xiangping Qu[1], Huijun Liu[1], Chi Liu[1], Meiling Tan[1], Jianxin Jiang[2], Yang Xiang[1]*
[1]*Central South University, China* [2]*Third Military Medical University, China*
**Email : xiangyang@csu.edu.cn*

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

ABS0531

Oxidative stress induces alteration of E-cadherin expression in lungs of mice

Shoumin Zhang[1], Ying Liu[1], Xiangping Qu[1], Xiaoqun Qin[1]*
[1]*Central South University, China*
**Email : qinxiaoqun@csu.edu.cn*

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

ABS0533

Effect of some stress infectors on the intracellular chloride concentration of 16HBE14O-, HEK293T, 3T3 and RaW

Lingzhi Liu[1], Chi Liu[1], Yang Xiang[1], Huijun Liu[1], Xiangping Qu[1], Yu Chen[1], Xiaoqun Qin[1]*
[1]*Central South University, China*
**Email : qiniaoqun@csu.edu.cn*

Chloride is the most abundant anion in mammalian cells, Since Cl^- flux is coupled with Na^+ and K^+ in several processes, including cell volume control, transepithelial transport, cell multiplication and so on. We have measured the effects of some stress factors such as LPS (20 mg/ml), H_2O_2 (1 mM), heat (42 ℃), cold (4 ℃), acid (pH = 6.4), Alkali (pH = 8.4) on $[Cl^-]i$ using the fluorescent chloride indicator N-(6-methoxyquinolyl) acetoethyl ester (MQAE) with Laser confocal fluorescence microscope. 16HBE14O-, HEK293T, 3T3, RaW was selected in our experiment. Fluorescence intensity which indicate $[Cl^-]i$ was measured by ImageJ software. After LPS (20 mg/ml) or H_2O_2 (1 mM) stimulate cells, Fluorescence intensity of cells decrease gradually. That is to say $[Cl^-]i$ will increase gradually after be stimulated by LPS (20 mg/ml) or H_2O_2 (1 mM). However, after be stimulated by heat (42 ℃), cold (4 ℃), acid (pH = 6.4), alkali (pH = 8.4), $[Cl^-]i$ will increase quickly and holding it for some time(about 10 seconds), then recovery to basal level. Our results showed a phenomenon that when cells be stimulated by some stress factors, $[Cl^-]i$ will change. Different stress factors produce different effect. But the interesting bit was that different cells have the same effect on the same stress infector. Maybe it can give us some hints that the intracellular chloride is a potential signaling molecules like Calcium ion response to stimulation and produce some cytological effect. (This work was supported by grants #81270065, #81370116 from NSFC and grant#2013JJ4030, #2015JJ2147 from Hunan Natural Science Foundation).

ABS0009

Smokers…. Stop!!!!!!!!!!!!!!…. analyse whats happening in you….

Shrinidhi.[1], Nishith Shetty[2]
[1]*Kanachur Institute of Medical Sciences, Mangalore - India, India 2Kasturba Medical College, Manipal, India*
**Email :*

Cigarette smoking is an established risk factor for cardiovascular disease. Endothelial dysfunction is a systemic disorder, which is critical element in the pathogenesis of cardiovascular disease and its complications. A noninvasive method of endothelial function assessment by ultrasound technique to evaluate brachial artery called Flow-Mediated Dilatation (FMD) has emerged as a marker of endothelial dysfunction. To study endothelial dysfunction by FMD method in smokers and non-smokers and to study the correlation of FMD with blood pressure (BP), lipid profile between smokers and non-smokers. The present study was done in young, age-matched 31 smokers and 31 non-smokers (all males). Resting state supine BP was recorded by a sphygmomanometer and brachial artery diameter (intima – intima) was recorded by ultrasonography following which BP cuff was inflated 50mmHg above systolic pressure for 5minutes and then deflated. Brachial artery diameter was again recorded for maximum dilation after deflation and FMD was calculated. Fasting lipid profile was also measured. FMD as an indicator of endothelial dysfunction was much less in smokers (7.33) compared to non-smokers (13.83). Systolic BP in smokers was 128.96 (±8) mmHg and 119.93(±7.6) mmHg in non-smokers. Dyslipidaemia was observed in smokers compared to non-smokers with a significant correlation for TG, HDL & VLDL. Significant endothelial dysfunction was observed in smokers as compared tonon-smokers. Smokers were associated with dyslipidemia, increased blood pressure all of which cause endothelial dysfunction a major trigger for cardiovascular disease. No COI.

ABS0083

Protective effects and mechanisms of rosmarinic acid against rotenone-induced Parkinson's disease models

Xixun Du[1], Wenting Jia[2], Huamin Xu[1], Hong Jiang[1], Junxia Xie[1]*
[1]*Qingdao University, China [2]Qingdao University, China*
**Email : jxiaxie@public.qd.sd.cn*

Parkinson's disease (PD) is a common neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantial nigra pars compacta (SNpc) and the appearance of fibrillar aggregates of α-synuclein called Lewy bodies (LBs). Rosmarinic acid (RA) is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, which is commonly found in various plant families. It has been demonstrated that RA has a number of interesting biological activities, e.g. antiviral, antibacterial, anti-inflammatory and antioxidant activities. Our previous studies demonstrate that RA could exert its neuroprotective effect in PD models. However, whether RA could act on α-synuclein aggregation involved in PD is still largely unknown. In the present study, we investigated the effects of RA on α-synuclein aggregation in rotenone-induced PD models. In the rotenone-treated mice, the number of nigral TH-positive neurons and the expression of TH were decreased, while pretreatment with RA could antagonize this effect. RA could also antagonize the rotenone-induced increased expression of α-synuclein. Similar results were obtained in in vitro studies, which showed that RA pretreatment partially restored cell loss and inhibited rotenone-induced increased α-synuclein expression in SH-SY5Y cells. The mechanisms might be related to its antioxidant effect by up-regulating the expression of SOD and promote the degradation of α-synuclein through HO-1 and HSP70 up-regulation. The up-regulation of the HO-1 might be connected with Nrf2 and HIF-1α. Intracellular signaling pathway MEK/ERK might participate in these processes. These results provide new findings and new strategies for the prevention and treatment of PD. No COI.

ABS0086

The effect of resistance training and aquarobics on bone remodelling and obesity

Siti Mukarromah[1]*, Hardhono Susanto[2], Ign. Riwanto[2]
[1]*Semarang State University, Indonesia* [2]*Diponegoro University, Indonesia*
**Email : sitibaitul@yahoo.co.id*

The prevalence of obesity is increased in premenopausal women. Condition of obesity provides the mechanical load on the bones and tends to cause excessive osteoporosis. Resistance training (RT) and aquarobic exercise (AE) can decrease level of obesity and increase the activity of bone remodeling in obese women. The purpose was to investigate the effect of different exercise RT and LA on the level of obesity and bone remodeling activity. Methods of research is experimental randomized pretest-posttest control group design performed in 36 obese women, aged 45-50 years, who were divided into 3 groups: RT group exercised at 75% RM, 3 sets, 12 reps, twice days for 8 weeks (n=12), AE group exercised at 75%HRmax, twice days for 8 weeks (n=12) and control group (n=12). Percentage of body fat, BMI, adiponectin levels, levels of CTX and N-MID Osteocalsin levels were examined before and after treatment. The percentage of body fat was higher in AE than the RT and control groups ($p<0.05$). IMT was higher in the RT group and lower in the AE group when compared to controls ($p<0.05$). Increased adiponectin levels were higher in the RT group than the LA group and the controls ($p<0.05$). Increased levels of IL-6 were higher in RT than the AE group and controls ($p<0.05$). Decreased levels of CTX was higher in RT than AE group and controls ($p<0.05$). Increased levels of N-MID Osteocalsin higher in RT and LA groups than in controls ($p<0.05$). RT is more dominant on increasing bone remodeling activity and AE is more dominant on reduction of obesity. No COI.

ABS0117

Screening of Thai medicinal plants for Alzheimer's disease

Ruedeemars Yubolphan[1], Sukanya Jaroenporn[1]*, Suchinda Malaivijitnond[1]
[1]*Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
**Email : Sukanya.ja@chula.ac.th*

The extracellular aggregation of amyloid-β (Aβ) protein which leads to amyloid plaque formation in the brain is a pathological hallmark of Alzheimer's disease (AD). To develop the anti-AD agents from natural-based chemicals, nine Thai herbal plants; Caesalpinia sappan (CS), Thunbergia laurifolia (TL), Rhinacanthus nasutus (RN), Tabernaemontana divaricata (TD), Cyperus rotundus (CR), Terminalia chebula (TC), Azadirachta indica (AI), Piper retrofractum (PR), and Asparagus racemosus (AR) were selected and screened in vitro for anti-Aβ aggregation and Aβ disaggregation potency and neuronal toxicity. For the anti-Aβ aggregation, 10 μM of Aβ1-42 was co-incubated with 0.01-100 μg/mL of each herbal extract for 28 h. For the disaggregation, Aβ1-42 was first allowed to assembly into fibrils for 28 h, and the extracts were added and incubated for another 28 h. After the incubation, the Aβ aggregation and disaggregation were monitored by Thioflavin-T binding assay. Only eight plants, except AR, elicited anti-Aβ aggregation and disaggregation properties, and proceeded for the protective efficacy against Aβ-induced neuronal toxicity. Human neuroblastoma (SH-SY5Y) cells were co-incubated with 0.01-100 μg/mL of each extract and 1 μM of Aβ1-42 for 48 hours and the cell viability was determined by MTT assay. Only four herbal extracts of RN (0.1 μg/mL), TD (10 μg/mL), TC (1 μg/mL) and PR (0.1 and 100 μg/mL) protected against Aβ-induced neurotoxicity, ranging by 91.74-96.87%. These effective concentrations of the four extracts were confirmed non-toxic to the cells after cytotoxicity test. From all above tests, RN, TD, TC and PR have a potential to be developed further as anti-AD agents for human use. No COI.

ABS0121

Effect of Fucoidan from Sargassum sp. on joint swelling and degree of pain in osteoarthritis rat

Aditya Mahendra[1]*, Maimun Zulhaidah[1], Khoirunnisah Hartanti[1], Alan Vahlevi[1], Surya Muhammad[1], Adrian Triwibawanto[1]

[1]*Brawijaya University, Indonesia*

**Email : adityaindram@gmail.com*

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

ABS0127

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

Shehani Delwatta[1], Melanie D Seneviratne[2], Dmmlb Dissanayaka[2], Ss Batagoda[2], Uah Udagedara[2], Wgpb Walpola[2], Mangala Gunatilake[2]*, Vera Baumans[3]

[1]*Faculty of Medical Sciences, University of Srijayawardanepura, Sri Lanka* [2]*Faculty of Medicine, University of Colombo, Sri Lanka* [3]*Utrecht University, Netherlands*

**Email : mangalagunatilake@hotmail.com*

Lack of availability of reference values for the Sprague-Dawley rat model led the researchers to have an additional group of rats as the control group in their experiments increasing the number of animals required for research. Hence, this project was conducted to establish a reference data base for selected physiological, haematological and biochemical parameters of Sprague-Dawley rats in the Animal House with the approval of the Ethics Review Committee of the Faculty of Medicine, Colombo. Healthy young male (n=10) and female (n=10) rats were group housed except on the days of urine collection and were fed ad libitum. Approximately 1 ml of blood was drawn from the tail vein of each rat, once a week for 15 consecutive weeks, for haematological and biochemical investigations in alternating weeks. EDTA anti-coagulated blood in haematological investigations and serum in biochemical investigations were used. Urine output, food and water intake were determined for 24 hours per week during the study period. The mean, median and ranges of the parameters were computerized separately for male and female rats. The Faculty Animal House has been supplying Sprague-Dawley rats since 1977, for various research projects through continuous breeding and the reference ranges are now established for the first time in the history. For animal models using the Sprague-Dawley rat strain, this will reduce the number of animals used in future research projects, thus applying 'Reduction' of the 3Rs concept of Russell and Burch. No COI.

ABS0148

Changes of blood pressure, blood flow and heart rate during 90° head-up tilt for 30 min in anesthetized rats

Hironobu Nishimura[1], Tetsuya Hiraiwa[1], Masao Yamasaki[1]*
[1]*Department of Physiology, Graduate School of Health Science, Fujita Health University, Japan*
**Email : yamasaki@fujita-hu.ac.jp*

It is well known that baroreceptor reflex (BR) is important to maintain systemic arterial pressure (BP) and blood flow (BF) toward head after transition from supine position (SP) to head-up posture. To clarify the role of BR and the changes of BP, BF and heart rate (HR) in response to transitions of 90° head-up tilt (HUT); from SP to HUT or from HUT to SP, in nerve-intact and sinoaortic denervation (SAD) rats, we measured BP, common arterial flow as BF and HR in anesthetized SD rats (urethane 1.0-1.5 g/kg, i.p.) under 15 min or 30 min HUT (approval #, H1442). After onset of HUT, BP and BF significantly decreased by -16.4% and -28.3% at 3.3 sec, respectively (mean, $p<0.001$: paired t-test, n=12), and then these parameters immediately increased and maintained throughout HUT; % control was -2.2% in BP or -19.9% in BF at 34.7±14.2 sec when BP was steady. After transition from 30 min, HUT, BP and BF increased by 14.6% and 44.8% at 1.6±1.1 sec (n=8), respectively, and then they decreased and increased in a short period until steady state. In the SAD rats, the changes of BP and BF during HUT showed similar pattern to nerve-intact, but each parameter was lower; % control was -7.3% in BP or -26.5% in BF at 24.1±6.0 sec (n=5). The decrease in BP after HUT in the nerve-intact rats led slightly higher level of HR, the change in HR at 34.3 sec was by 11.3±13.5 beats/min and statistically significant compared with SP control. However, this response disappeared in SAD rats. These results indicate that initial decreases or increase in BP due to the 90° transition is produced by the hydrostatic pressure gradient and the increase in HR during HUT, which is basically caused by BR, suggesting that BR is important to maintain BP and BF during long-term HUT. No COI.

ABS0154

Protective effect of Na-DNA on pressure ulcer and elucidation of its mechanism

Jiadai Liu[1], Mami Noda[1]*
[1]*Labolatory of Pathophysiology, Pharmaceutical Science, Kyushu University, Japan*
**Email : noda@phar.kyushu-u.ac.jp*

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

ABS0155

High concentration carbohydrate mouth rinse improves endurance exercise capacity

Naoyuki Hashimoto[1], Naoyuki Hashimoto[1], Sumire Onitsuka[1], Ryo Ishikawa[1], Souichi Ando[2], Hiroshi Hasegawa[1]*

[1]Hiroshima University, Japan [2]University of Electro-Communications, Japan

**Email : hasehiro@hiroshima-u.ac.jp*

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

ABS0158

The effects of low salt and high salt intakes on insulin sensitivity in healthy subjects

Nyein Aye[1]*, Ohnmar Ohnmar[2], Phyu Khin Phyu [3], Mya Twin Mya[3]

[1]Physiology, Myanmar [2]Department of Physiology, UM1, Myanmar [3]Department of Physiology, UM2, Myanmar

**Email : kaunghtet1183@gmail.com*

It was well known that high salt intake was associated with not only hypertension but also insulin resistance. But the association between restriction of salt intake and insulin resistance was still unclear. The present study aimed to investigate the effect of low salt and high salt intakes on insulin sensitivity in healthy subjects. This study was undertaken in 51 apparently healthy male subjects (Age 22.22 ± 3.26 years, BMI 26.51 ± 4.88 kg/m^2). All participants were maintained on a low salt intake (<50 mmol/day sodium) and a high salt intake (>165 mmol/day sodium) for one week each, in random order. Fasting blood samples were collected at the end of each intervention. Fasting blood glucose was measured by glucose oxidase method. Serum insulin level was measured by ELISA kit method. Insulin sensitivity was calculated by HOMA-IR. The fasting plasma glucose level and serum insulin level in low salt and high salt intakes were 5.32 ± 0.50 mmol/l vs 5.2 ± 0.42 mmol/l and 17.32 ± 8.78 μIU/ml vs 12.68 ± 5.69 μIU/ml respectively. The fasting plasma glucose levels after two interventions were within the normal range and it was found that normal fasting plasma glucose level was maintained by compensatory hyperinsulinaemia in healthy subjects. HOMA-IR was increased during low salt intake than high salt intake (4.1 ± 2.14 vs 2.9 ± 1.32, P<0.001). Insulin sensitivity was significantly lower in low salt intake than in high salt intake in healthy male subjects. Salt loading and salt restriction can induce insulin resistance. The effects of low salt diet on insulin resistance was found to be higher than in high salt diet. No conflict of interest. Key words: Insulin sensitivity, Low salt intake, High salt intake and Homeostasis model assessment (HOMA-IR). No COI.

ABS0177

Bacopa monnieri (Brahmi) on spatial memory and long-term potentiation effects

Charkriya Promsuban[1], Namphung Thongta[1], Unchalee Wattarakorn[1], Kanokwan Tilokskulchai[1], Narawut Pakaprot[1]*

[1]*Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand*

**Email : sinarawut@mahidol.ac.th*

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

ABS0222

Fibroblast growth factor 21 (FGF21) improved cognitive impairment in obese-insulin resistant rats.

Piangkwan Sa-nguanmoo[1], Pongpan Tanajak[1], Wasana Pratchayasakul[1], Nipon Chattipakorn[1], Siriporn Chattipakorn[2]*

[1]*Cardiac Electrophysiology Research and Training Center, Department of Physiology, Faculty of Medicine,* [2]*Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand*

**Email : scchattipakorn@gmail.com*

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

ABS0238

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

Nifareeda Samerphob[1], Surapong Chatpun[2], Ekkasit Kumarnsit[1]*
[1]*Department of Physiology, Faculty of Science* [2]*Institute of Biomedical Engineering, Faculty of Medicine, Prince of Songkla University, Thailand*
**Email : ekkasit.k@psu.ac.th*

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

ABS0240

Low gamma wave oscillations in mice striatum following morphine administration

Chayaporn Reakkamnuan[1], Ekkasit Kumarnsit[1]*, Siriphun Hiranyachattada[1]
[1]*Department of Physiology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand*
**Email : kumarnsit.e@gmail.com*

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