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. Neural control of breathing in aquatic vertebrates (Naofumi Kimura)
3. Intracellular functions of endozepine (Yuji Ohno)
4. Study of purinergic receptors in human-derived mast cells (Haruhisa Nishi)
5. Significance of cerebellar sagittal zones in cerebrocerebellar communication (Taro Ishikawa, Misa Shimuta)
6. The basic mechanism underlying the anticonvulsant effects of a ketogenic diet (Masahito Kawamura)

Research Activities

Synaptic transmission and its modulation in the basal ganglia and basal forebrain
Electrophysiological studies using slice patch-clamp recording techniques were performed to analyze synaptic transmission and its modulation by neuromodulators, such as dopamine and serotonin, and their developmental changes in the nigrostriatal or mesolimbic dopaminergic system and in the cholinergic system of the basal forebrain. These systems are involved in various psychological functions as well as their disorders, including Parkinson's disease and Alzheimer's disease. Electrochemical analyses were also performed with a new biosensor material, carbon nanotube, to elucidate the mechanisms of catecholamine release in the midbrain. Furthermore, optogenetic activation techniques for neurones in these brain areas are being introduced to analyze local neural circuits.
Another issue is the regeneration of synapses and local circuits after basal ganglia-related disorders. Electrophysiological, morphological, and behavioral studies were performed to elucidate the mechanisms and time course of the reconstruction of synaptic organization and transmission and the functions of whole animals in Parkinson's disease model rats. In addition, the function of physiologically released dopamine has been analyzed in the regulation of synaptic transmission as well as in behavior, using dopamine receptor knockout mice.
These basic analyses could lead to the identification of the mechanisms underlying the related disorders mentioned above, as well as to the development of novel therapeutic tools.

Neural control of breathing in aquatic vertebrates

Yawning in mammals has been considered a respiration-related behavior because of the accompanying long-lasting inspiration and brief expiration. However, aquatic turtles, amphibians, and air-breathing fishes with lungs, unlike mammals, never open their glottis during yawning. Sharks, which lack lungs, also show yawning-like behavior. "Yawning" in the sharks is characterized by their stretching their jaws (considered to be derived from the first gill arch) and the remaining gill arches. An act similar to yawning in sharks was examined in more primitive jawless fish, the lamprey. Lampreys, when they stopped sucking the wall of the tank, occasionally stretched their branchial arches and the rostral part of their bodies. "Yawning" in vertebrates may be redefined as a stretching movement of the branchial arches or of derived structures (such as the jaw and pharyngolarynx).

Intracellular functions of endozepine

In the central nervous system, endozepine is known as an endogenous anxiogenic peptide that can suppress GABA binding to $GABA_A$ receptor through the association of the peptide with a benzodiazepine receptor. In addition to this extracellular or intercellular function, we have suggested, in bovine adrenocortical cells, that the peptide could promote steroidogenesis through intramitochondrial cholesterol transport. To obtain the protein, we extracted the messenger RNA from bovine adrenocortical cells and the complementary DNA of endozepine was amplified with the polymerase chain reaction to insert in expression vectors of *Escherichia coli*. However, the peptide expressed by *E. coli* could not exert sufficient function. In view of posttranslational modification including glycosylation, it should be expressed by mammalian cells, such as HEK 293. After the isolation and purification of endozepine, we would like to examine whether the protein could enhance steroidogenesis in adrenocortical mitochondria.

Study of purinergic receptors in human-derived mast cells

The role of purinergic receptors in mast cell degranulation was investigated in leukocyte adhesion deficiency 2 cells, a human-derived mast cell line. Particular attention was focused on the purinergic receptors for $P2Y_{11}$ and $P2Y_{14}$ and their properties. Our findings are as follows:

1. Stimulation of mast cells by adenosine 5′-O-(3-thio)triphosphate (ATPγS), a $P2Y_{11}$-selective agonist, induced both intracellular Ca^{2+} mobilization and phosphorylation of phosphatidylinositol 3-kinase (PI3K) and Akt. Stimulation of ATPγS also resulted in synergistic enhancement of allergic degranulation following stimulation of Fc epsilon receptor I, a high-affinity immunoglobulin E receptor, while the agonist did not induce degranulation by itself.
2. Uridine diphosphate-glucose, a $P2Y_{14}$-selective agonist, did not induce Ca^{2+} mobilization, and neither PI3K nor Akt phosphorylation was observed. However, the agonist did induce degranulation without any allergic stimulation of the cells.

These results show that mast cells express multiple purinergic receptor subtypes linked to degranulation via different intracellular pathways. These findings show the feasibility of targeting distinct purinergic receptors on mast cells as part of therapeutic strategies to reduce allergic symptoms.

This study was presented at the 88th Annual Meeting of the Pharmacological Society (Nagoya, March 2015).

Significance of cerebellar sagittal zones in the cerebrocerebellar communication
The cerebellar cortex receives descending signals from the cerebral cortex as well as sensory signals from the periphery. However, how these 2 pathways of inputs are integrated and the role of such interaction are poorly understood. Moreover, it is unclear if input pathways are different for individual sagittal zones, which are recognized by the expression of a glycolytic enzyme aldolase C, in the cerebellar cortex. To address these issues, we used knock-in mice that express Venus fluorescent protein in aldolase C-positive cells (provided by Professor Izumi Sugihara, Tokyo Medical and Dental University). After visually indentifying the sagittal zones in these mice, we simultaneously recorded field potentials from the cerebellar granule cell layer in crus II and the cerebral somatosensory cortex in anesthetized mice. We found that the cerebellar response has biphasic peaks and that the first and second peaks corresponded to the direct pathway from the peripheral and the indirect pathway from the cerebral cortex, respectively. We also found that the direct signal was strongest in the aldolase 5− band and that that the ratio of the indirect signal was highest in the most lateral 7+ band. Furthermore, we made whole-cell patch-clamp recordings from individual granule cells in vivo and found that some granule cells have biphasic excitatory postsynaptic currents, indicating that mossy fiber inputs from 2 pathways converge onto single granule cells. On the basis of these findings, we are conducting research to clarify the mechanism of cerebrocerebellar communication.

The basic mechanism underlying the anticonvulsant effects of a ketogenic diet
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. To elucidate these mechanisms, we used a complementary approach that included in vivo dietary treatment followed by the electrophysiological characterization of acute brain slices. We fed rats and mice a ketogenic diet or a control diet for 2 to 3 weeks, prepared acute hippocampal slices, and performed electrophysiological and pharmacological studies in the seizure-prone CA3 region of the hippocampus. Slices from animals fed a ketogenic diet showed reduced excitability, and seizure propensity depended on maintaining a reduced extracellular glucose level. This reduced excitability was not observed in rats and mice fed a control diet. The effects of the ketogenic diet could be reversed with blockers of adenosine A_1 receptors and were absent in slices obtained from mice lacking adenosine A_1 receptors fed a ketogenic diet. These results suggest that the reduction of neuronal activity through activation of adenosine A_1 receptors is a key mechanism underlying the anticonvulsant effects of a ketogenic diet.

Publications

Nakamura T[1], Sato A[2], Kitsukawa T[1], Momiyama T, Yamamori T[1], Sasaoka T[2] ([1]Natl Inst Basic Biol, [2]Kitasato Univ). Distinct motor inpairments of dopamine D1 and D2 receptor knockout

mice revealed by three types of motor behavior. *Front Integr Neurosci*. 2014; **8:** 56.
Kawamura M Jr, Ruskin DN[1], Geiger JD[2], Boison D[3], Masino SA[1] ([1]Trinity Coll, [2]Univ North Dakota, [3]Legacy Res Inst). Ketogenic diet sensitizes glucose control of hippocampal excitability. *J Lipid Res*. 2014; **55:** 2254–60.

Reviews and Books

Masino SA[1], Kawamura M Jr, Ruskin DN[1] ([1]Trinity Coll). Adenosine receptors and epilepsy: current evidence and future potential. *Int Rev Neurobiol*. 2014; **119:** 233–55.