Laboratory of Pharmacology

Professor: Shigeo Ito
Associate Professor: Toshio Ohta

We teach veterinary pharmacology including lecture and laboratory on pharmacological science. The goal of research works in our laboratory is to understand signal transduction mechanisms involved in catecholamine secretion, muscle contraction and chemoreceptor activities. Experiments are performed using whole animals and various isolated preparations such as perfused adrenal glands and smooth muscle segments, single skeletal muscle fibers, isolated smooth muscle cells and cultured adrenal chromaffin cells and rat PC12 cells. The experimental techniques we use are chemical assays of neurotransmitters, hormones and polypeptides, tension recordings of small bundles of intact smooth muscles and single skeletal muscle fibers, simultaneous measurements of contraction and intracellular Ca^{2+} concentration, simultaneous measurements of released catecholamine and ATP, measurements of ionic concentrations in a single cell, and measurements of ionic currents and membrane potentials using patch-clamp techniques.

The projects currently underway are as follows:

1. Mechanism of adrenal catecholamine secretion induced by muscarinic and nicotinic receptor activation: We found that muscarinic receptor activation caused the release of Ca^{2+} from intracellular Ca^{2+} stores through inositol 1,4,5-trisphosphate production and produced hyperpolarization followed by depolarization. The hyperpolarization was elicited by activation of Ca^{2+} dependent K^+ currents, the depolarization by activation of non-selective cation channels. Catecholamine secretion induced by nicotinic receptor activation exclusively depended on depolarization-induced Ca^{2+} entry, while that induced by muscarinic receptor activation both depolarization-induced Ca^{2+} entry and inositol 1, 4, 5-trisphosphate-induced Ca^{2+} release from Ca^{2+} stores.

2. Interaction between releases of adrenocortical and adrenomedullary hormones: In this project, we examined the effect of cortisol and dexamethasone on catecholamine secretion from perfused adrenal gland of the guinea-pig. These steroids inhibited catecholamine secretion induced by nicotine but not excess KCl and greatly inhibited nicotine-induced inward currents without any effects of voltage-dependent Na^+ and Ca^{2+} currents. In order to know interaction between the cortex and medulla of the adrenal gland, we now try to examine the effect of endogenous cortisol released by ACTH on catecholamine secretion evoked by various secretagogues and splanchinic nerve stimulation.

3. Diversity of voltage-dependent Ca^{2+} channels in adrenal chromaffin cells: The aim of this project is to identify the subtypes of voltage-dependent Ca^{2+} channels in chromaffin cells and to clarify the mechanisms of channel modulation in porcine and guinea-pig adrenal chromaffin cells. We showed that N-, L- and P / Q-types of Ca^{2+} channels are present in porcine adrenal chromaffin cells and that N-and L-type channels mainly contribute to increases in intracellular Ca^{2+} concentration and catecholamine secretion induced by excess KCl. The N-type channel is under inhibitory control by G-proteins which is relieved by depolarization.

4. Simultaneous releases of catecholamine and ATP from adrenal chromaffin cells and PC12 cells: In cultured porcine adrenal chromaffin cells, ACh, excess KCl and $BaCl_2$ caused simultaneous releases of catecholamine and ATP, indicating that the molar ratio of catecholamine to ATP is constant in releasable secretory vesicles. The studies of effects of catecholamine reuptake inhibitors on the molar ratio in vesicles is in progress.

5. Capacitative Ca^{2+} entry in smooth muscle cells : A rise in cytosolic Ca^{2+} is essential for evoking smooth muscle contraction. Voltage-dependent Ca^{2+} channels and receptor-linked Ca^{2+} channels play a key role in smooth muscle contraction. Recently, we reported the presence of the third Ca^{2+} entry pathway in smooth muscles, called capacitative Ca^{2+} entry, which is activated by the depletion of Ca^{2+} in intracellular stores. The involvement of tyrosine kinase in the activation of this Ca^{2+} entry pathway and pharmacological properties of the pathway are investigating.

6. Chemoreceptor function of the aortic body : This research project is to examine the properties of epithelioid cells containing 5-hydroxytrypramine (5-HT) in the chicken thoracic aorta. We recently found that the epithelioid cells in the chicken aorta are chemoreceptors which sense a decrease in Po_2 and then release 5-HT through activation of L- and N-types of voltage-dependent Ca^{2+} channels. Now, the epithelioid cell is subjected to the whole cell voltage-clamp to examine channels responsible for chemoreceptor function.

References

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