Department of Pharmacology

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General Summary

The research interests of the Department of Pharmacology include the following.
1. Synaptic transmission and its modulation in the basal ganglia and basal forebrain (Toshihiko Momiyama)
2. Neural control of breathing in aquatic vertebrates (Naofumi Kimura)
3. Peripheral benzodiazepine receptors on adrenal cells (Yuji Ohno)
4. Purinergic receptor-linked up-regulation of melatonin synthesis-related enzymes in mast cells (Haruhisa Nishi)
5. Analysis with optogenetics of cerebrocerebellar interaction (Taro Ishikawa and Misa Shimuta)
6. Mild hypothermia-mediated neuroprotection for experimental ischemia through adenosine receptors (Masahito Kawamura)
7. Nanoscale distribution of synaptic vesicles at a central presynaptic terminal (Yukihiro Nakamura)
8. Cholinergic modulation of central synaptic transmission (Etsuko Suzuki)

Research Activities

Synaptic transmission and its modulation in the basal ganglia and basal forebrain
Electrophysiological studies with slice patch-clamp recording techniques were performed to analyze synaptic transmission and its modulation, mediated by dopamine, serotonin, and muscarine receptors, in the nigrostriatal or mesolimbic dopaminergic system and in the cholinergic system of the basal forebrain. Developmental changes in the modulation are also under investigation. These systems are involved in various psychological functions and in their disorders, including Parkinson's disease and Alzheimer's disease. Furthermore, optogenetic activation techniques for neurons in these brain areas have been introduced to analyze neuron type-specific synaptic transmission and its modulation. These basic analyses can lead to the identification of the mechanisms underlying the related disorders mentioned above and to the development of novel therapeutic tools.

Neural control of breathing in aquatic vertebrates
The neural respiratory output of the isolated brainstem of *Xenopus laevis* displayed 2 motor patterns: the lung ventilation-like large bursts and the functionally unidentified small bursts. The lung ventilation-like bursts were abolished by bath application of the low concentration (0.1 μM) of the μ-opioid receptor agonist DAMGO ([D-Ala(2), N-Me-Phe(4), Gly(5)-ol]-enkephalin) and restored by 1 to 5 μM naloxone. In contrast,

the small bursts were resistant to the low concentration of DAMGO. The small bursts might have a common origin with the buccal rhythm of terrestrial frogs.

Peripheral benzodiazepine receptors on adrenal cells

Peripheral benzodiazepine receptors localize in the outer mitochondrial membrane, transfer cholesterol in steroidogenic organs under physiological conditions, and are readily upregulated under various pathological conditions, such as cancer, inflammation, and neurological disease. We would like to investigate whether endozepine and its metabolite, which we prepared from bovine adrenocortical cells, are related to these pathological conditions.

Purinergic receptor-linked up-regulation of melatonin synthesis-related enzymes in mast cells

Recent investigations of cells involved in human immune system regulation have suggested that mast cells play important roles in maintaining homeostasis. That mast cells are unique in their ability to release melatonin and that melatonin has beneficial antioxidative effects are not widely recognized. Furthermore, the detailed mechanism of melatonin production in mast cells is unclear.

The present study focused on mast cell melatonin synthesis because of its roll in immune responses. Cells of LAD2, a human-derived mast cell line, were analyzed for their expression of both messenger (m) RNA and proteins for the 2 key enzymes of melatonin synthesis: aralkylamine N-acetyltransferase and hydroxyindole O-methyltransfase. The intracellular cascades involved in enzyme synthesis were also examined.

The LAD2 cells were positive for the mRNA expression of both enzymes. The mRNA levels were enhanced by stimulation of the G-protein coupled $P2Y_{11}$ receptor with no degranulation; in contrast, stimulation of the $P2X_7$ receptor (ligand-gated ion channel) did not enhance mRNA levels but did induce degranulation.

These results suggest that melatonin release from mast cells is involved in maintaining homeostasis and is not involved in allergic responses.

Analysis of the cerebrocerebellar interaction using optogenetics

Cerebrocerebellar communication is important in a wide range of brain functions, including sensory information processing. We investigated the somatosensory-signaling pathways to the cerebellar cortex, using transgenic mice whose cerebral cortex can be suppressed by light illumination, and revealed that direct signals from the trigeminal nucleus and indirect signals via the somatosensory cortex are integrated in Purkinje cells and in granule cells in the cerebellar cortex. Recently, we have also found that the primary somatosensory cortex is linked to both the climbing fibers and the mossy fibers projecting to the cerebellar cortex.

Mild hypothermia-mediated neuroprotection for experimental ischemia through adenosine receptors

The therapeutic hypothermia for acute stroke might play an important role in neuroprotection; however, the key mechanism of this therapy is still undetermined. We examined

the role of adenosine in hypothermia-induced neuroprotection by using extracellular and patch clamp recordings. Mild hypothermia (32°C) causes protection for ischemia-induced loss of synaptic transmission through activation of adenosine A_1 receptors, but deep hypothermia (28°C)-induced neuroprotection is not caused by adenosine receptors. This study might reveal the involvement of adenosine in the therapeutic hypothermia (usually 32°C-33°C) for acute stroke.

Nanoscale distribution of synaptic vesicles at a central presynaptic terminal

The probability and timing of neurotransmitter release are determined by the coupling distance between voltage-gated calcium channels and synaptic vesicles. To investigate the spatial distribution of ready-releasable vesicles, we performed simultaneous patch-clamp recording from the calyx of Held and postsynaptic medial nuclei of trapezoid body neurons in acute brainstem slices of mice. Excitatory postsynaptic currents were evoked by strong depolarization and were then analyzed with deconvolution. The comparison of this result with simulations of vesicular release reveals that synaptic vesicles are widely distributed over 10 to 25 nm but are most concentrated around 50 nm from calcium channels in the active zone.

Cholinergic modulation of central synaptic transmission

Acetylcholine is a neurotransmitter involved in learning and memory. In the central nervous system, acetylcholine has been shown by several studies to modulate synaptic transmission and the firing properties of neurons. We elucidated the cholinergic modulation in striatum using an electrophysiological technique. In the striatum, we have found that GABA release from striatal medium spiny neurons onto cholinergic interneurons is inhibited by the activation of presynaptic intracellular muscarinic M1 receptors.

Publications

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