Department of Pharmacology

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General Summary

The research interests of the Department of Pharmacology include:
1) Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
2) Respiratory neural activities in *Xenopus* (Naofumi Kimura)
3) Design of secretory proteins (Yuji Ohno)
4) Analysis of the mechanisms underlying histamine release from human-derived mast cells (Haruhisa Nishi)
5) High-frequency firing of cerebellar mossy fibers (Taro Ishikawa)
6) The basic mechanism of a ketogenic diet: purinergic autocrine regulation of CA3 pyramidal neurons (Masahito Kawamura)
7) Visual response in the cerebellar paraflocculus (Misa Shimuta)

Research Activities

Synaptic transmission and its modulation in the basal ganglia and basal forebrain
Slice-patch-clamp electrophysiological studies were performed to analyze synaptic transmission, its modulation by neuromodulators, and their developmental change in the nigrostriatal or mesolimbic dopaminergic system and the cholinergic system of the basal forebrain. These systems are involved in various psychological functions and their disorders, including Parkinson disease and Alzheimer disease. The regulation of output from these systems to the cerebral cortex was also studied.
Another issue is the regeneration of synapses and local circuits after basal ganglia-related disorders. Electrophysiological, morphological, and behavioral studies were performed to clarify the mechanisms and time course of the reconstruction of synaptic organization and transmission and the functions of whole animals in Parkinson disease model rats and cerebral ischemia model rats. In addition, the role of the phosphatidylinositol system in basal ganglia synaptic transmission was analyzed.
These basic analyses could lead to the identification of the mechanisms underlying the related disorders mentioned above and to the development of novel therapeutic tools.

Respiratory neural activities in Xenopus
Aquatic pipid frogs have several 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 the 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 the cranial nerves V and X, the hypoglossal nerve, and the third spinal nerve innervating the "diaphragm." The buccal ventilation-like activity occurred in the 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 31 was secreted from human embryonic kidney cells when the protein was obligatorily expressed in cells transfected with a mammalian expression plasmid with the 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, N-terminal sequences of interleukin 31 from signal peptides to the first glycosylation site (SG sequences) could be crucial. Furthermore, we examined the fusion proteins of p53, which has nuclear localization signal sequences with SG sequences, and aquaporine, which is a membrane protein with SG sequences. We were able to design some secretory proteins associated with SG sequences.

Analysis of the mechanisms underlying histamine release from human-derived mast cells

To further study the function of extracellular purines and their purinergic receptors and the effects they may have on type I allergies, LAD2, a human mast cell-derived cell line, was introduced and successfully cultured in our laboratory. LAD2 cells were used in the following types of experiments: studies of cell activation by definitive antibodies and antigens, detection of messenger RNA for purinergic receptors, pharmacological assays of the effects of extracellular purines on both mast cell products (histamine and beta-hexosaminidase) and intracellular Ca^{2+} mobilization, and RNA interference studies of knockdown procedures of intracellular proteins using originally constructed short hairpin RNA plasmids.

The results of these experiments suggest that activation of the FcεRI (allergic stimulation) in LAD2 cells followed by increases in intracellular Ca^{2+} is enhanced by activation of phosphoinositide 3 kinase. This finding suggests a role for purinergic receptors in allergic stimulation in LAD2 cells.

High-frequency firing of cerebellar mossy fibers

Somatosensory signals from the facial area of rodents are delivered to the cerebellum via pontocerebellar and trigeminocerebellar pathways. Projection fibers of these pathways form mossy fibers, which terminate in the granule cell layer of the cerebellar cortex. It has been previously reported that somatosensory stimulation to the whiskers and the perioral skin triggers burst firings of the mossy fibers and that the instantaneous frequency of such burst action potentials can exceed 700 Hz. What is not known, however, is which of the cerebellar afferent pathways conducts such high-frequency firings. Therefore, we investigated firing properties of projection neurons of the pontine nuclei (PN) and the tri-

geminal nuclei (TrgN) in acute slice preparations. During depolarizing pulses, PN neurons did not fire the high-frequency action potentials but showed regular firings with moderate accommodation. The maximum instantaneous frequency of individual PN cells did not exceed 700 Hz. In contrast, TrgN neurons showed burst firing, which had a high instantaneous frequency exceeding 700 Hz in a subset of cells. These results suggest that the high-frequency firings of cerebellar mossy fibers are direct signals from the TrgN but not from the PN.

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

A ketogenic (low-carbohydrate high-fat) diet has been used successfully 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 concentration in the central nervous system and causes 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. Under 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 A_1 receptors. This autocrine regulation might be an important mechanism underlying the success of a ketogenic diet.

Visual response in the cerebellar paraflocculus

Our previous studies have revealed that the cerebellar paraflocculus receives remarkable visual signals and that around 80% of the granule cells in this area respond to visual stimuli. However, responses of the Purkinje cells in this area have not been investigated. Thus, we made recordings from the Purkinje cells of anesthetized rats. The results indicate that visual stimuli trigger a change in the frequency of "simple spikes" of Purkinje cells. We are now investigating what kind of visual stimulation can evoke "complex spikes" of Purkinje cells.

Publications

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