

Water Deprivation Does Not Enhance Pressor Responses to Handgrip Exercise in Humans or Sciatic Nerve Stimulation in Rats

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Water deprivation elevates plasma osmolality (pOsm) and activates baseline sympathetic nerve activity (SNA) to maintain blood pressure (BP). We tested the hypothesis that water deprivation elevates pOsm and consequently augments BP responses to handgrip exercise (humans) and sciatic afferent nerve stimulation (rats). To test this hypothesis, we induced modest dehydration in humans and severe dehydration in rats. Seven young men (26 ± 1 yrs; 25 ± 1 kg/m²; BP $114\pm 2/77\pm 3$ mmHg) completed two protocols: 16 hrs water deprivation (WD) and normal hydration (NH). In humans, beat-to-beat BP was collected during isometric handgrip (HG) exercise at 40% of maximal voluntary contraction. pOsm (291 ± 2 vs. 288 ± 1 mOsm/kg H₂O) and hematocrit (42 ± 1 vs. $41\pm 1\%$) were higher during WD compared to NH ($p < 0.05$ for both). However, mild dehydration did not alter the BP response to HG exercise (Δ mean BP 17 ± 4 vs. 16 ± 3 mmHg, $p > 0.05$). Six male Sprague-Dawley rats were WD for 48 hrs, and six were given water ad libitum. BP and lumbar SNA were recorded during sciatic afferent nerve stimulation. pOsm (310 ± 4 vs. 295 ± 1 mOsm/kg H₂O) and hematocrit (47 ± 1 vs. $41\pm 1\%$) were higher during WD compared to NH ($p < 0.05$ for both). Activation of sciatic afferents produced frequency-dependent increases in BP and SNA, but there were no between group differences at any frequency (Δ mean BP at 5 Hz: 21 ± 2 vs. 18 ± 3 mmHg; Δ lumbar SNA at 5 Hz: 129 ± 11 vs. $139\pm 11\%$; $p > 0.05$ for both). These preliminary data suggest that mild dehydration in humans and severe dehydration in rats both elevate pOsm but do not augment handgrip or sciatic nerve stimulation pressor responses.

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Developmental influences on sympathetic circuits regulating energy expenditure and susceptibility to obesity

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Mounting evidence indicates that maternal and other early developmental exposures can “program” later obesity risk by influencing the establishment of metabolic set-points at a young age. It is widely believed that the activity of neuronal circuits regulating energy balance is tuned in response to early cues, which allows the fetus or neonate to adapt to the predicted postnatal environment. To date, most mechanistic studies of developmental programming of obesity risk focused on hypothalamic circuits regulating food intake. We are exploring possible contributions from influences on the development of sympathetic innervation from the stellate ganglion to brown adipose tissue (BAT). There is a growing appreciation of the contribution of BAT to resting energy expenditure and metabolic function in humans and conversely, the possibility that impaired BAT function could contribute to obesity. We took advantage of the fact that BAT function is modulated by changes in the ambient temperature, without adverse impacts on health or metabolism, to study the impact of early BAT activity levels on developing efferent sympathetic systems. We identified a time window when the number of sympathetic neurons innervating BAT is increased in order to meet enhanced metabolic needs imposed by a cold environment. We have evidence that the stellate ganglion retains this ability to adapt to a cold stress for several weeks. Defining the time window and source of this plasticity may also have important implications for the capacity for adaptations in the stellate ganglion in response to stresses in other target organs, such as the heart.

Serum ganglionic acetylcholine receptor antibodies in patients with chronic intestinal pseudo-obstruction

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Background: Several autoantibodies against specific neuronal antigens are found in patients with autoimmune gastrointestinal dysmotility, e.g. chronic intestinal pseudo-obstruction (CIPO). Activation of autoimmune pathways has been implicated as a contributing mechanism to the pathogenesis in patients with CIPO. **Aim:** To elucidate the clinical characteristics of CIPO with serum ganglionic acetylcholine receptor relationship between CIPO and anti-ganglionic acetylcholine receptor antibodies (gAChR Abs). **Methods:** Serum samples were obtained from teaching and general hospitals throughout Japan between January 2012 and December 2016. We listed 14 serum samples of patients with CIPO and measured Abs against gAChR α 3 and gAChR β 4 by a luciferase immunoprecipitation system. We analyzed the clinical features of anti-gAChR Abs positive CIPO cases. **Results:** Seven patients (50%) with CIPO were found to have anti-gAChR Abs. Single seropositivity for anti-gAChR α 3 antibodies was observed in 5 patients, while 2 were positive for both antibodies. Autonomic manifestations were widespread in seropositive CIPO; especially, sicca complex and bladder dysfunction were frequently observed (71.4%, respectively). **Conclusions:** A half proportion of patients with CIPO have antibodies against gAChR. CIPO is a dysmotility syndrome that presents with symptoms and signs of intestinal obstruction and radiographic evidence of dilated bowels, but no anatomic obstruction could be found. As patients with anti-gAChR Abs have generally cholinergic dysfunction including gastrointestinal symptoms, it seemed plausible CIPO could be limited variant of autoimmune autonomic ganglionopathy.

EVIDENCE OF A GHRELIN-INDEPENDENT ACTIVATION OF GHRELIN RECEPTORS IN THE SPINAL CORD

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Colon activity is voluntarily controlled from the cortex which sends signals via the spinal cord defecation centre to the colorectum. We have previously demonstrated that ghrelin receptor agonists, that cross the blood/CNS barrier, enhance colorectal propulsion and lead to defecation. We now show that water avoidance stress (WAS) causes a defecation response in rats that is antagonized by the GHSR1a antagonist, YIL781, indicative of a physiological role of GHSR1a in defecation control. However, ghrelin itself, the only known ligand for GHSR1a, is absent from the CNS. We have investigated these responses in ghrelin knockout rats compared to wild type littermates. Animals were placed on a small platform surrounded by water and pellets expelled were counted each 15 min for an hour. In wild-type rats, there was a substantial increase in pellets in the first 15 minutes after WAS, which was reduced to < 50% by YIL-781 (3 mg/kg i.p., 10 min before WAS), compared to vehicle control (P=0.035). Interestingly, in ghrelin knockout rats, we observed a substantial decrease in stimulatory role of WAS: control 5.3±1.1 and KO 3.3±1.1 pellets in the first 15 min, which was reduced to 61.5% by YIL-781 (P=0.5). The total amount of pellets in both groups of animals after 60min was similarly reduced by the GHSR1a antagonist to 63.8% (P=0.02) and 60.7% (P=0.1) in WT and mutant rats respectively. The results indicate that there is a potential unidentified endogenous ligand in the spinal cord which activates ghrelin receptors, directly or indirectly, during defecation.

Medullary reticular nuclei control metabolism and food intake during starvation

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Hypothalamic release of neuropeptide Y (NPY) in response to hunger simultaneously induces hyperphagia and reduces energy expenditure, such as brown adipose tissue (BAT) thermogenesis. However, the central neural circuit mechanisms of these hunger responses have been unknown. We hypothesized that NPY-triggered neural signaling from the hypothalamus inhibits sympathetic premotor neurons in the rostral medullary raphe (rMR) that control BAT thermogenesis. Our neural tracing studies in rats and mice revealed that many GABAergic neurons in the intermediate and parvocellular reticular nuclei (IRt/PCRt) of the medulla oblongata directly innervate sympathetic premotor neurons in the rMR. NPY-triggered neural signaling activated rMR-projecting GABAergic neurons in the IRt/PCRt. Selective stimulation of GABAergic IRt/PCRt neurons using the DREADD technology inhibited skin cooling-induced BAT thermogenesis. On the other hand, inactivation of IRt/PCRt neurons eliminated the inhibitory effect of hypothalamic NPY on BAT thermogenesis, indicating that GABAergic IRt/PCRt neurons mediate NPY-induced hypometabolism. Intriguingly, stimulation of IRt/PCRt neurons elicited mastication and increased food intake as well as inhibited BAT thermogenesis. Furthermore, neural tracing studies revealed many GABAergic IRt/PCRt neurons that directly innervate both masticatory motor region and BAT sympathetic premotor region in the rMR. These results demonstrate that GABAergic neurons in the IRt/PCRt, which are activated by hypothalamic NPY-triggered hunger signaling, play key roles to survive starvation by both inhibiting metabolic thermogenesis and promoting food intake.

Enhancement of neurogenesis in enteric nervous system by c-Kit inhibition

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Introduction: Neurogenesis in enteric nervous system (ENS) is rarely observed in adult intestine, although there are precursor cells which have potential to differentiate into new neurons in gut wall. In our previous study, we demonstrated that neurogenesis of ENS were prominently enhanced in *c-kit* mutant (W/W^l) mice after the ablation of myenteric plexus using benzalkonium chloride (BAC). Aim: In this study, we compared the morphological characteristics of the newly appeared neurons among wild type mice, W/W^l mice, and wild type mice with c-Kit inhibitor administration. Methods: Using the ablation model described above, the NADPH-diaphorase staining, immunohistochemistry, transmission electron microscopy were performed. Results: One week after BAC ablation, the infiltration of new fibers from intact area to damaged area were observed both in wild type and W/W^l mice. Three weeks after ablation, new neurons appeared and they were abundantly observed in W/W^l mice. Most of these new neurons located ectopically: i.e. in the longitudinal muscle layer and the subserosal layer. The ectopic new neurons were differentiated into several neuronal subtypes and composed ganglion like structure at least under ultrastructural observation. In addition, wild type mice with *Imatinib* administration, which were c-Kit inhibitor, showed extremely abundant ectopic new neurons comparing to those without the administration. Conclusion: It is likely that c-Kit activity negatively regulates the neurogenesis of ENS in wild type mice.

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Astrocytes play a role in the generation and spread of seizure activity and eventual death in a severely hypoxic condition

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Recently, it has been elucidated that astrocytes play active roles in various brain functions and are closely involved in evolution of abnormal network excitability in various neurological disorders such as epilepsy. Severe hypoxia induces a seizure, which reduces ventilation, further worsens hypoxia, and could cause death. Thus, a hypoxia-induced seizure is a serious threat in patients with epileptic diseases. However, its precise pathophysiological mechanisms remain unclear. We aimed to elucidate the role of astrocytes in the generation of seizure activity and eventual death in a severely hypoxic condition. Twenty four conscious, spontaneously breathing, adult male mice were used in experiments. We measured ventilation by whole body plethysmography. We also measured the O₂ concentration in the recording chamber. After recording ventilation in room air, the gas in the chamber was switched to 5% O₂ (N₂ balanced) until seizures and ventilatory depression occurred. Ventilatory responses to hypoxia were tested in two groups (n=12 in each group), one without and the other with administration of arundic acid, an inhibitory modulator of astrocytic activation. Severe hypoxia initially increased ventilation, followed by occurrence of seizures and reduction of ventilation in all mice. Death followed frequently in a group without arundic acid. However, arundic acid delayed the occurrence of seizures and prevented death. We suggest that astrocytes play a crucial role in the generation of seizure activity and eventual death in a severely hypoxic condition. Astrocytic activation antagonism may prevent seizures and death under severe hypoxia, which would be worthy to investigate in a clinical trial.

Can self-care using contact needles based on the gentle tactile stimuli applied to the skin relieve menstrual pain? A randomized, placebo-controlled, double-blind, comparative study.

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Tactile stimulation with the contact needles which have elastic micro cones induced a morphine-like effect that selectively inhibited the C-reflex of cardiac sympathetic nerve reflexes evoked by stimulation of somatic afferent C-fibers in previous animal study. We conducted a randomized, placebo-controlled, double-blind, comparative study in order to evaluate the efficacy of tactile stimulation with this contact needle for menstrual pain relief. 19 women who experienced menstrual pain were randomly assigned to a contact needle or placebo needle group. Participants were self-care by settling contact or placebo needles on acupuncture points (BL37, BL55, and BL56) in their lower extremities on the first and second menstrual days of 4 menstrual cycles. The primary outcome was the change in menstrual pain intensity which was assessed by using a Visual Analogue Scale (VAS) that was compared between the groups. In the 14 participants (contact needle group: n = 8, mean of VAS at baseline = 48.63 mm / placebo needle group: n = 6, mean of VAS at baseline = 55.08 mm) who completed the study, the mean change in menstrual pain: -17.31 mm in the contact needle group and -18.83 mm in the placebo needle group. There was no intergroup difference ($p = 0.87$, Cohen's $d = 0.09$). Thus, given that its effect of contact needle on menstrual pain was equivalent to placebo needle, the pain relief observed in this study might have been due to non-specific effects, such as placebo effects and acupressure effects.

Outcome of Orthostatic Dysregulations in Psychosomatic Medicine

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Background: Orthostatic dysregulations (OD) are often comorbid with mental disorders and functional somatic syndromes (FSS), including chronic fatigue syndrome, irritable bowel syndrome, and fibromyalgia. Outcomes of OD patients with these comorbidities are not unclear. Aim: to investigate the impact of clinical factors on outcome of OD patients with psychosomatic comorbidities. Methods: A retrospective chart review was performed on OD patients who were diagnosed by active standing tests and the DSM-IV criteria in our psychosomatic outpatient clinic in 2016. Results: Among 56 participants, 38 were female and mean age was 35.5±17.8 years old. Postural orthostatic tachycardia syndrome was the most common OD subtype (n=45, 80.4%). Major comorbid mental disorders were panic disorder (n=15, 26.8%), other anxiety disorders (n=15, 26.8%) and depressive disorders (n=12, 21.4%). Thirty participants had FFS (14 migraine, 4 functional gastrointestinal disorders, 3 chronic fatigue syndrome, 3 fibromyalgia, etc.). Pharmacological treatments were SSRI (n=28), Midodrine (n=13), fludrocortisone (n=9), and beta-blockers (n=8). In 38 (67.9%) of Participants, orthostatic physical symptoms were improved within 3.90±3.02months. In a comparison between treatment methods, patient educations and exercise were related with these improvements. Age, sex, OD subtypes, mental disorders were not significantly related with outcomes. However, participants with FFS had a significantly lower improvement rate than without FFS (50.0% vs. 84.6%, p=.0148). Conclusion: Comorbid FFS may have a negative impact on outcome OD, rather than comorbid mental disorders.

The effect of direct current acupuncture stimulation on waist dorsal blood flow response

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Background: Back pain in Japan has been over 10 million people. Treatment is increasing blood flow in the sympathetic nerve block. Aim: This study was to investigate the effect of Direct Current Electro-Acupuncture (DCEA) to ST36 (Zusanli) on the blood flow of Lw4-5. Methods: This study was a randomized trial, assigning healthy adults (80 males and females, 21.7 ± 0.6 years old). The optimum was compared by Heart Rate (HR). The polarity assigned 16 people to the (+) group (-) group of $75\mu\text{A}$. Intensity was assigned to 64 people in the Control group, Manual group, $-25\mu\text{A}$ group, and $-75\mu\text{A}$ group. The blood flow in the back was oxy hemoglobin (HbO^2) and compared. 64 people were assigned to the Control group of Lw4-5, $-75\mu\text{A}$ group, Control group of Th7-8, $-75\mu\text{A}$ group. Intervention was 5 minutes, after intervention it was 5 minutes. Results: Polarity (-) group decreased HR than (+) group ($P=0.0028$). The intensity decreased in the $-75\mu\text{A}$ group than in the other groups. Control group ($p<0.001$), Manual group ($p<0.001$), $-25\mu\text{A}$ group ($p<0.01$). The blood flow of Lw4-5 was HbO^2 ($P<0.05$) in comparison between the $-75\mu\text{A}$ group and the Manual group. In the $-75\mu\text{A}$ group, the amount of HbO^2 increased at rest and 1 min after intervention, and the increase persisted even after intervention ($P<0.05$). There was no difference in the amount of HbO^2 in Th7-8 in comparison with the $-75\mu\text{A}$ group, Manual group. Conclusion: The DCEA $-75\mu\text{A}$ of ST 36 may increase the blood flow of Lw4-5.

Does cardiac intrinsic innervation depend on animal species?

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The heart rate of mammalian species is proportional to body mass: small mammals have higher, whereas larger mammals have lower resting heart rates. Previous studies have identified a relationship between structural components of the autonomic nervous system and the structural parameters of the heart. We examined heart ventricles of four different mammals – mouse, rabbit, pig and sheep – and compared different neurohistochemical marker distributions between their epicardial and myocardial nerve structures. Transverse sections of 2 mouse, 10 rabbit, 4 pig and 3 sheep hearts were stained immunohistochemically for protein gene product 9.5 (PGP9.5), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), neuronal NO synthase (nNOS), substance P (SP) and calcitonin gene-related product (CGRP). Positively stained epicardial and myocardial neuronal structures were analysed by calculating the area, nerve fibre composition and density. Cross-sectional nerve area increases with increasing mammal size. Small animals have better myocardial innervation: the density of myocardial PGP9.5(+) fibres in the mouse was twice as large than in the sheep and six times larger than in the pig. In the mouse, the comparative amount of myocardial TH(+) fibres was twice as large than in the sheep. Meanwhile, the comparative amount of ChAT(+) fibres was largest in the pig, five times less in the rabbit and absent in the mouse. nNOS(+) were prominent in the rabbit, scarce in the sheep and absent in the mouse. SP and CGRP did not present patterns of innervation between species. The present study shows evidence of species related autonomic nervous system regulation of heart function.

Tetanic stimulation of the hypothalamus evokes persistent excitation in the ventrolateral medulla: Spatiotemporal dynamics analyzed by voltage-sensitive dye imaging

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The dorsomedial hypothalamic nucleus (DMH) and the paraventricular nucleus (PVN) of the hypothalamus play crucial roles in central cardiovascular regulation. Although electrophysiological and histological analyses have revealed the functional and anatomical connection from the hypothalamus to the ventrolateral medulla, spatiotemporal dynamics how the hypothalamus innervates the ventrolateral medulla have not been elucidated. We hypothesized that prolonged excitation of the hypothalamus induces persistent activation of the ventrolateral medulla, and aimed to reveal the functional connectivity from the hypothalamus to the ventrolateral medulla. The hypothalamus, lower brain stem and spinal cord were isolated en bloc under deep anesthesia from the neonatal (P0-P2) rat. The preparation was dyed with a voltage-sensitive dye (Di-2-ANEPEQ), fixed with the ventral side up in a recording chamber, and superfused with an oxygenated artificial cerebrospinal fluid. The viability of the preparation was confirmed by recording its neural respiratory output. Using a CMOS sensor array (MiCAM Ultima, BrainVision, Tokyo) connected with an epifluorescence microscope, we imaged neural activity of the ventral medulla in response to electrical stimulation of the hypothalamus and analyzed the spatiotemporal dynamics. Single pulse stimulation (pulse duration 3 msec, 0.5 mA) of the hypothalamus evoked brief excitation in the ventrolateral medulla, but tetanic stimulation (10 Hz, for 10 sec, 0.4mA) induced excitation that persisted nearly 10 sec after the cessation of the stimulation. Observed activation of the ventrolateral medulla partly explains the mechanism of persistence and augmentation of the sympathetic nervous activity induced by hypothalamic excitation. This neural mechanism may underlie the pathophysiology of hypertension.

Sympathetic intestinal innervation regulates murine dextran sodium sulphate (DSS)-induced colitis.

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Background: Vagus nerve stimulation is currently clinically evaluated as a treatment for inflammatory bowel disease (IBD). However, whether vagus nerve activity directly regulates colitis, remains unclear. Firstly, we determined the effect of intestine-specific vagotomy or intestine-specific denervation of the superior mesenteric nerve (SMN), on dextran sodium sulphate (DSS)-induced colitis in mice. Secondly, we determined the effect of SMN stimulation on DSS-induced colitis in rats. **Methods:** We achieved intestine-specific vagotomy, SMN denervation or a combination of both denervations surgically. Chronic SMN stimulation was achieved by implantation of a cuff electrode, and stimulation was done bidaily for 5 minutes. We gave a biphasic pulse at 10 Hz of 200 ms, 200 μ A. Disease activity index (DAI) was used as a clinical parameter for DSS-induced colitis severity in rat and mouse. Furthermore, we assessed colonic cytokine levels and histology and endoscopy of the colon. **Results:** Intestine-specific vagotomy had no effect on the severity of DSS-induced colitis. However, SMN denervation alone or in combination with vagotomy caused a significantly higher DAI. Next, we assessed the effect of SMN stimulation on DSS-induced colitis. Noteworthy, the stimulus was well tolerated. SMN stimulation led to a significantly improved DAI compared to sham stimulation. However, colonic cytokines, endoscopy outcome and histological outcome did not change. **Conclusions:** We conclude that the vagus nerve innervating the intestine does not affect DSS-induced colitis. Alternatively, our data imply that the SMN ameliorates colitis in our DSS-induced colitis models. This study warrants further investigation into nerve stimulation as treatment for IBD.

Sympathetic activity regulates macrophages via the β 2-adrenergic receptor.

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Background: The autonomic nervous system is an important immune regulator. It has been established *in-vitro* that norepinephrine, the main sympathetic neurotransmitter, has anti-inflammatory effects on myeloid cells. We have shown that Rag1^{-/-} mice, lacking mature T- and B-cells, develop spontaneous colitis upon sympathetic denervation of the intestine (ISAN conference 2015, Stresa, Italy). Here, we investigated the effect of the sympathetic activity on macrophages, *in-vitro* and in the intestine of Rag1^{-/-} mice. **Methods:** First, bone-marrow derived macrophages (BMDM) were stimulated with norepinephrine or salbutamol, a β 2-adrenergic receptor agonist, prior to LPS stimulation. After 24 hours, cytokine levels were determined in the supernatant with ELISA and at mRNA level in cell lysates with qPCR. Second, in Rag1^{-/-} mice, a sympathetic denervation of the intestine was accomplished by cutting the superior mesenteric nerve (sympathectomy). Two weeks after sympathectomy, intestinal macrophages were sorted and analysed on flow cytometry and cytokine expression levels at qPCR. **Results:** Pre-treatment of LPS-stimulated BMDM with norepinephrine or salbutamol led to a decrease in pro-inflammatory cytokines such as TNF- α and IL-6 and upregulation of the anti-inflammatory M2-marker arginase 1 compared to no pre-treatment or pre-treatment combined with the beta-blocker propranolol. Norepinephrine levels were lower in the intestine of Rag1^{-/-} mice after sympathectomy compared to sham operated Rag1^{-/-} mice. Subsequently, macrophages in Rag1^{-/-} colons shifted towards a more inflammatory phenotype upon sympathectomy. **Conclusion:** We show that β 2-adrenergic receptor activity regulates macrophages both *in-vitro* and *in-vivo* and that the sympathetic nervous system is critical in regulating the intestinal myeloid immune compartment.

Correlation of seizure-associated central apneic episode durations with their frequency of occurrence in a rat model

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Respiratory derangements, including irregular, tachypnic breathing and central or obstructive apnea can be consequences of seizure activity. Periods of seizure-associated central apnea suggest that seizures spread to brainstem respiratory regions to disrupt breathing. Recently, we showed that seizure-associated central apnea episodes are associated with 1) a reset of the respiratory rhythm, and 2) activation of brainstem regions serving the diving reflex to suppress respiratory behavior (Villiere et al., 2017). Using a rat model, we demonstrated a sequence of events ending in death that begins with obstructive apnea caused by seizure-induced laryngospasm (Nakase et al., 2016). We sought to determine if patterns of central apnea behavior (duration and/or frequency of occurrence) correlated with the tendency for obstructive apnea to occur. There were not statistically significant differences in central apneic episode duration or number of recorded episodes in animals that eventually showed obstructive apnea compared with animals that never showed obstructive apnea. There was, however, a clear positive correlation of the average apneic episode duration with the number of apneic episodes recorded ($R^2=0.54$) and their crude frequency of occurrence (events/min of recording; $R^2=0.58$). Whereas the frequency or duration of central apneic episodes do not seem to be useful predictors of eventual obstructive apnea, the correlation of central apneic episode duration with frequency suggests that these episodes may reflect an attempt by brainstem networks to protect core physiology. A deeper understanding of seizure spread into brainstem regions may permit additional biomarkers to be established for identifying risk of severe systemic consequences of epileptic seizures.

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Circadian rhythm of autonomic function and sleep patterns in the elderly

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[Purpose] The quality of sleep is said to deteriorate with aging. To examine the cause of this deterioration, we studied the circadian rhythm of autonomic function rhythm and sleep patterns in the elderly. [Subjects and method] Twenty-two healthy elderly men with independent activities of daily life (ADL) were recruited. The controls were 12 healthy young males. We calculated the hours of sleep, sleep latency, number of waking episodes, waking time, and the sleep efficiency using an Actigraphy record (AMI, USA). We then calculated the parasympathetic-nerve function (HF) and the sympathetic nerve function (LF/HF) using a frequency analysis of the R-R interval of an ECG recorded using a Holter ECG. [Result and Discussion] The waking time and the sleep latency of the elderly group were significantly longer than those of the control group. Elderly individuals cannot fall asleep easily during a hypnagogic state or after awakening. Therefore, the sleep efficiency of elderly individuals is considered to be significantly low. The parasympathetic nerve function of the control group exhibited a significant circadian rhythm, increasing during sleep and decreasing when awake. In the elderly, this circadian rhythm disappeared. A circadian rhythm of sympathetic nerve function was not observed in either the elderly or control groups. [Conclusion] In the elderly, we thought that neither energy pooling nor somatic reparation is thought to be performed sufficiently, because the circadian rhythm of parasympathetic nerve function decrease at night. And the quality of sleep decreases because the circadian rhythm of parasympathetic nerve function is reduced.

Location and characteristics of the central thermal sudomotor pathways in human spinal cord

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Though the central sudomotor pathways in human spinal cord were studied by several investigators, their results are inconsistent. To elucidate the question, thermal sweating was investigated in 45 subjects with spinal lesions visualized by MRI (cervical: 23, upper thoracic: 12, lower thoracic: 6, and lumbo-sacral: 4) Thermal sweating was qualitatively assessed by a modified Minor's colorimetric test, and sweating patterns were photographed at least two different occasions during sweat test. Seventeen patients with extensive lesions showed anhidrosis or marked hypohidrosis below the lesion level. Twenty-four subjects exhibited finally generalized discoloration, and 21 out of them showed sweating laterality of various extents. Three subjects with lesions limited to the postero-medial portion of the cord showed normal sweating. In one subject with a lesion limited to lateral and superficial portion of the cervical cord showed normal sweating except ipsilateral hypohidrosis on the face. Unilaterally dominant lesions hardly resulted in anhidrosis of the lower extremities. The comparison of sweating patterns and extents of lesions suggested following points. 1) The hypothalamo-spinal pathways related to thermal sweating may pass ipsilaterally through areas anterior to the lateral cortico-spinal tracts which correspond to the reticulo-spinal tracts. 2) There must be some crossing fibers. 3) There may be somatotopic arrangement.

Systematic study of the contribution of cutaneous afferent A β , A δ , and C fiber groups on the inhibition of the rhythmic micturition contractions of the urinary bladder in rats

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We have recently found that a gentle mechanical stimulus to the perineal skin applied by a soft roller can strongly inhibit rhythmic micturition contractions in anesthetized rats, and that the stimulation induces a low-frequency (range, 0.03–11 Hz) excitation of low-threshold mechanoreceptive A β , A δ , and C fibers in the cutaneous branch of the pudendal nerve. We aimed to systematically study the contribution of cutaneous afferent A β , A δ , and C fiber groups on the inhibition of the rhythmic micturition contractions of the urinary bladder. The experiments were performed in 10 male rats anesthetized with urethane. The bladder was filled with saline to produce isovolumic bladder rhythmic micturition contraction. Electrical stimulation (pulse duration: 0.5 ms) was applied to cutaneous branch of the pudendal nerve at frequencies of 0.1, 1, and 10 Hz for 1 min. Nerve fiber groups were defined by recording compound action potentials from the cutaneous nerve. Activation of only A β fibers (0.2 V) produced an inhibition of rhythmic micturition contractions at 7–11 min after the onset of stimulation (late inhibition), at any tested frequency. Additional activation of A δ fibers (1 V) produced additional early inhibition (immediately after stimulation) at 1 and 10 Hz. Furthermore, additional activation of C fibers (10 V) at 10 Hz completely stopped rhythmic micturition contractions for 10 min. This strong inhibition persisted after local application of capsaicin to the stimulating cutaneous nerve. We conclude that activities of A β , A δ , and C afferent fibers, without capsaicin-sensitive channels, can contribute to the inhibition of bladder contractions.

Metaboreflex Regulation of Sympathetic Activity at Altitude in Lowlanders and Sherpa

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Muscle sympathetic nerve activity (SNA) is elevated during chronic hypoxia and the SNA response to exercise is augmented during acute hypoxia. Thus, we hypothesized increases in SNA and blood pressure during isometric handgrip (IHG) would be augmented at altitude in Lowlanders but not in long term high-altitude residents (Sherpa). Lowlanders (n=16) were studied at 344m and after acclimatization at 5050m and Sherpa (n=6) were tested at 5050m. SNA (microneurography), heart rate (ECG) blood pressure (MAP; Finometer) and cardiac output (CO; Modelflow) were recorded continuously during 2 min of IHG (30% max). Total peripheral resistance (TPR) was calculated as MAP/CO. In Lowlanders, resting SNA burst frequency (13 ± 5 to 31 ± 11 bursts/min; $p < 0.001$) and incidence (23 ± 6 to 50 ± 19 bursts/100 beats; $p < 0.001$) were increased after acclimatization; with resting incidence exceeding that of Sherpa (29 ± 17 bursts/100 beats; $p < 0.05$). Burst frequency was highest in acclimatized Lowlanders across all conditions (versus low altitude and Sherpa; main effect $p < 0.01$). However, the increase in burst frequency during IHG ($+17 \pm 9$, $+18 \pm 13$ and $+16 \pm 12$ bursts/min) were the same between groups (Lowlanders at 334m and 5050m and Sherpa at 5050m respectively). Although increases in blood pressure during IHG were similar between groups, Sherpa had a smaller increase cardiac output and greater increase in resistance (both $p < 0.05$). These data indicate the SNA and pressor responses to exercise is preserved at altitude and similar between acclimatized lowlanders and Sherpa. However, Sherpa have a greater neurovascular transduction ($\Delta TPR/\Delta SNA$) compared to lowlanders. Funded by NSERC (CDS & PNA) and a CRC (PNA).

DISTRIBUTION AND LOCALISATION OF GFP UNDER TPH1 CONTROL AND ITS RELATION TO 5-HT IN THE MOUSE GIT

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Background: Although enteroendocrine cells (EECs) are known to secrete multiple regulatory molecules, the lack of a transcriptional profile of individual types has limited our understanding of their precise functions and roles in gastrointestinal pathologies. To overcome this challenge, a bacTRAP transgenic mouse line where the L10a ribosomal subunit is tagged with GFP and placed under the control of the *tph-1* promoter was generated. Tryptophan hydroxylase (TPH) catalyzes the rate limiting step in the biosynthesis of serotonin. **Aim:** This study immunohistochemically characterised the bacTRAP transgenic mouse line to determine the specificity of the reporter for EECs. **Method:** The numbers of cells immunopositive for GFP and 5-HT, as well as their morphologies and distribution patterns were determined. Cells were counted at 63x from each GIT region of 3 bacTRAP mice. **Results:** The morphology and staining pattern of cells differed along the GIT. Numerous EECs with long processes were observed in the distal colon, but long processes on 5-HT cells were not discernible in duodenal samples. The majority of *tph-1* EECs contained 5-HT. However, in the stomach there were two distinct groups of 5-HT immunoreactive cells, with cells at the base of the glands staining positive for *tph-1* and 5-HT, and those further towards the lumen staining positive for 5-HT only. **Conclusions:** Although GFP-Tph-1 staining has made it possible to distinguish the morphology of 5-HT cells more clearly, further characterisation is required to establish if another Tph enzyme is responsible for 5-HT synthesis in cells that do not express GFP.