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Laboratory of Pharmacology

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The teaching staff consisits of a professor and two associate professors shown above. We teach the veterinary pharmacology including lecture and laboratory on pharmacological science. The goal of the research works in our laboratory is to understand the signal transduction mechanisms involved in catecholamine secretion, muscle contraction and chemoreceptor activities. Experiments are performed using whole animals and various isolated preparations such as isolated adrenal glands and smooth muscle segments, single skeletal muscle fibers, isolated smooth muscle cells and cultured adrenal chromaffin cells. The techniques employed to accomplish the experiments are chemical assays of neurotransmitters, hormones and polypeptides, tension recording of small bundles of intact smooth muscles and single skeletal muscle fibers, simultaneous measurement of contraction and intracellular Ca^{2+} concentration, simultaneous measurement of released catecholamine and ATP, measurement of the ionic concentration in a single cell, and measurement of ionic currents and membrane potential using patch-clamp technique.

The projects currently underway in our laboratory are as follows:

1. Mechanism of adrenal catecholamine secretion induced by muscarinic receptor activation. This research project started since we first found that muscarinic and nicotinic receptor activation stimulated catecholamine release through different mechanisms with respect to both voltage-dependence and/or Ca^{2+} requirements in perfused adrenal glands of cat (1984) (Fig. 1) and guinea pig (1988). Subsequent works showed that adrenal chromaffin cells possess intracellular Ca^{2+} stores from which muscarinic receptor activation causes release of Ca^{2+} resulting in catecholamine secretion. Catecholamine secretion induced by nicotinic receptor activation exclusively depended on depolarization-induced Ca^{2+} entry, while that by muscarinic receptor activation on both depolarization-induced Ca^{2+} entry and Ca^{2+} mobilization from inositol 1, 4, 5-trisphosphate-sensitive intracellular stores.

2. Capacitative Ca^{2+} entry in smooth muscle cells. A rise in cytosolic Ca^{2+} is essential for evoking smooth muscle contraction, proliferation and differentiation. Plasmalemmal voltage-dependent Ca^{2+} channels and receptor-linked Ca^{2+} channels have been known to play a key role as the Ca^{2+} entry pathway in smooth muscle cells. Recently, we have reported the third Ca^{2+} entry mechanism, called "Capacitative Ca^{2+} entry" which is activated by the depletion of Ca^{2+} in intracellular stores by Ca^{2+}-releasing agonists or SR-ATPase inhibitors in smooth muscle cells. The studies on the involvement of protein tyrosine kinase in the intracellular signaling for the activation of this Ca^{2+} entry mechanism and its pharmacological properites are now in progress.

3. Effect of ATP on membrane properties and its receptor classification. In this project, the effect of extracellular ATP (adenosine 5'-triphosphate) on membrane properties and its receptor classification were studied in guinea pig adrenal chromaffin cells and rat gastric circular muscle. The results obtained were that ATP activates non-selective cation channels, and Ca^{2+} entrered through these channels causes the inactivation of voltage-dependent Ca^{2+} channels, and that ATP modulates Ca^{2+} channels via the pathway related to G-proteins. In rat gastric circular muscle, at least three subtypes of purinoceptor mediating relaxation and contraction are present. It was also demonstrated that some purinoceptors and pyrimidinoceptors are located on either the chromaffin cell or the smooth

muscle cell. Thus, ATP and its related compounds may play a role as a transmitter in these tissues.

4. 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 present in chromaffin cells and to clarify the mechanisms modulating the properties of these channels. We showed that N-, L- and P/Q-type Ca^{2+} channels are present in porcine adrenal chromaffin cells and N- and L-type channels mainly contribute to increase in intracellular Ca^{2+} concentration and catecholamine secretion induced by high-K^+ stimulation. N-type channels are under inhibitory control by G-proteins which is relieved by depolarization. This mechanism is involved in depolarization-induced facilitation of Ca^{2+} channel currents in chromaffin cells.

5. Chemoreceptor function. This research project is concerned with the properties of epithelioid cells containing 5-hydroxytryptamine (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 in response to Ca^{2+} infux through L- and N-types of voltage-dependent Ca^{2+} channels. Now, the epithelioid cell is subjected to the whole cell voltage-clamp to examine Ca^{2+} channels responsible for chemoreceptor function.

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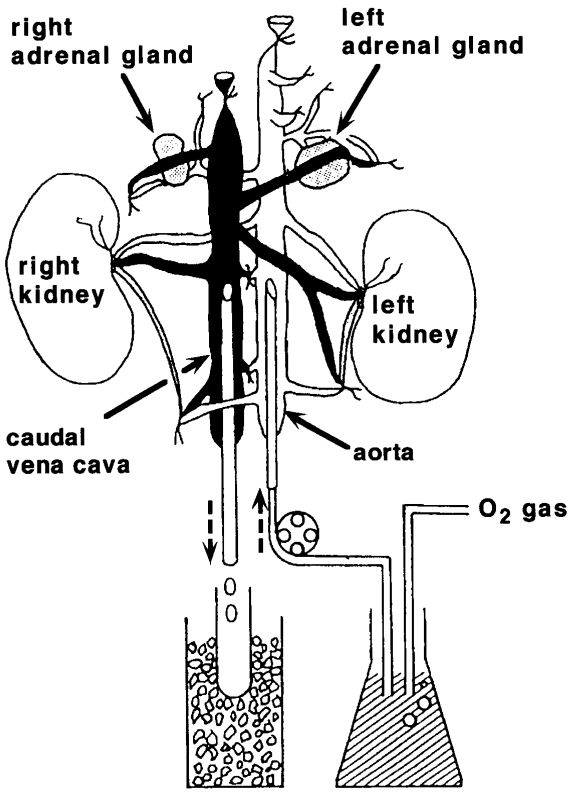

Fig. 1. Schematic illustration of perfusion of cat adrenal glands. Both adrenal glands were perfused with Locke's solution at a flow rate of 0.4 to 0.5 ml/min by a peristaltic pump. The perfusion fluids were infused into the lower aorta through a polyethylene cannula and were collected from polyethylene cannula inserted in the caudal vena cava.

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