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) Design of secretory proteins (Yuji Ohno)
4) i) Study of the mechanisms involved in the modification of allergic degranulation via purinergic receptors in a cell line derived from human mast cells (Haruhisa Nishi)
ii) Study of glucocrticoid production by activation of purinergic receptors in a human-derived adrenocortical cell line (Haruhisa Nishi)
5) Firing patters of pontine neurons in cerebrocerebellar interaction (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
Electrophysiological studies using slice patch-clamp recording were performed to analyze synaptic transmission, its modulation by neuromodulators, and their developmental changes in the nigrostriatal and mesolimbic dopaminergic systems and the cholinergic system of the basal forebrain. These systems are involved in various psychological functions and their disorders, including Parkinson's disease and Alzheimer's disease. Electrochemical analyses with carbon nanotube, a new biosensor material, have also been performed to clarify the mechanisms of catecholamine release in the midbrain.
Another issue is the regeneration of synapses and local circuits after basal-ganglia-related disorders. In these studies, electrophysiological, morphological, and behavioural studies were performed to clarify the mechanisms and time course of the reconstruction of synaptic organization, transmission, and functions of whole animals in Parkinson's disease model rats or 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.

Neural control of breathing in aquatic vertebrates
Yawning is a common behavioral event in almost all vertebrates. In this study a video

camera was used to investigate yawning in amniotes and nonamniotes. Yawning in turtles (which originated later than in mammals) and in vertebrates more basal than mammals (amphibians, lungfish, ray-finned fishes, and sharks) consisted of maximal opening of the mouth and lowering of the oropharyngeal (buccal) floor. Interestingly, aquatic turtles, amphibians, and air-breathing fishes (lungfish, gar, and polypterus) never open their glottis during yawning, unlike mammals. These observations indicate that yawning is a common behavior in jawed vertebrates and is more primitive than lung ventilation. Opening of the glottis would be a characteristic for yawning in some specific vertebrates, such as mammals. A possible reason mammals open their glottis during yawning is that the ancestral mammalian used part of the nerve activity that lowers the buccal floor to drive the diaphragm in the evolutionary process.

Design of secretory proteins

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

Study of the mechanisms of the modification of allergic degranulation via purinergic receptors in a cell line derived from human mast cells

The aims of the present study were to investigate the function and intracellular mechanisms of purinergic receptors in LAD2 human-derived mast cells and to determine the possibility of controlling degranulation via purinergic systems. The findings suggest that purinergic receptors modulate the intracellular enzymatic cascade between phosphatidylinositol-3 kinase and Akt, a Ser/Thr kinase, and have some effects on kinase activity downstream of EcεRI activation leading to enhancement or inhibition of FceRI-induced allergic degranulation. The findings also suggest the possibility that controlling purinergic systems on mast cells is a new therapeutic approach for type I allergy.

Study of glucocrticoid production by activation of purinergic receptors in human-derived adrenocortical cell line

The human adrenocortical cell line H295R was used to investigate the function of purinergic systems in human adrenocortical steroidgenesis. The results suggest that H295R cells express multiple and functional purinergic receptors for intracellular Ca^{2+}-mobilization and that the $P2Y_1$ subtype of the purinergic receptor is linked to the store-operated Ca^{2+} entry activation, leading to Ca^{2+}-influx which might be necessary for glucocorticoid production. On the other hand, some purinergic receptors expressed on H295R cells were found to be linked to production of cyclic adenosine monophosphate. The present

findings suggest that some functional purinergic systems and the crosstalk of intracellular second messengers for steroidogenesis are present in human adrenal cortex.

Firing patterns of pontine neurons in cerebrocerebellar interaction

Somatosensory signals from the cerebral cortex of rodents are delivered to the cerebellum via the pontocerebellar pathway. Somatosensory stimulation to the whiskers and the perioral skin triggers high-frequency burst firing of the projecting mossy fibers. However, the cellular mechanisms that generate this high-frequency firing are not known. Therefore, we investigated firing properties and synaptic currents in projection neurons in the pontine nuclei in acute slice preparations and in vivo in anaesthetized animals. The results indicate that the pontine nuclei neurons can fire at a high frequency by depolarization but that low-frequency synaptic inputs do not trigger firing. We are investigating the firing properties of pontine nuclei neurons in further detail.

The basic mechanism of a ketogenic diet: 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 increases ATP concentrations 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 from 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 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 showed that the cerebellar paraflocculus receives visual signals and that most granule cells in this area respond to visual stimuli. However, responses of Purkinje cells in this area have not been investigated. Thus, we recorded from the Purkinje cells of anesthetized rats. The results indicated that the visual stimuli trigger a change in the frequency of "simple spikes" of the Purkinje cells but do not trigger "complex spikes." We are now exploring stimulation methods that can evoke simple and complex spikes independently by using direct electrical stimulation of the cerebral cortex.

Publications

Mitsumori T*[1]*, Furuyashiki T*[1]*, Momiyama T, Nishi A*[2]*, Shuto T*[2]*, Hayakawa T*[1]*, Ushikubi F*[3]*, Kitaoka S*[1]*, Aoki T*[1]*, Inoue H*[1]*, Matsuoka T*[1]*, Narumiya S*[1] *(*[1]Kyoto Univ,*** [2]***Kurume Univ,*** [3]***Asahikawa Med Coll).*** Thromboxane receptor activation enhances striatal dopamine release, leading to suppression of GABAergic transmission and enhanced sugar intake. *Eur J Neurosci.* 2011; **34:** 594-604.

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Reviews and Books

Masino SA*[1]*, Svedova J*[1]*, Kawamura M Jr., Dimario FJ Jr.*[2]*, Eigsti IM*[3] *(*[1]*Trinity Coll,* [2]*UConn Health Center,* [3]*UConn). Adenosine and Autism—Recent Research and a New Perspective. In: Eapen V, editor. Autism—a neurodevelopmental journal from genes to behaviour. Rijeka: InTech; 2011. p. 103–22.