Hiroshi and Aya Irisawa Memorial Award Symposium

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Kisspeptin neuron, a central regulator of reproductive function, and sex steroids

(March 28, 9 : 00–10 : 30, Room B)

2MS2B-1

Postnatal changes in the expression of *Kiss1* and its regulation by gonadal steroids in rat hypothalamus

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Kisspeptins, encoded by *Kiss1* gene, play pivotal roles in the development and regulation of reproductive functions. In rodents, kisspeptin neurons localize in two hypothalamic nuclei ; anteroventral periventricular nucles (AVPV) and arcuate nucleus (ARC), and are involved in gonadal steroid feedback regulation of gonadotropin release. To clarify the postnatal ontogeny of kisspeptin neurons and its regulation by gonadal steroids, we determined the expression of *Kiss1* mRNA during postnatal development in intact and hormonally manipulated rats by in situ hybridization. In intact rats, *Kiss1* mRNA expressing neurons in AVPV first appeared during postnatal week 1-3, whereas *Kiss1* neurons were present in ARC from postnatal day 3. The number of *Kiss1* neurons in both regions increased along puberty in both sexes. These results indicate that the *Kiss1* neurons in ARC emerge earlier than those in AVPV and that the increase in *Kiss1* expression across puberty might be involved in the onset of puberty. At neonatal and prepubertal stages, clear sex differences in the number of ARC *Kiss1* neurons were observed ; females had a significantly greater number of *Kiss1* neurons than the males. However, gonadectomy at those stages resulted in significant increases in the *Kiss1* neuron number and the sex differences disappeared, indicating that ARC *Kiss1* expression is negatively regulated by gonadal steroids from early postnatal stages and the sex difference in ARC *Kiss1* expression might be attributed to the difference in circulating gonadal steroid levels.

2MS2B-2

Indispensable role of kisspeptin in controlling gonadotropin–releasing hormone release in mammals

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Gonadotropin-releasing hormone (GnRH) release is responsible for initiation of puberty and normal reproductive performance in mammals. Recent progress in kisspeptin biology has provided clue for the mechanism driving the two modes of GnRH release, pulses and surges. The present paper focuses on the role of kisspeptin neurons in controlling the two modes of GnRH release in mammals. Kisspeptin, a potent candidate for afferent inputs to the GnRH neurons, emerged from genetic linkage analyses of the patients of hypogonadotropic hypogonadism. Kisspeptin neurons are mainly localized in the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) of rodents, which are candidate regions of the centers for GnRH pulses and surges, respectively. Recently, we have generated *Kiss1* KO rats to prove the indispensable role of kisspeptin to control GnRH pulses and surges. Male and female *Kiss1* KO rats showed no puberty and complete suppression of pulsatile luteinizing hormone (LH) release. *Kiss1* deficiency also abolished estrogen-induced LH surges in females. These results indicate that kisspeptin neurons are indispensable for two modes of GnRH/LH release to regulate puberty and normal reproductive function in rats. This work was supported in part by the Research Program on Innovative Technologies for Animal Breeding, Reproduction, and Vaccine Development.

2MS2B-3

Functional and evolutionary diversity of vertebrate kisspeptin neuron systems

Kanda, Shinji; Oka, Yoshitaka (*Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan*)

Hypothalamic neurons that produce a peptide kisspeptin (kisspeptin neurons) are supposed to be essential for reproduction in mammalian species. In addition to *Kiss1*, a gene that encodes the mammalian kisspeptin, *Kiss2*, which is produced by gene duplication early in the vertebrate evolution, has recently been found in various vertebrate species. Here, we will introduce recent advancement in the understanding of vertebrate kisspeptin systems from the viewpoint of evolution and diversity of their physiological functions. Although the function of kisspeptin neurons have not yet been clearly shown in nonmammalian vertebrates, recent studies are beginning to show that some of the kisspeptin neurons in teleost brain also show steroid sensitive *kiss1/kiss2* expressional variation at the cellular level. By carefully analyzing physiology and anatomy of steroid sensitive *kiss1/2* neurons in teleosts and those in mammals, we now have a working hypothesis on the evolution of *kiss1* and *kiss2* neurons in vertebrates.

In addition to the reproductive functions in mammals, we have recently found in medaka morphological evidence for the expression of kisspeptin receptors in the isotocin and vasotocin (oxytocin and vasopressin homologs in teleosts, respectively) neurons, which implies possible novel functions of kisspeptin neurons. Furthermore, we have recently established transgenic medaka whose kisspeptin receptor-expressing cells are visualized by GFP. We will also introduce this recent approach toward the comprehensive understanding of the physiological functions of kisspeptin neurons in vertebrates.

Hiroshi and Aya Irisawa Memorial Award Symposium
Tactile stimuli and emotions and an autonomic response

(March 28, 10 : 30–12 : 00, Room B)

2MS3B-1

Effects of gentle skin stimulation on somato-cardiovascular reflexes and contribution of emotions

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Somatosensory stimulation may elicit not only sensations and emotions, but also analgesia and autonomic responses. We reported that gentle mechanical cutaneous stimulation (touch) inhibited the somatocardiac sympathetic C-reflex in anesthetized rats, depending on the texture of contacting objects. Recently, we studied the effect of touch on noxious heat-induced cardiovascular responses in conscious humans and anesthetized rats. In humans, changes in heart rate (HR) and the amplitude of finger pulse wave were evoked by heat stimulation applied to the right plantar foot. Heat-induced cardiovascular responses were inhibited by touch applied to the right medial malleolus. The inhibitory effects were dependent on the texture of touch while a difference in textures was not recognized. In deeply-anesthetized rats, heat stimulation was applied to the lower back. Heat-induced HR responses were inhibited by touch applied to a unilateral inner thigh without affecting basal HR. These results in humans and rats are consistent, suggesting that touch may inhibit cardiovascular responses via common mechanisms in conscious humans and anesthetized animals. Since it was assumed that the inhibitory effect of touch was spinal segmental, naloxone or μ-opioid receptor antagonist CTOP was intrathecally injected in anesthetized rats and the touch effect was abolished, indicating touch activates spinal opioid receptors. These results suggest that gentle mechanical cutaneous stimulation inhibits nociceptive transmission into autonomic reflex pathways via the spinal opioid system, which is independent of cognition and emotions.

2MS3B-2

Tactile skin stimulation increases dopamine release in the nucleus accumbens in rats

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We have shown that tactile stimulation of the skin affects various autonomic functions including arterial pressure, adrenal catecholamine secretion, and spinal cord blood flow. Tactile stimulation also produces psychological effects such as relaxation, the alleviation of anxiety and depression. That the psychological effects evoked by touch therapy involve stimulation of dopamine (DA) or serotonin secretion is suggested, however, there is no direct evidence of their increased release in the brain. The present study aimed to answer this unsolved question. For this purpose, we applied tactile stimulation in rats, and measured DA release in the nucleus accumbens, which is thought to play an important role in the pathophysiology of anxiety and depression. The present study demonstrate that innocuous tactile stimulation, but not noxious pinching stimulation, of the skin increases DA release in the nucleus accumbens both in conscious and in anesthetized rats. Our results show that innocuous mechanical stimulation can directly stimulate DA release in the nucleus accumbens in the absence of conscious perception or emotion. Furthermore, the increases of DA release can be generally observed in response to tactile stimulation of the various segmental skin areas, but it was only produced by contralateral stimulation to the site where DA release was measured. These results underlie the clinical effects of tactile stimulation on anxiety and depression, and provide strong evidence that touch therapy is useful for relieving the anxiety and depression.

2MS3B-3

Tickling alters emotional responses in adolescent rats

Hori, Miyo (*Foundation for Advancement of International Science*)

Play behaviors in adolescence is considered to facilitate normal cognitive and social development, whereas social isolation is noxious and can cause stress vulnerability. Adolescent rats emit 50-kHz ultrasonic vocalizations (USVs), which reflect positive emotion, such as rough-and-tumble play or tickling. The emission of 50-kHz USVs is suggested to be mediated by dopamine release in the nucleus accumbens, however, there is no direct evidence supporting this hypothesis. Thus, we examined whether tickling can trigger dopamine release in the nucleus accumbens with 50-kHz USVs. Tickling stimulation for 5 min increased dopamine release in the nucleus accumbens. Conversely, light-touch, as a discernible stimulus, did not change dopamine release. In addition, 50-kHz USVs were emitted during tickling, but not light-touch. Further, tickling-induced 50-kHz USVs were blocked by the direct application of SCH23390 (D1 receptor antagonist) and raclopride (D2/D3 receptor antagonist) into the nucleus accumbens.

Next, we examined whether repeated tickling could reverse stress vulnerability, occurred by socially isolation. We conditioned rats to fear an auditory tone which was initially paired with a mild foot-shock, and retention test was conducted 48 h and 96 h after conditioning. We found that prior tickling treatment diminished fear-induced freezing. And tickled rats showed reduced concentrations of both plasma adrenaline and noradrenaline. Current study demonstrates that tickling stimulation increases dopamine release in the nucleus and repeated exposure to tickling can modulate fear-related behavior and sympatho-adrenal stress responses.

Hiroshi and Aya Irisawa Memorial Award Symposium

Role of the enteric nervous system in coordinating the gastrointestinal functions

(March 28, 16 : 00–18 : 00, Room B)

2MS4B-1

Interaction between the protease–signalings and the mucosal nerves in regulation of colonic epithelial Cl^- secretion

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Serine proteases are versatile signaling molecules and often exert this function by activating the proteinase-activated receptors (PARs). We elucidated the roles of serine proteases in regulating Cl^- secretion in the mouse cecum. A mucosa-submucosal sheet of the cecum was mounted in Ussing chambers, and the short-circuit current (I_{sc}) was measured. PAR_1 activating peptide (AP) and PAR_2-AP both induced the Cl^--dependent I_{sc} increase when added from the serosal side, but had no effect from the luminal side. The I_{sc} increase induced by PAR_1-AP was abolished by tetrodotoxin (TTX), indicating that it occurred through activation of PAR_1 on the submucosal secreto-motor neurons. On the other hand, the PAR_2-mediated response was TTX-insensive, thus probably occurred by activating epithelial basolateral PAR_2. Trypsin, a typical serine protease, added to the serosal side induced a TTX-sensitive I_{sc} increase. This response was inhibited in part by a pretreatment of the tissue with PAR_1-AP, but not by PAR_2-AP. These results suggest that serine proteases released from subepithelial inflammatory cells induce Cl^- secretion, thereby help hosts to wash out the luminal noxious agents. PAR_1 on the subepithelial secreto-motor neuron is partially responsible for the response.

2MS4B-2

Luminal chemosensing and regulation of large intestinal motility and fluid secretion

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Intestinal lumen is the external environment for the internal milieu in the body. Especially in the large intestinal lumen, at least more than 1,000 species and more than 100 trillion numbers of commensal bacteria live symbiotically. These commensal bacteria metabolize (ferment) a variety of indigested and unabsorbed components from diet *etc*, and produce differ compounds. Short-chain fatty acids (SCFAs), 2-6 carbon-carboxyl acids, are the most predominant fermented products in the large intestine. The SCFAs not only are absorbed as nutrients, but also stimulate large intestinal mucosa inducing a smooth muscle contraction and a transepithelial anion secretion. These physiological responses to SCFAs are induced partially via neural pathways. We further found that the SCFA receptors, FFA2 and FFA3, which are deorphanized GPCRs, GPR43 and GPR41, respectively, were expressed in enteroendocrine L cells containing PYY and GLP-1. In addition, we have found that the bitter taste receptors (T2Rs) and olfactory receptors (ORs) are expressed in the colonic mucosa. We further reported that a bitter tastant, 6-propyl-2-tiouracil, and an odrant, thymol, induced an anion secretion in the human and rat colon. Therefore, our studies have suggested that the large intestinal epithelia survey the luminal chemical environment by enteroendocrine cells, brush cells, and surface epithelial cells, and the luminal chemosensing mechanism have a role for physiological and pathophysiological regulation of the large intestine and the host-defense.

2MS4B-3

Subepithelial Fibroblasts and Afferent Neurons in the Intestinal Villi Interact Mutually via ATP and Substance-P to Regulate Villous Movement and Other Functions

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Intestinal villi are a unique structural and functional unit for the luminal sensing, digestion, absorption, secretion and immune defense in the small intestine. Subepithelial fibroblasts of the intestinal villi, which form a contractile network beneath the epithelium, are in close contact with epithelial cells, neurons, capillaries, smooth muscles and immune cells. They seem to play pivotal roles in the villous functions. Villous subepithelial fibroblasts possess purinergic receptor P2Y1 and tachykinin receptor NK1. ATP and substance-P (SP) induce increase in intracellular Ca^{2+} and cell contraction. They make synapse-like structures with varicosities of SP and/or non-SP neurons, mostly intrinsic afferents nerve terminals. They are highly mechano-sensitive and release ATP, which spreads to and activates the surroundings via P2Y1 and the afferent neurons (IPANs) via P2X (2, 3, 2/3) ('auto-/paracrine pathway'). The activated IPANs may spread electrical signal to the subsequent varicosities and also propagate action potential to neighbor villi, and then release SP, which activates subepithelial fibroblasts again via NK1 ('neural pathway'). These mutual interactions may play essential roles in the signal transduction of mechano reflex pathways including a coordinate villous movement, and also in the maturation of the structure and function of the intestinal villi.

2MS4B-4

Cooperative gut motility requires the network of pacemaker cells

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Smooth and elaborate motions of various biological systems require cooperative activities of cellular members contained. In the gut, it is well known that a network of intrinsic neurons simultaneously induces ascending contraction and descending relaxation of smooth muscle, leading to peristaltic movements. Relatively recent studies have revealed that special interstitial cells, referred to as interstitial cells of Cajal (ICC) act as pacemaker cells for the basal electric activity. Under physiological conditions, these cells appear to also play a crucial role in spatial organization of gut excitability through their network of long processes. Furthermore, it is likely that these cells undergo pharmacological modulations and pathological alterations.
In this presentation, we first show several important features of ICC pacemaker activity. For example, unlike the network of enteric neurons, the propagation direction of pacemaker potential is reversible. Next, we carefully explain how we measure electrical activity of ICC using microelectrode array. Namely, low impedance microelectrodes are preferred to record slow oscillating electric potentials in a small region of ～1-4 mm^2. Thirdly, we show examples of alterations of ICC pacemaker activity through neurotransmitters and immune signals, which are possibly related with important diseases, such as irritable bowel syndrome and inflammatory bowel disease. Lastly, we assess what mechanisms couple electrical activity of ICC, which are thought to be Ca^{2+} oscillators.

2MS4B-5

Nervous control on physiological function of the distal gut–defecation reflex mechanism

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Important physiological function of the distal gut is the defecation reflex. Moderate rectal distension elicits rectal (R-R) reflex contractions and simultaneous internal anal sphincter (R-IAS) reflex relaxations that together comprise the defecation reflex. Both reflexes are controlled by pelvic nerves, lumbar colonic nerves, and enteric nervous system (ENS). Lateral pontine reticular formation suppresses lumbar colonic nerves during defecation. In addition, in the rodent, ENS such as cholinergic ascending and nitrergic descending nerves play an important role on defecation reflex. To reveal the role of ENS, Takaki et al. established the distal gut model where intrinsic nitrergic descending nerves were injured. The rectum 30 mm oral from anal verge was transected without damage to extrinsic nerves, and subsequent anastomosis was performed. R-IAS reflex relaxations were abolished without changes in R-R reflex contractions after the transection and anastomosis. Eight weeks after sectioning of intrinsic reflex nerve pathways in the rectum, R-IAS reflex recovered to the control level, accompanied with regeneration of reflex pathways. The result indicated that nitrergic descending nerves participate in the descending R-IAS reflex relaxations. Furthermore, Takaki et al. found a small molecular compound, mosapride citrate facilitated recovery of the R-IAS reflex relaxations and associated reflex pathways mediated via enteric 5-HT_4 receptors. The possibility for neurogenesis in the ENS and the rescue of defecation dysfunction by this drug is promising.
Key word : defecation reflex, internal anal sphincter, enteric nervous system, enteric 5-HT_4 receptors