

Loss of bone mass due to short sleep is related to the leptin-sympathetic nervous system activity

N. Kuriyama^{a*}, M. Inaba^c, E. Ozaki^a, Y. Yoneda^a, D. Matsui^a, K. Hashiguchi^a, T. Koyama^a, K. Iwai^a, I. Watanabe^a, F. Niwa^b, Y. Watanabe^a

^a Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan

^b Department of Neurology, Kyoto Prefectural University of Medicine, Kyoto, Japan

^c Department of Metabolism, Endocrinology, and Molecular Medicine, Graduate School of Medicine, Osaka City University, Osaka, Japan

Background: Sleep, a component of lifestyle, has been reported to be an important factor in bone metabolism, and sympathetic nervous system activity has been reported to regulate bone metabolism. **Aim:** In this study, we evaluated the association between sleep, sympathetic nervous system activity, and bone mass. **Methods:** The study subjects were 221 middle-aged individuals (108 males; 113 females; 55.1 years) divided into two groups: those who slept for less than 6 hours daily (short sleep [SS] group), and those who slept 6 hours or longer (normal sleep [NS] group). The groups were compared with regard to lifestyle, cortical bone thickness, cancellous bone density, bone metabolism markers, blood leptin levels, and sympathetic nervous system activity as evaluated by heart rate variability analysis. **Results:** Significant differences were observed between the two groups in cortical bone thickness, blood TRACP-5b (bone resorption markers), and leptin levels. The L/H ratio (an index of sympathetic nervous system activity) was higher in the SS group than in the NS group. Significant negative correlations were observed between cortical bone thickness and both the L/H ratio and leptin levels, and a significant positive correlation was observed between and the L/H ratio and leptin levels. **Conclusions:** Short sleep was associated with the decline of cortical bone thickness due to promotion of bone resorption and sympathetic nervous system hyperactivity in middle-aged group. Since leptin levels and cortical bone thickness were found to be closely related, cortical bone mass is suggested to be regulated via interaction with the leptin-sympathetic nervous system.

Sympathetic neurograms with characteristics of both muscle and skin sympathetic nerve activity in a patient with pure autonomic failure

K. Shindo, M. Tsuchiya, T. Sato, Y. Ichinose, N. Yamashiro, T. Nagasaka, Y. Takiyama
Department of Neurology, University of Yamanashi, Japan

Background and Aim: Muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA) can usually be recorded separately. **Subject:** A 69-year-old man presented to our outpatient clinic with frequent episodes of orthostatic dizziness and heat intolerance in summer. He had mild orthostatic hypotension and hypohidrosis of the face and body. Pure autonomic failure (PAF) was diagnosed because cardiac uptake of MIBG was reduced. **Methods:** Sympathetic neurograms were obtained by microneurography. Blood pressure and the chest electrocardiogram (ECG) or the skin sympathetic response and skin blood flow (laser Doppler method) were recorded simultaneously at rest and under various conditions, including mental stress, the Valsalva maneuver, and electrical stimulation (ES). After measurement of the latency between the R wave on the ECG and sympathetic bursts, the latency between reflex bursts after ES and the R-R interval on the ECG, or the latency between one burst and the next, histograms were made to display the data. **Results:** The frequency of spontaneous sympathetic bursts was increased by mental stress and the Valsalva maneuver. Single reflex sympathetic bursts were induced by ES. The latency between the R wave and sympathetic bursts was relatively constant on the histograms, while the latency of reflex bursts varied after ES. R-R intervals were more constant than the burst-burst latencies. **Conclusions:** In patients with PAF, it may be necessary to perform careful confirmation of the characteristics of sympathetic activity due to the possibility that MSNA and SSNA cannot be recorded separately.

MORPHOLOGY OF THE MYENTERIC PLEXUS AT DIFFERENT GUT SEGMENTS OF HUMAN FOETUSES

S. Bhukya, A. Shariff, S. Singh, T.C. Nag, J.A. Quadri, S. Saba
Department of Anatomy, All India Institute of Medical Sciences, INDIA

Introduction: The Neural Crest Cells derived Enteric Nervous System is the intrinsic innervation of gastro intestinal tract (GIT) which consists of neurons and enteric glia cells in the myenteric ganglia. Because of its autonomic control over the GIT, it is also called the “second brain”¹. ENS consists mainly of submucosal and myenteric plexuses. Clinical studies revealed that congenital malformations of the ENS seriously affect the gut motility, gastric acid secretion, and water and electrolyte transport². Scarcity of existing literature on the development of myenteric plexus at different segments of the GIT, which are sites of various diseases, motivated us to initiate this study. **Aim:** To determine the morphology of the myenteric plexus of foregut (oesophagus), midgut (ascending colon) and hindgut (descending colon) with increasing age of gestation (12 – 30 weeks). **Materials & Methods:** Tissue samples from 5 aborted foetuses aged 12– 30 weeks of gestation (WG) were processed for immunohistochemistry. The neurons, enteric glia and ICC were visualized using PGP 9.5, vimentin and S-100 antibodies.

Observation: The number of neurons and enteric glial cells appeared lowest in the oesophagus compared to the ascending and descending colon. The size of the neurons and appearance of neuronal processes within the myenteric ganglia increased remarkably with increasing gestational age. **Conclusion:** The knowledge of the development of the innervation of different segments of the gut in humans from 12 to 30WG may help in understanding the pathophysiology of various congenital disorders affecting ENS.

References:

1. GERSHON, MD. The Second Brain: A Groundbreaking New Understanding of Nervous Disorders of the Stomach and Intestine. Harper Collins, 1998.
2. UEDA, T. and OKAMOTO, E. Embryogenesis of intramural ganglia of the gut its relation to Hirschprung disease. Journal of Paediatric Surgery, 1967, vol. 2, p. 437-441

Electrical microstimulation of peripheral sympathetic nerve fascicle enhances glucose uptake independently of insulin action in rats

D. Sato^a, R. Yamaguchi^a, R. Suzuki^a, H. Sasaki^a, M. Kusunoki^b, Z. Feng^c, T. Nakamura^a

^a Department of Biomedical Information Engineering, Graduate School of Medical Science, Yamagata University, Yamagata, Japan

^b Research Center of Health, Physical Fitness and Sports, Nagoya University, Nagoya, Japan

^c Department of Bio-Systems Engineering, Graduate School of Science and Engineering, Yamagata University, Yonezawa, Japan

Background: It was reported that increase in sympathetic nerve activity enhanced glucose uptake in peripheral tissue. We previously reported that electrical microstimulation of peripheral sympathetic nerve fascicle via a microelectrode (0.05-0.10 V lower than muscle contraction threshold) induced transient blood glucose reduction (from 68 ± 4 to 64 ± 3 mg/dL, $P < 0.01$, mean \pm SD) while plasma insulin changed little (from 2.0 ± 0.7 to 1.7 ± 0.7 ng/mL) in anesthetized rats. **Aim:** In the present study, we quantitatively evaluated glucose uptake induced by the microstimulation. **Methods:** To locate the tip of microelectrode in a sympathetic fascicle in the rat sciatic nerve, we microneurographically detected peripheral sympathetic nerve signal first. And then electrical stimulation was applied via the microelectrode at a rate of 2 bursts/s; each burst comprised 10 pulses of 0.25-ms width at 25-ms intervals. Glucose uptake was assessed as glucose infusion rate (GIR) of euglycemic clamp measured before, during, and after the microstimulation. **Results:** The microstimulation increased GIR from 12.9 ± 1.5 to 15.2 ± 1.5 mg/kg/min ($P < 0.01$). In addition, even after the termination of microstimulation, GIR remained significantly higher (14.7 ± 1.8 mg/kg/min) than that observed before the stimulation ($P < 0.05$). Plasma insulin changed little throughout the euglycemic clamp. **Conclusions:** The results suggest that the microstimulation may enhance non-insulin-mediated glucose uptake via sympathetic nervous system, and that the effect could last even after terminating the microstimulation.

Parkinson's disease and prostate hyperplasia: which is more contributing to overactive bladder?

R. Sakakibara^a, O. Takahashi^b, F. Tateno^a, M. Kishi^a, Y. Tsuyusaki^a, Y. Aiba^a, H. Lee^c, M. Yano^c, H. Suzuki^c, C. Shibata^d, T. Uchiyama^d, T. Yamanishi^d, T. Yamamoto^d, Y. Suzuki^a

^aNeurology, Internal Medicine, Sakura Medical Center, Toho University, Sakura, Japan

^bClinical Physiology Unit, Sakura Medical Center, Toho University, Sakura, Japan

^cUrology, Sakura Medical Center, Toho University, Sakura, Japan

^dNeurology, Chiba University, Chiba, Japan

^eContinenace Center, Dokkyo Medical College, Tochigi, Japan

Objective – To investigate which is more contributing to elderly overactive bladder (OAB) / urinary incontinence in Parkinson's disease (PD)/ benign prostatic hyperplasia (BPH). **Methods** – We had 177 patients. Regarding the prostate volume, we divided them into 5 groups: BPH without PD, 12; male PD with large BPH (ultrasound prostate volume >30 ml), 20; male PD with small BPH (20-30 ml), 11; male PD without BPH, 46; and female PD, 88. We performed a urinary symptom questionnaire and a urodynamics. **Results** – 1) Frequency of detrusor overactivity (DO) in PD without BPH (male 61.3%, female 73.3%) was almost the same with that in BPH without PD (male 60%). However, 2) bladder volume at the first sensation ($p<0.01$), at the normal desire to void ($p<0.01$) and at bladder capacity ($p<0.01$, 0.05) in PD without BPH were significantly smaller than that in BPH without PD. 3) Phasic DO in PD without BPH (41.9-64.3%) was more common than that in BPH without PD (20%), while terminal DO occurred equally. 4) 40.2% of male PD had concurrent BPH (mean volume 39 ml; almost the same with that [45 ml] in pre-surgical BPH without PD). 5) In PD, disease duration and HY motor grade had positive impact on smaller bladder capacity ($p<0.05$ and $p<0.01$, respectively), while age did not affect LUT function. **Conclusion** –BPH and PD might occur together. Bladder volume in PD is significantly smaller than that in BPH, indicating that PD is more contributing to elderly OAB/ urinary incontinence. Phasic DO might suggest PD.

Primary afferent fibers are involved in partial recovery of urinary function in neonatally spinalized rats.

M. Fujioka^a, M. Takiguchi^b, K. Funakoshi^b

^aYokohama City University School of Medicine, Japan

^bDepartment of Neuroanatomy, Yokohama City University School of Medicine, Japan

Complete spinal transection in adult rats results in poor recovery of hind limb function and severe urinary bladder dysfunction. In contrast, neonatal rats that received a spinal cord transection show not only some recovery of hind limb function, but also spontaneous urination, which is sufficient to obviate the manual bladder expression required by adult spinal-transected rats. We hypothesized that primary afferent fibers not only contribute to the improvement of hind limb locomotor function, but are also involved in ameliorating urinary bladder dysfunction. In this study, we labeled primary afferent fibers by injecting an anterograde tracer into the lumbar sixth (L6) dorsal root ganglion (DRG) and examined whether primary afferent fibers are involved in micturition in neonatally spinalized rats. In intact rats, primary L6 afferent fibers projected to the L6 dorsal horn, intermediate zone, and ventral horn. Moreover, these fibers projected both ipsilaterally and contralaterally, which is not observed when an anterograde tracer is injected into the L5 DRG (Takiguchi et al., 2015). In neonatally spinalized rats, we observed more primary afferent fiber projections to the intermediate zone on the both sides. In addition, primary afferent fibers from L6 DRG projected to the lateral horn of the L6 spinal cord and made terminal button structures on parasympathetic preganglionic cholinergic neurons in neonatally spinalized rats. The findings of the present study indicate that primary afferent fibers might be involved in the recovery of both hind limb locomotor function and urinary bladder function after neonatal spinal cord transection.

Disparate phenotypes of sympathetic nervous system activation in essential hypertension: Origin of the Obesity Paradox in hypertension?

Murray Esler

Baker IDI Heart and Diabetes Institute, Melbourne, Australia

Applying isotope dilution methodology, to determine sympathetic nerve noradrenaline release and reuptake, and single fibre recording in sympathetic efferents directed to skeletal muscle vasculature, my colleagues and I have found there is a remarkable disparity in sympathetic nervous system biology between normal weight- and obesity-hypertension.

Phenotypes of Sympathetic Activation in Lean and Obese Patients with Essential Hypertension

Lean

- cardiac sympathetic activity increased
- *single sympathetic fibres*: increased firing rates, plus salvos of multiple firing
- reduced neuronal noradrenaline reuptake
- sympathetic nerve adrenaline co-release

Obese

cardiac sympathetic activity normal or low
single sympathetic fibres: recruitment of fibres which fire at normal rate, without salvos
noradrenaline reuptake normal
no sympathetic nerve adrenaline co-release

I propose this is the basis for the Obesity Paradox in essential hypertension (American Journal of Medicine 2007;120:863-870), increased longevity in hypertensive patients who are overweight and obese. The neuroscience formulation is that cardiac sympathetic nerve biology is “toxic” in normal-weight essential hypertension, compared with that of obese hypertensives. The cardiac sympathetic outflow is preferentially activated, single sympathetic fibres commonly have multiple firings in a cardiac cycle (salvos) causing high junctional noradrenaline concentrations, and the effect of this nerve firing characteristic is intensified by a fault (apparently epigenetic) in neuronal reuptake of noradrenaline. Additionally, adrenaline isotope dilution analysis, with coronary sinus sampling, documents cardiac sympathetic nerve adrenaline co-release in normal weight essential hypertension. This is potentially arrhythmogenic, due to greater cardiac myocyte β_1 adrenoceptor affinity to adrenaline than noradrenaline. In comparison, cardiac sympathetic nerve biology is “benign” in obesity-hypertension.

Carotid body chemosensory potentiation is crucial for the autonomic dysfunction and hypertension induced by intermittent hypoxia mimicking obstructive sleep apnea

R. Iturriaga^a, D.C. Andrade^{a,b}, R. Del Rio^b

^aLab. Neurobiology, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, ^bLab. of Cardiorespiratory Control, Universidad Autónoma de Chile, Santiago, Chile.

Chronic intermittent hypoxia (CIH), a feature of obstructive sleep apnea, elicits autonomic dysfunction, increased sympathetic outflow and hypertension, attributed to systemic oxidative stress. Notwithstanding, CIH enhanced carotid body (CB) chemosensory discharges, contributing to the hypertension. Antioxidant treatment prevents the enhanced CB discharges and hypertension, making unfeasible to establish any causal relationship. Accordingly, to assess the CB contribution to the hypertension, we eliminated both CBs in conscious hypertensive CIH-rats. Male Sprague-Dawley rats (200g) were exposed to CIH (5% F_iO₂, 12 times/h, 8 h/day). After 3 weeks, the CBs were cryo-destroyed under isoflurane anesthesia and rats were exposed one more week to CIH. We studied the effects of CB ablation on arterial blood pressure (BP) measured by telemetry, baroreflex sensitivity (BRS), heart rate variability (HRV), arrhythmia index and oxidative stress. CIH increases BP (~10 mmHg) in 4 days, reduced BRS, produces HRV alterations toward sympathetic predominance and increased cardiac arrhythmic episodes. CB ablation normalized the elevated BP, BRS and HRV, and reduces cardiac arrhythmic episodes. Systemic oxidative stress was unaffected by CB ablation. Our results show that the CB plays a crucial role in autonomic alterations and the hypertension induced by CIH. CB ablation reduced the elevated BP and restored the autonomic balance after 21 days of CIH. Furthermore, CB ablation reduces CIH-induced cardiac arrhythmogenesis. Together, our results strongly support that functional CBs are required for the maintenance of the neurogenic hypertension during CIH.

Supported by FONDECYT 1150040.

Facial skin blood flow decreases during exposure to pleasantly charged movie in humans

R. Asahara^a, K. Endo^a, M. Yoshikawa^a, S. Kusunoki^a, K. Matsukawa^a

^a Department of Integrative Physiology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Japan

The changes in regional facial skin blood flow and vascular conductance have been assessed for the first time with noninvasive two-dimensional laser speckle flowmetry during audiovisually elicited emotional challenges for 2 min (comedy, landscape, and horror movie) in humans. Limb skin blood flow and vascular conductance and systemic cardiovascular variables were simultaneously measured. The extents of pleasantness and consciousness for each emotional stimulus were estimated by the subjective rating of pleasantness and consciousness from -5 (the most unpleasant; the most unconscious) to +5 (the most pleasant; the most conscious). Facial skin blood flow and vascular conductance, especially in the lips, decreased during viewing comedy and horror movies, whereas they did not change during viewing a landscape movie. The decreases in facial skin blood flow and vascular conductance were the greatest with the comedy movie. The changes in lip, cheek, and chin skin blood flow negatively correlated ($P < 0.05$) with the subjective ratings of pleasantness and consciousness. The changes in lip skin vascular conductance negatively correlated ($P < 0.05$) with the subjective rating of pleasantness, while the changes in infraorbital, subnasal, and chin skin vascular conductance negatively correlated with the subjective rating of consciousness. However, none of the changes in limb skin blood flow and vascular conductance and systemic hemodynamics correlated ($P > 0.05$) with the subjective ratings. These findings suggest that the more emotional status becomes pleasant or conscious, the more neurally mediated vasoconstriction may occur in facial skin blood vessels.

Anxiety and Fear: Quantifying the Mind Using EKG with mDFA

T. YAZAWA

Biological Science, Tokyo Metropolitan University, Japan

The heartbeat represents momentarily varying inner emotional tension, which is directly cooperated with the functioning of the autonomic nerve function. This psychological variations of the inner world is detectable and quantifiable using a long-time electrocardiogram (EKG), from the invertebrate animals to humans. We made our own EKG amplifiers for being enable us to record a stable EKG: A perfect EKG where the EKG trace never jump-out from the PC monitor screen. Using this amplifier, we captured approximately 2000 heartbeats without missing a single beat. For the analysis of the EKGs, we used “modified detrended fluctuation analysis (mDFA)” technique, which we have recently developed by our group [1]. The mDFA calculates the scaling exponent (SI, scaling index) from the R-R interval time series. The mDFA can distinguish between a normal and an abnormal heart: a normal healthy heartbeat exhibits an SI of around 1.0, comparable to the fluctuations exemplified as the $1/f$ spectrum. The heartbeat recorded from subjects who have stress and anxiety exhibited a lower SI. Arrhythmic heartbeats and extra-systolic heartbeats both also exhibited a low SI ~ 0.7 , for example. We report that the mDFA technique is a useful computation method for checking health.

[1] T. Yazawa, 2015, “mDFA.” ASME monograph book, USA, 2015, Amer. Soc. Mechanical Engineers.

Nitric oxide-mediated S-nitrosylation can be involved in brain nicotinic acetylcholine receptors-mediated activation of central adrenomedullary outflow in rats

T. Shimizu^a, Y. Higashi^a, M. Yamamoto^a, K. Tanaka^b, K. Nakamura^a, S. Shimizu^a, S. Zou^a, T. Yawata^c, T. Ueba^c, K. Yuri^b, M. Saito^a

^aDepartment of Pharmacology, Kochi Medical School, Kochi University, Japan.

^bDepartment of Neurobiology and Anatomy, Kochi Medical School, Kochi University, Japan.

^cDepartment of Neurosurgery, Kochi Medical School, Kochi University, Japan.

We previously reported that intracerebroventricularly (icv) administered (\pm)-epibatidine, a nicotinic acetylcholine receptor (nAChR) agonist, evokes secretion of noradrenaline and adrenaline from the rat adrenal medulla by brain $\alpha 4\beta 2$ nAChRs- and cyclooxygenase-mediated mechanisms. Cyclooxygenase can be activated by nitric oxide (NO) and NO exerts its effects through not only activation of soluble guanylate cyclase (GC) but also S-nitrosylation, a posttranslational modification of protein cysteine residues. Therefore, we investigated which NO-mediated mechanism in the brain is involved in (\pm)-epibatidine-induced activation of central adrenomedullary outflow. Anesthetized male Wistar rats were icv pretreated with L-NAME (non-selective NO synthase (NOS) inhibitor), 3-bromo-7-nitroindazole (selective neuronal NOS inhibitor), BYK191023 (selective inducible NOS (iNOS) inhibitor), carboxy-PTIO (NO scavenger), ODQ (GC inhibitor) or dithiothreitol (non-selective S-nitrosylation inhibitor) before (\pm)-epibatidine administration (5 nmol/rat, icv) and plasma noradrenaline and adrenaline were subsequently measured electrochemically after HPLC. Furthermore, we examined an effect of (\pm)-epibatidine on the immunoreactivity of S-nitrosylated cysteine residues (SNO-Cys) in spinally projecting neurons of the hypothalamic paraventricular nucleus (PVN, a regulatory center of central adrenomedullary outflow). Icv administered (\pm)-epibatidine-induced elevation of plasma noradrenaline and adrenaline was attenuated by icv pretreated L-NAME, BYK191023, PTIO and DTT, but not by 3-bromo-7-nitroindazole or ODQ. In the hypothalamic PVN, (\pm)-epibatidine induced the immunoreactivity of SNO-Cys in nAChR $\alpha 4$ subunit-positive spinally projecting neurons, and this induction was abolished by pretreatment with BYK191023. These results indicate that brain iNOS-derived NO-induced protein S-nitrosylation can be involved in central adrenomedullary outflow induced by stimulation of brain nAChRs in rats.

Seasonal differences in cardiac autonomic nervous activity during exercise in obese men

M. Sato^a, H. Hayashi^a, T. Ikemoto^b, T. Ushida^b, S. Iwase^a, M. Sato^a

^aDepartment of Physiology, Aichi Medical University School of Medicine

^bInstitute of Physical Fitness, Sports Medicine and Rehabilitation, Aichi Medical University School of Medicine

【Background】 Light is the strongest synchronizer controlling circadian rhythms. The intensity and duration of light change throughout the year, thereby influencing body weight, food preferences, and melatonin secretion in humans and animals. In Japan, obesity is defined by a BMI of 25 kg/m² or over; 28.6 % of men and 20.6 % of women are obese. Obese people have an increased incidence of developing cardiovascular, renal, and hormonal diseases and sleep disorders. We have previously reported that the seasonal differences of thermoregulation and melatonin secretion during sleep in obese subjects. **【Aim】** In this study, we investigated that the seasonal changes in autonomic nervous activity in obese and non-obese subjects during exercise. **【Methods】** Six of non-obese men and 5 of obese men participated in summer and winter. Electrocardiogram (ECG) was recorded continuously using a standard device and R–R intervals were analyzed by spectral analysis using the maximal entropy method (MemCalc/Win, GMS, Tokyo, Japan). **【Results】** The heart rate was increased during exercise in obese than in non-obese in summer and winter. High frequency (HF), cardiac parasympathetic nerve activity, was decreased in obese after exercise. LF/HF, cardiac sympathetic nerve activity, was not different between obese and non-obese.

Development of measurement and advanced analysis systems to examine the effect of autonomic nervous activity

Y. OKITA^a, K. OHARA^b, I. TAKAHASHI^a, M. KIMURA^a, H. NAKAMURA^b

^aDepartment of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka, Japan

^bGraduate School of Human Development and Environment, Kobe University, Hyogo, Japan

Autonomic nerve activity (ANA) has been widely used in many fields. It has been reported that ANA in heart rate variability (HRV) have only the effects of either raised parasympathetic or decreased sympathetic activity after intake of GABA (functional foods), but it was not simultaneously measured by Electrocardiogram (ECG) and Electrogastrography (EGG). There are few reports to the effects of ANA by simultaneous measurement of ECG and EGG at this time. On the other hand, ECG and EGG sequences contain motion artifacts and electromyographic activity. To solve these problems, we developed systems for simultaneous measuring and analyzing ANA in ECG and EGG. We propose a signal processing method for eliminating motion artifacts and muscle signal activity from the ECG and the EGG, which was enabled by a new filter design of the system. The subjects were 3 healthy male. All subjects gave written informed consent to participate in this study. In preliminary study, the subjects participated in two kinds of experiments: (1) Control with water, and (2) GABA with water. Physiological data (ECG, EGG) were continuously obtained until 60 min after intake. The developed system enables simultaneous measuring and analyzing ANA in ECG and EGG with high time-frequency resolution. In ANA analysis in ECG and EGG, we confirmed that GABA or Control intakes have gastric myoelectrical activity and sympathetic and parasympathetic modulation in a normal range. The results indicate that the newly developed system would be useful for physiological interpretation and functional food science of HRV and EGG.

Effects of traditional kampo medicine, Kamikihito on acute/chronic nociceptive stress in rats

T. Maruyama, M. Yoshimura, Y. Motojima, S. Sonoda, H. Ueno, R. Saito, Y. Ueta
Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Japan

Kamikihito (KKT), traditional kampo medicine, is well known for the clinical effects for psychiatric disorder such as anxiety, insomnia and depression. Although some clinical effects have been recognized, the basic mechanism how KKT acts remains unknown. In the present study, behavioral and neuroendocrine-based evaluations were performed to investigate the effects of KKT on acute/chronic nociceptive stress in adult male/female rats. After KKT or vehicle solutions were orally administered once a day in adult male and female rats for 7 days, von Frey test and hot plate test was performed to evaluate the efficacy for acute analgesic effect. On the other hand, formalin test was used to evaluate for chronic pain. In addition, forced swim test was used for evaluation for anti-depression effect. Although chronic oral administration of KKT did not affect acute analgesia in male and female rats but show significant increases of pain threshold in formalin treated female rats. In forced swim test, mild anti-depressant effects of KKT were observed in male rats. It is worth noting that KKT medication has no influence for the feeding and growth in all rats. These results indicate that KKT might act on the central nervous system for regulation of pain control and stress responses in rats.

A novel *in vivo* method is useful for investigating central mechanisms of esophageal peristalsis in rats.

K. Horii, T. Shiina, K. Naitou, H. Nakamori, Y.Sano, H. Shimaoka, Y. Shimizu
Laboratory of Veterinary Physiology, United Graduate School of Veterinary Sciences,
Gifu University, Japan

Background: Esophageal peristalsis is controlled by the brainstem via vago-vagal reflex. However, the regulatory mechanisms of esophageal peristalsis are not fully understood. For elucidation of central mechanisms, it is essential to keep the neuronal connection between the esophagus and the brainstem during experiment. Therefore, the aim of present study was to establish a novel *in vivo* method, which is useful to investigate the central mechanism. Methods: A balloon tipped catheter was placed in the esophagus of a rat anesthetized with urethane through the oral cavity. The balloon was inflated to induce esophageal peristalsis. For evaluating esophageal peristalsis, we measured the distance and the velocity of the balloon movements. Results: Balloon moved to aboral direction in response to its inflation. Similar balloon movements, in terms of the distance and the velocity of the movements as well as the balloon volume triggering the movements, were reproducibly induced at least three times in the same animal. Vagotomy abolished the induction of balloon movements, showing that our novel method can evaluate the esophageal peristalsis mediated by the central mechanism. Balloon inflation failed to induce the esophageal peristalsis when a nitric oxide (NO) synthase inhibitor, L-NAME was intravenously applied. This result suggests that NO plays important roles in esophageal peristalsis. Conclusion: We devised a novel *in vivo* method for investigating central mechanisms of esophageal peristalsis in rats. By using the novel method, we elucidated essential roles of NO in the regulation of esophageal peristalsis.

A case of autoimmune autonomic ganglionopathy presenting as a unilateral Adie's tonic pupil

J.H. Cho

Department of Neurology, National Health Insurance Service Ilsan Hospital, Goyang, Korea

Background : Autoimmune autonomic ganglionopathy (AAG) is a very rare autonomic disease associated with antibodies to nicotinic acetylcholine receptors. AAG is characterized by systemic autonomic failure including orthostatic hypotension, gastrointestinal dysmotility and tonic pupil. **Aim** : We present a case of AAG presenting as a unilateral Adie's tonic pupil. **Method** : A 41-year-old man developed acute near-vision disturbance of right eye. He had decreased sweating and salivation, diarrhea and orthostatic dizziness over three weeks. The right pupil was dilated and showed hypersensitivity to pilocarpine. There was no evidence of sensory and motor dysfunction. Head-up tilt test showed orthostatic hypotension. The supine plasma norepinephrine concentration was reduced (29 pg/mL; normal > 70 pg/mL) and failed to increase on standing. Sudoscan showed very low electrochemical skin conductance in hands (12.7 μ S; normal > 60 μ S) and feet (12.8 μ S; normal > 60 μ S) suggesting severe sudomotor dysfunction. Serologic testing demonstrated low titer of circulating antibody to the ganglionic acetylcholine receptor (0.04 nmol/l; normal \leq 0.02). **Result** : He felt less dizzy and the right pupil was dilated less after treatment of IVIG. **Conclusions** : AAG should be considered in the differential diagnosis of patient who presents with Adie's tonic pupil and has generalized autonomic function.

Serum uric acid and non-motor symptoms in de novo Parkinson's disease

C. Toyoda, T. Umehara, A. Nakahara, H. Matsuno, H. Oka

Department of Neurology, The Jikei University School of Medicine, Tokyo, Japan

Background: The association between low serum uric acid (UA) and Parkinson's disease (PD) has been studied. But the relationship between UA and non-motor symptoms in de novo PD has been poorly investigated. Aim: Our aim is to assess the relationship between serum UA levels and clinical features including autonomic symptoms in de novo PD. Methods: We enrolled 51 patients with de novo PD (21 men and 30 women, 72.3 ± 7.9 years, disease duration 1.5 ± 1.4 years); and measured serum UA, albumin, calculated body mass index (BMI), and examined the Unified Parkinson's Disease Rating Scale (UPDRS), Mini-mental State Examination (MMSE), Frontal Assessment Battery (FAB), The odor stick identification test for Japanese (OSIT-J), Gastrointestinal Symptom Rating Scale (GSRS), [123 I]meta-iodobenzylguanidine (MIBG) myocardial scintigraphy, and coefficient variation of RR intervals (CVR-R). We investigated the correlations between serum UA levels and other parameters. Results: Mean serum UA, albumin, and BMI were 4.7 ± 1.4 mg/dl, 4.1 ± 0.3 g/dl, 21.7 ± 3.2 kg/m², respectively. Serum UA levels had a strong correlation with BMI ($p < 0.01$). Mean early heart-to-mediastinum (H/M) ratio, delayed H/M ratio, and washout rate (WR) of MIBG myocardial scintigraphy were 1.7 ± 0.3 , 1.4 ± 0.3 , and $35.4 \pm 7.4\%$, respectively. Serum UA levels had correlated with WR ($p < 0.05$) except with MIBG uptake. There were no correlations between serum UA levels and other parameters. Conclusions: The serum UA level is directly proportional to the BMI. This applies to the general population as well. The serum UA level didn't affect motor, cognitive, and olfactory functions and gastrointestinal complaints in de novo PD patients.

Validation of electroacupuncture effect for work efficiency

Waki H^{a,b}, Suzuki T^c, Tanaka Y^c, Tamai K^{c,d}, Minakawa Y^{a,b}, Imai K^{a,b}, Uebaba K^{a,b}, Hisajima T^{a,b}.

^a Faculty of Health Care, Teikyo Heisei University, Japan

^b Research institute of oriental medicine, Japan

^c Graduate School of Health Sciences, Teikyo Heisei University, Japan

^d Faculty of Health Care and Medical Sports, Teikyo Heisei University, Japan

Background: Electroacupuncture (EA) for first division of the trigeminal nerve was decreased HR, and it was increased parasympathetic nerve activity and CBF of both side. The autonomic nervous system (ANS) and cerebral blood flow (CBF) of functional decline were decreased work efficiency. Aim: This study was to examine the effect of EA for the trigeminal nerve on work efficiency, CBF of the prefrontal cortex (PFC) and ANS function. Methods: This study was a randomized crossover study for 2 weeks. Five healthy volunteers (aged 23.40 ± 1.34 years) were assigned to one of two groups: a control group and an EA group. All experiments were repeated 1 min rest period and 1 min Uchida-Krapelin test (UKT) periods five times after rest (control group) or EA (EA group) for 10 min. In addition, refreshment, work efficiency and the number of answers were evaluated after intervention or UKT. Acupuncture points were chosen the medial ends of the eyebrows and 1 cm off the front hairline midpoint. EA group received EA at 100Hz with stainless steel needles. Measurements of the ANS and CBF in both groups were made 1min rest period and UKT periods. Results: Compared with the control group, the EA group showed a higher refreshment score, work efficiency score and the number of answers ($p < 0.05$). However, the comparison between the groups showed no significant differences in the ANS and CBF during UKT. Conclusion: EA for first division of the trigeminal nerve may improve work efficiency.

Pathogenesis of the sigmoid diverticular disease is manifested by the calcitonin gene-related peptide expression

A.G. Pauza^a, K. Rysevaite-Kyguoliene^a, M. Malinauskas^b, J.I. Lukosiene^c, E. Stankevicius^b, J. Kupcinskas^c, Z. Saladzinskas^d, A. Tamelis^d, N. Pauziene^a

^a Institute of Anatomy, Lithuanian University of Health Sciences, Lithuania

^b Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Lithuania

^c Clinic of Gastroenterology, Lithuanian University of Health Sciences, Lithuania.

^d Department of Surgery, Lithuanian University of Health Sciences, Lithuania

With high prevalence in the elderly population, diverticular disease (DD) is one of the most common maladies of the large intestine. Calcitonin gene-related peptide (CGRP) is a potent gastrointestinal smooth muscle relaxant acting through nitric oxide. It is eminent that smooth muscle relaxation is a subject to alteration in DD. The aim of the study was to investigate CGRP signalling in symptomatic (SDD) and asymptomatic (ADD) diverticular disease patients. Specimens obtained from patients undergoing surgery for colorectal carcinoma served as control (n=10) and ADD (n=10); sigmoid resection after recurrent attacks of diverticulitis as SDD (n=10). Full-thickness colon sections were processed for CGRP, its receptors CRLR (calcitonin receptor-like receptor) and RAM1 (receptor activity-modifying protein 1) and NOS (nitric oxide synthase) immunohistochemistry and analysed by means of quantitative fluorescence microscopy within ganglia of sigmoid colon. Isometric smooth muscle activity was recorded *in vitro* using organ bath technique. CGRP was found primarily innervating nitrergic neurons. CGRP expression decreased up to 40%, however CRLR - increased up to 25% in SDD in all divisions of enteric nervous system. ADD patients showed intermediate values for CGRP and CRLR. RAMP1 – expression was found to be unaltered. DD smooth muscle displayed 40% ($p<0.05$) reduced relaxation responses. CGRP induced relaxation was significantly improved and only 14-17% weaker compared to control samples. Our results suggest that CGRP signalling pathway is subjected to alteration in DD. We suggest that upregulation of CRLR and hypersensitivity to CGRP may develop as a compensatory mechanism for gradually declining levels of CGRP in DD.

Stimulation of the superior laryngeal nerve promotes calcitonin and thyroxine secretion, without changes in parathormone secretion, from the thyroid and parathyroid glands

H. Hotta^a, A. Onda^a, H. Suzuki^a, P. Milliken^b, A. Sridhar^b

^aDepartment of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

^bBioelectronics R&D, GlaxoSmithKline plc., London, United Kingdom

The thyroid gland receives vagal innervation mainly via the superior laryngeal nerve (SLN), which contains both afferent and efferent fibers. The majority of myelinated fibers in rat SLNs (96%–99%) are afferent. This study examined the effect of stimulating the SLN on the secretion of calcitonin, thyroxine, and parathormone from the thyroid and parathyroid glands in anesthetized rats. Nineteen male rats were used in this study. A thin catheter was inserted into a thyroid vein, and plasma hormone levels in the collected blood were measured by ELISA to examine changes in the secretion rate of hormones from the glands before, during, and after stimulation. To define the role of unmyelinated efferent nerve fibers, cut peripheral segments of SLNs or cervical sympathetic trunks (CSTs) were stimulated with supramaximal intensity (10 V). To examine the role of myelinated fibers (typically afferent), intact SLNs were stimulated with a subthreshold intensity for unmyelinated fibers (4–60 μ A). Nerves were stimulated bilaterally (pulse duration, 0.5 ms) at various frequencies up to 40 Hz. During SLN stimulation, in cut and intact nerves, the secretion of calcitonin and thyroxine increased at 40 Hz, but the secretion of parathormone was unaffected. In contrast, during CST stimulation at 20 Hz, the secretion of calcitonin and thyroxine decreased, but the secretion of parathormone increased. These results suggest that the excitation of myelinated afferents in the SLN promotes the secretion of calcitonin and thyroxine from the thyroid gland, potentially via the reflex activation of parasympathetic efferent nerve fibers in the SLN.