Department of Pharmacology (I)

Masahiro Kawamura, *Professor*
Noboru Nakamichi, *Lecturer*
Seiji Hori, *Professor*
Yuji Ohno, *Lecturer*

General Summary

Our main interests are the physiological roles of nucleotide receptors and the mechanisms of regulation of intracellular Ca^{2+} concentrations in several kinds of cells, including bovine adrenocortical fasciculate cells (BAFCs), 3T3-L1 preadipocytes, Madin-Darby canine kidney (MDCK) cells, and brain astrocytes. We are also interested in the convulsant effect of theophylline, the nonantibacterial effects of quinolones, and the effect of the urocortin family on the cardiovascular system.

Research Activities

Cross-talk between ACTH receptors and ATP receptors in BAFCs

Extracellular ATP and uridine triphosphate (UTP) bind to P2 receptors to regulate several cell functions in many kinds of cell. P2 receptors are divided into 2 families: the ligand-gated P2X and the G-protein coupled P2Y. The P2Y family has 14 subfamilies. We have previously reported that BAFCs contain Gq-protein-coupled P2Y2. Both ATP and UTP bind to P2Y2 to stimulate Ca^{2+} influx via IP3 production from the extracellular space. ACTH is a physiological stimulator of adrenocortical steroidogenesis via cAMP production. We found that ATP and UTP potentiated both ACTH-induced steroidogenesis and cAMP production. Our findings suggested that the Ca^{2+} influx pathway or the Bγ subunit of Gq-protein (which links to P2Y2) or both are involved in the cross-talk between the ACTH receptor and P2Y2. Under our experimental conditions, the Ca^{2+} influx pathway was not involved in the event. Furthermore, agents that disturb the actin cytoskeleton did not affect the cross-talk. Therefore, movement of the Bγ subunit of Gq-protein might not participate in this cross-talk between the ACTH receptor and P2Y2 in cAMP production.

Study of the intracellular Ca^{2+} dynamics

Ca^{2+} is an important regulator in many cellular functions. Therefore, we studied intracellular Ca^{2+} dynamics and its physiological functions in BAFCs, 3T3-L1 preadipocytes, MDCK cells, and brain astrocytes through the use of samples loaded with a fluorescent calcium indicator.

1. Store-operated Ca^{2+} entry in BAFCs

Store-operated Ca^{2+} entry (SOCE), i.e., Ca^{2+} entry triggered by the depletion of intracellular Ca^{2+} stores, plays a physiological role in nonexcitable cells. Gq-protein-coupled receptor agonists stimulate IP3 production followed by Ca^{2+} release from the endoplasmic reticulum and depletion of luminal Ca^{2+}. Then Ca^{2+} enters from the extracellular space through the activation of SOCE. However, the precise mechanism of SOCE

remains obscure. Three hypotheses for SOCE activation have been proposed: the conformational coupling model, the secretion-like model, and the diffusible messenger model. However, in all cases, an SOCE channel in the plasma membrane is necessary for Ca^{2+} to enter from the extracellular space. One candidate for the SOCE channel is the transient receptor potential protein (TRP), especially, the TRPC subtype. The plasma membrane of BAFCs reportedly contains TRPC. Therefore we studied the possible involvement of TRPC in SOCE in BAFCs. Although SOCE is high-Ca^{2+}-selective, TRPC is not. Under our experimental conditions, the extracellular Ca^{2+} enters the cells after treatment with cyclopiazonic acid, a sarcoplasmic/endoplasmic reticulum Ca^{2+} (SERCA) pump inhibitor, to deplete its luminal Ca^{2+} but not Sr^{2+} or Ba^{2+}. The results suggest that TRPC is not the SOCE channel in BAFCs.

2. SOCE in 3T3-L1 preadipocytes

3T3-L1 preadipocytes differentiate to adipose cells under controlled culture conditions. Intracellular Ca^{2+} mobilization is believed to be an important factor in this process. Therefore, we studied the mechanism of Ca^{2+} influx in 3T3-L1 preadipocytes. We found that these cells have SOCE that is activated by prostaglandin (PG) F2α via PG receptors and thapsigargin, a SERCA pump inhibitor. The 3T3-L1 preadipocytes biosynthesize and secrete PGF2α. Thus, these results suggest the possible involvement of this SOCE-activation factor in the adipose differentiation of 3T3-L1 preadipocytes in an autocrine/paracrine fashion.

3. Intracellular Ca^{2+} dynamics in MDCK cells

MDCK cells are often used to study the mechanism of polycystic kidney formation, a process in which Ca^{2+} has been proposed to play an important role. However, intracellular Ca^{2+} dynamics in MDCK cells is not fully understood. We found that a calcium oscillation phenomenon occurs in MDCK cells either in the absence or presence of extracellular Ca^{2+}. The results suggest that the intracellularly stored Ca^{2+} plays a pivotal role in this phenomenon. The oscillation was inhibited by probenecid, but the mechanism of inhibition has not been identified.

4. Extracellular ATP-induced modulation of astrocytic calcium oscillations in rat hippocampal slice cultures

Activation of various P2 receptors increases the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of astrocytes in primary cultures. To analyze the role of extracellular ATP in astrocytic Ca^{2+} wave oscillations *in situ*, fluorometric measurements were made from rat hippocampal slice cultures. ATP induced a transient increase in $[Ca^{2+}]_i$, which was followed by an increase in the frequency of spontaneous Ca^{2+} oscillations. The increase in the frequency of spontaneous Ca^{2+} oscillations was mediated by the activation of adenosine receptors by adenosine, which was produced via extracellular breakdown of ATP. The transient increase in $[Ca^{2+}]_i$ was induced by the activation of either adenosine receptors or P2 receptors. These results suggest that extracellular ATP plays a dual role in astrocytic Ca^{2+} wave propagation through the activation of P2 receptors and adenosine receptors in the rat hippocampus.

Study of the non-antibacterial effects of qunolones

1. Convulsant activity of quinolones and their interaction with anti-inflammatory

drugs
Quinolones have potent convulsant activity, which is enhanced by concurrent administration of anti-inflammatory drugs. We studied the convulsant activity of respiratory quinolones, which have excellent antibacterial activities against organisms causing respiratory tract infections, and examined whether the convulsant activity is affected by anti-inflammatory drugs. Each respiratory quinolone had different convulsant potency. The order of potency was gatifloxacin＞levofloxacin＞moxifloxacin＝sparfloxacin≫tosufloxacin. The convulsant activity of these quinolones was not enhanced by concurrent administration of anti-inflammatory drugs.
2. Evaluation of intestinal absorption and drug interaction of quinolones *in vitro* (In collaboration with the Department of Practical Pharmacy, Kyouritsu University of Pharmacy)
In vivo studies have shown that the intestinal absorption of some quinolones is inhibited by the co-administration of divalent metal ions. We established the everted intestinal sac method to study in greater detail the mechanism of absorption of quinolones *in vitro*. Everted intestinal sacs were prepared from male Wistar rats. Norfloxacin and levofloxacin accumulated in the sac in a dose-dependent manner. Aluminum ion inhibited the absorption of the quinolones, whereas calcium and magnesium ions had no effect. These results suggest that each quinolone has a different manner of intestinal absorption and a different manner of drug interaction with metal ions.
3. Convulsant activity of theophylline and its metabolites
Subcutaneous injection of theophylline induces convulsions in a dose-dependent manner in mice. To examine the convulsant effects of thephylline and its metabolites, we administered these agents to the right lateral ventricles of mice. 1-Methylxanthine, a physiological metabolite of theophylline, showed strong convulsant activity, but theophylline itself had weak convulsant activity. These results suggest that 1-methylxanthine is the compound responsible for thophylline-induced convulsions.
4. Anti-inflammatory activity of theophylline (In collaboration with the Department of Practical Pharmacy, Kyoritsu University of Pharmacy)
Theophylline is considered to have anti-inflammatory activity as well as bronchodialatory activity. However, few reports have demonstrated the anti-inflammatory activity in animal models. We studied the effects of theophylline and its metabolites on carrageenan-induced edema in rat foot pad. Theophylline, but not its metabolites, reduced edema. The effect of theophylline was inhibited by pretreatment with mifepristone, a glucocorticoid receptor antagonist. These results suggest that the glucocorticoid receptor system is involved in the anti-inflammatory effects of theophylline.

Studies of the effects of cardiovascular regulatory substances on rat cardiomyocyte function
We have studied the effects of cardiovascular regulatory factors and agents on primary cultured neonatal rat cardiomyocytes. However, some factors and agents have little effect on isolated cardiomyocytes but have more prominent effects on cardiomyocytes in the diseased human heart or in in vivo animal models. In pathological conditions of the heart, the ratio of non-cardiac myocytes to cardiomyocytes increases. Therefore, the

cross-talk between cardiomyocytes and non-cardiac myocytes should be clarified. We prepared a cardiomyocyte/non-cardiac myocyte co-culture system as an experimental model of pathological conditions of the heart and evaluated the physiology of cardiomyocytes under such conditions. We showed that the secretory pattern of atrial and brain natriuretic peptides changed as the ratio of non-cardiac myocytes to cardiomyocytes increased. The results suggest that non-cardiac myocytes play an important role in heart disease. We also studied the involvement in cardiac disease of urocortin, a peptide related to cortiocotropin-releasing hormone, and other peptides of its family in HL-1 cardiomyocytes, a cardiomyocyte cell line from the mouse atrium.

Clinical studies of human blood rheology with the falling needle rheometer

The onset of ischemic cardiac disease is closely related to the viscosity of blood. A new type of rheometer, the falling needle rheometer, was developed jointly by the Department of Chemical Energy and Environmental Engineering, Kansai University, and the Department of Legal Medicine, Dokkyo Medical University. This new type of rheometer can quickly measure multiple apparent viscosity without the addition of anticoagulants. However, its clinical usefulness must be evaluated. Therefore, we studied the performance of the rheometer in adult volunteers. We demonstrated the blood fluidity analysis by multiple apparent viscosity and, in a few volunteers, the difference in fluidity with or without anticoagulant. The results show that blood coagulation ability is a factor in changes in blood fluidity.

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