Department of Pharmacology

Toshihiko Momiyama, *Professor*
Naofumi Kimura, *Professor*
Haruhisa Nishi, *Assistant Professor*
Seiji Hori, *Professor*
Yuji Ohno, *Assistant Professor*
Taro Ishikawa, *Assistant Professor*

General Summary

The research interests in the Department of Pharmacology include the following.

1) Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
2) Effects of new quinolones on body temperature and blood pressure *in vivo* (Seiji Hori)
3) Respiratory neural activities in *Xenopus* (Naofumi Kimura)
4) Design of secretory proteins (Yuji Ohno)
5) Purinergic receptors on human adrenocortical cells and mechanisms of histamine release modification by purinergic receptors in human lung mast cells (Haruhisa Nishi)
6) Cerebrocerebellar interactions (Taro Ishikawa)
7) Cardiovascular endocrinology (Keiichi Ikeda)
8) The basic mechanism of a ketogenic diet: purinergic autocrine regulation of CA3 pyramidal neurons (Masahito Kawamura)

Research Activities

Synaptic transmission and its modulation in the basal ganglia and basal forebrain

Electrophysiological studies using slice patch-clamp recording are performed to analyze synaptic transmission, its modulation by neuromodulators, and their developmental change in the nigrostriatal or mesolimbic dopaminergic system, and the cholinergic system in the basal forebrain. These systems are involved in various psychological functions as well as their disorders, including Parkinson's disease and Alzheimer's disease. The regulation of output from these systems to the cerebral cortex is also studied.

Another issue is the regeneration of synapses and local circuits after basal ganglia-related disorders. In these disorders, electrophysiological, morphological, and behavioral studies are performed to clarify the mechanisms and time course of reconstruction of synaptic organization and transmission and the functions of whole animals in Parkinson's disease model rats or cerebral ischemia model rats.

Effects of new quinolones on body temperature and blood pressure in vivo (collaborative study with Department of Practical Pharmacy, Keio University School of Pharmacy)

We studied the effect of new quinolones on body temperature in mice. We showed that new quinolones deceased body temperature in rats in a dose-dependent manner.

Gatifloxacin had potent body temperature-decreasing activity, but levofloxacin had weak activity. Our *in vivo* results suggest that each new quinolone has its own body temperature-decreasing activity and that we should be aware of this activity, especially when a new quinolone is used for a patient with compromised renal function.
We also studied the effects of new quinolones on blood pressure in rats. In our *in-vivo* experiments, new quinolones decreased blood pressure in a dose-dependent manner. Garenoxacin had stronger activity, and levofloxacin had weaker activity. This *in vivo* result suggests that each new quinolone has potent blood pressure-decreasing activity. We are studying the precise mechanisms of these activities of new quinolones.

Respiratory neural activities in Xenopus
Aquatic pipid frogs have some interesting characteristics from the viewpoint of comparative respiration physiology. Unlike other anurans, pipids lack the buccal ventilatory cycle, exhale air from the lung before aspirating air into the buccal cavity, and have inherent muscles that may be homologous to the mammalian diaphragm. To study the mechanism of the lack of the buccal cycle, respiratory motor activities were recorded from the isolated brainstem-spinal cord preparations of *Xenopus laevis*. The intermittent lung ventilation-like burst complexes occurred in cranial nerves V and X, the hypoglossal nerve, and the third spinal nerve innervating the "diaphragm." The buccal ventilation-like activity occurred in cranial nerves V and X, but did not appear in the hypoglossal nerve or the third spinal nerve of *Xenopus*. These results suggest that the brainstem of *Xenopus* is capable of buccal oscillation but partly lacks the motor output.

Design of secretory proteins
We found that almost all mouse interleukin (IL) 31 was secreted from human embryonic kidney cells when the protein was obligatorily expressed in cells transfected by an mammalian expression plasmid with a cytomegalovirus promoter. We then confirmed that the fusion protein of enhanced green fluorescent protein with the cytokine was also efficiently secreted. As we investigated the secretory sequences, the N-terminal sequences of IL-31 from signal peptides to the first glycosylation site (SG-sequences) could be crucial. Furthermore, we examined the fusion proteins of p53 and aquaporine, which has nuclear localization signal sequences and is a membrane protein, respectively, with SG-sequences. We could design secretory proteins associated with SG-sequences.

Purinergic receptors on human adrenocortical cells and mechanisms of histamine release modification by purinergic receptors in human lung mast cells
The expression and pharmacological function of purinergic receptors in H295R, a cell line derived from human adrenocortical cells, were studied as a model of these receptors in human adrenocortical cells. H295R expressed G-protein coupled P2Y receptors, suggesting that human adrenocortical cells express multiple purinergic receptors linked to their steroidogenic function. These results suggest that Ca^{2+} influx is important for the steroidogenesis of H295R cells.
In collaboration with Dr. E.S. Schulman of Drexel University (Philadelphia, PA, USA),

we synthesized several plasmids with the construct of short hairpin RNA (shRNA)-interference for the knock-down of histidine decarboxylase and P2Y2 receptors (a subtype of P2Y receptors). The gene knock-down systems were transfected with a lentivirus to LAD2 cells, a cell line derived from human mast cells. The results suggest that multiple purinergic receptors are related to the modification of IgE receptor-mediated histamine release in human lung mast cells.

Cerebrocerebellar interactions

The pontine nuclei in the brainstem receive signals from the cerebral cortex and relay them to the cerebellum. We have started a project that is aimed to clarify the rules of signal conversion in the pontine nuclei and the underlying cellular mechanisms through a combination of *in vivo* (anaesthetized animal) and *in vitro* (brain slice) experimental systems. Using the in vitro system, we made patch-clamp recordings from principal cells, which are excitatory projecting neurons, in slices of the pontine nuclei and investigated cellular properties, including the spontaneous firing rate and current-voltage relationships. Spontaneous and stimulation-evoked excitatory synaptic currents were also recorded, and their facilitation and depression patterns were analyzed. While developing the *in vivo* experimental system, we could make patch-clamp recordings from the pontine nuclei of anaesthetized animals via a ventral approach.

Cardiovascular endocrinology

We have been studying cardiovascular endocrinology in 2009. On the basis of a previous study of the secretion of adrenocorticotropic hormone from the hypertensive heart and the expression of corticotropin-releasing hormone type 2 receptor in cardiomyocytes, we studied the secretion of adrenocorticotropic hormone from cardiomyocytes using the HL-1 mouse atrial cardiomyocyte cell line.

The basic mechanism of a ketogenic diet: a purinergic autocrine regulation of CA3 pyramidal neurons

A ketogenic (low-carbohydrate, high-fat) diet has successfully been used to treat pediatric and medically refractory epilepsy. The mechanisms underlying the success of ketogenic diet therapy, however, are not well understood. A ketogenic diet has been reported to increase ATP concentrations in the central nervous system and to cause mild hypoglycemia. To clarify the role of extracellular purines underlying the anticonvulsant effect of the ketogenic diet, whole-cell voltage clamp recordings were made from CA3 pyramidal neurons in acute hippocampal slices of rats. In conditions of reduced extracellular glucose and high intracellular ATP concentrations, CA3 pyramidal neurons hyperpolarize themselves via direct ATP release through pannexin-1 channels with the subsequent activation of adenosine A1 receptors. This autocrine regulation might be an important mechanism underlying the success of a ketogenic diet.

Publications

Ogino H*[1]*, Yamada K*[1]*, Yuhara M*[1]*, Tsuchida S*[1]*, Maezawa K*[1]*, Kizu J*[1]*, Hori S (*[1]Keio Univ).*** Fluoroquinolones reduce carrageenan-induced edema in rats and the involvement of the glucocorticoid receptor system. *J Infect Chemother* 2009; **15:** 113–7.

Akiyama N, Ohno Y, Fukuda T, Manome Y, Saito S. Enhancing activity of N-glycosylation for constitutive proteins secretions in non-polarized cells. *Biochem Biophys Res Commun* 2009; **381:** 612–8.

Ogino H*[1]*, Fujii M*[1]*, Ono M*[1]*, Maezawa K*[1]*, Hori S, Kizu J*[1] *(*[1]Keio Univ).*** In vivo and in vitro effects of fluoroquinolones on lipoplysaccharide-induced pro-inflammatory cytokine production. *J Infect Chemother* 2009; **15:** 168–73.

Ikeda K, Tojo K, Inada Y, Takada Y, Sakamoto M, Lam M*[1]*, Claycomb WC*[1]*, Tajima N (*[1]Louisiana State Univ Health Sci Center).*** Regulation of urocortin I and its related peptide urocortin II by inflammatory and oxidative stresses in HL-1 cardiomyocytes. *J Mol Endocrinol* 2009; **42:** 479–89.

Hori S. Convulsant activity of sitafloxacin and its interactions with anti-inflammatory drugs in mice. *J Infect Chemother* 2009; **15:** 266–8.

Tanaka Y*[1]*, Furuyashiki T*[1]*, Momiyama T, Namba H*[2]*, Mizoguchi A*[3]*, Mitsumori T*[1]*, Kayahara T*[3]*, Shichi H*[4]*, Kimura K*[3]*, Matsuoka T*[1]*, Nawa H*[2]*, Narumiya S*[1] *(*[1]Kyoto Univ Grad Sch Med,*** [2]***Brain Res Inst Niigata Univ,*** [3]***Mie Univ Grand Sch Med,*** [4]***Wayne State Univ).*** Prostaglandin E receptor EP1 enhances GABA-mediated inhibition of dopaminergic neurons in the substantia nigra pars compacta and regulates dopamine level in the dorsal striatum. *Eur J Neurosci* 2009; **30:** 2338–46.

Hoffman AF*[1]*, Laaris N*[1]*, Kawamura M, Masino SA*[2]*, Lupica CR*[1] *(*[1]NIH,*** [2]***Trinity Coll).*** Control of cannabinoid CB1 receptor function on glutamate axon terminals by endogenous adenosine acting at A1 receptors. *J Neurosci* 2010; **30:** 545–55.

Kimura N. Fictive lung ventilation in the isolated brainstem preparation of the aquatic frog, Xenopus laevis. *Adv Exp Med Biol* 2010; **669:** 9–12.

Kawamura M Jr., Ruskin DN*[1]*, Masino SA*[1] *(*[1]Trinity Coll).*** Metabolic autocrine regulation of neurons involves cooperation among pannexin hemichannels, adenosine receptors, and KATP channels. *J Neurosci* 2010; **30:** 3886–95.

Reviews and Books

Momiyama T. Developmental changes in calcium channels and D1-like receptors involved in the glutamatergic transmission onto rat basal forebrain cholinergic neurons. In: Fisher A, Hanin I, editors. New Trends in Alzheimer and Parkinson Related Disorders: ADPD 2009. Prague: Medimomd S.r.l. International Proceedings; 2009. p. 299–305.