Phospholipid Composition and Ca^{2+} Uptake Properties of Plasma Membrane Isolated from Canine Stomach Smooth Muscle

Takayoshi ITO and Yasushi SAKAI

2nd Department of Physiology Showa University, School of Medicine. 1-5 Hatanodai, Tokyo 142, Japan

Abstract

Ito, T. and Sakai, Y. *Phospholipid composition and Ca^{2+} uptake properties of plasma membrane isolated from canine stomach smooth muscle.* Japanese Journal of Smooth Muscle Research, 22(1), 11-19. — The Ca^{2+} uptake and phospholipids influencing it in plasma membrane isolated from canine stomach smooth muscle was investigated. The major phospholipids in plasma membrane were phosphatidylethanolamine (PE, 26%), phosphatidylcholine (PC, 20%) and phosphatidylserine+phosphatidylinositol (PS+PI, 15%). The cholesterol/phospholipid ratio in the membrane was 0.64. ATP-dependent Ca^{2+} uptake was inhibited by exogenous PE, PC and PS, but increased by PI. Saponin and phospholipase C inhibited ATP-dependent Ca^{2+} uptake and accelerated release of Ca^{2+} from actively loaded plasma membrane vesicles. In the absence of ATP, PE slightly and PI significantly increased Ca^{2+} uptake, but PC and PS did not affect the Ca^{2+} uptake. Release of Ca^{2+} from actively loaded vesicles was 5 times greater in the presence of PI than in its absence. Results suggest PI to be the most active of the tested phospholipids in influencing Ca^{2+} movement across plasma membrane.

Introduction

Investigation of Ca^{2+} movement across the isolated plasma membrane is the most direct approach to determine the capability of smooth muscle membrane to regulate intracellular Ca^{2+} and thus control muscle function. We have previously reported that plasma membrane isolated from canine gastric corpus smooth muscle can control intracellular Ca^{2+} (Sakai *et al.*, 1981) and determined several of the factors that contribute to this control (Sakai *et al.*, 1983, Yasuda and Sakai, 1984). Phospholipids are important in physiological functions of the cell (Coleman, 1973, Cossins and Prosser, 1978), and interrelate structurally with proteins and other membrane components. It has been reported that the activity of adenylate cyclase in plasma membrane of mammalian cells depends on its interactions with anionic lipids, particularly phosphatidylinositol (PI) and phosphatidylserine (PS) (Birnbaumer, 1973; Levey, 1972). Hauser and Dawson (1968) found that the displacement of bound Ca^{2+} by alkali metal ions or

Accepted for publication, February 5, 1986.

伊藤隆義, 坂井 泰: 昭和大学医学部 第二生理学教室, 〒142 東京都品川区旗の台 1-5

some local anesthetics was affected by the molecular conformation of the lipid monolayers. In view of the different functions of the membrane that require specific phospholipids, the distribution of various phospholipids may be important in the control and integration of any vectorial process occurring in the membrane (Dominski *et al.*, 1983). It is therefore important to determine the composition of phospholipids in the membrane and to investigate the effects of excess of individual phospholipids. This latter procedure can be carried out by introducing various individual phospholipids exogenously. The present study was undertaken to investigate the role of phospholipids in the transport of Ca^{2+} by plasma membrane isolated from canine gastric corpus smooth muscle.

Materials and Methods

Membrane preparation

Plasma membranes were prepared from mongrel dogs (12–20 kg) by the method of Sakai *et al.* (1981) with slight modifications. After submucosa and longitudinal muscle were removed, the circular muscle was cut into small pieces, and finely minced. The tissue was homogenized 3 times for 5 sec each time in ice-cold sucrose-MOPS (250 mM sucrose and 20 mM morpholinopropane sulfonic acid-NaOH pH 7.0 1 g/10 ml) using a motor driven homogenizer (B. Braun Model ET-20), and briefly centrifuged at $1,000 \times g$ for 10 min to remove the cell debris and nuclei. The post-nuclear supernatant (PNS) was then centrifuged at $12,000 \times g$ for 10 min to sediment the mitochondrial fraction (MIT) and subsequently at $140,000 \times g$ for 60 min to separate the crude microsomal fraction. The crude microsomal fraction was then resuspended and centrifuged at $10,000 \times g$ again for 10 min to obtain the refined microsomal fraction as the supernatant. This was placed on top of a discontinuous sucrose density gradient (18%, 32%, 40%, 60%) and centrifuged at $112,000 \times g$ for 2 hr. The plasma membrane enriched fraction was obtained from the interphases of the sucrose layers. These fractions have been previously characterized (Sakai *et al.*, 1981).

Lipid extraction from isolated plasma membrane

The lipids were extracted from the suspended plasma membrane buffer with a 3-fold volume of chloroform/methanol (2:1, v/v) as described by Folch *et al.* (1968). The lipid extract was dissolved in chloroform, washed with 0.9% NaCl, and the upper layer was discarded. The lower layer was then evaporated under N_2 gas at 30–32°C. The lipids were separated by thin-layer chromatography using a silica-gel G plate (Merck, Darmstadt) and developed with petroleum ether/ether/acetic acid (80:30:1, v/v). Total phospholipids were visualized by exposure to iodine vapour. The spots were scraped from the plates and extracted three times with chloroform/methanol (2:1) and once with ether. The extract was evaporated under N_2 gas at 30–32°C then dissolved with chloroform again. Specific phospholipids were then separated by thin-layer chromatography using a silica-gel G plate and developed with chloroform/methanol/acetic acid/water (25:15:4:2, v/v). Phospholipids were visualized by exposure to iodine vapour. The spots were scraped from the plates and analyzed for phosphate content by the method of Bartlett (1959). Cholesterol content was measured by the method of Sperry and Webb (1950).

Ca²⁺ uptake measurement

Ca²⁺ accumulation by isolated subcellular membrane fractions was assayed using a filtration technique previously described (Yasuda and Sakai, 1984). A typical reaction mixture contained 250 mM sucrose, 40 mM imidazole-HCl, 5 mM Na₂ ATP, 5 mM MgCl₂ and 100 μ M CaCl₂ labelled with ⁴⁵CaCl₂ (0.2–1 μ Ci) at pH 7.0. This gave a calculated concentration of about 20 μ M free Ca²⁺ (Janis *et al.*, 1977). When Na₂ ATP was not used, only 20 μ M CaCl₂ was used. To study the effects of saponin and phospholipase C, these compounds were added to the Ca²⁺ reaction medium. The typical Ca²⁺ assay procedure was as follows: With constant shaking, 50 μ l of membrane fraction containing 20–50 μ g of protein was added to 200 μ l medium. After 10 min incubation at 37°C, the 200 μ l suspension was filtered on Millipore filters (TOYO TYPE TM-2 0.45 μ m pore size) which had been stored in 0.1 M KCl solution and washed just before the experiment with sucrose/IM solution. The filters were then washed twice with 5 ml of the sucrose/IM solution. Radioactivity on the filters and in the incubation medium was measured in Bray's solution (Bray, 1960). The radioactivity on the filters without membrane protein was subtracted. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Materials

Saponin was purchased from Wako Pure Chemical Industry. Na₂-ATP, MOPS and phosphatidylinositol were purchased from Sigma. Phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine were purchased from Tokyo Kasei Kogyo. ⁴⁵CaCl₂ was purchased from New England Nuclear. All other reagents were purchased in the purest form available from standard commercial sources.

Results*Phospholipid composition of plasma membrane*

The content of phospholipids and cholesterol in the membrane was determined in five

Table 1. Composition of phospholipids in the plasma membrane isolated from canine stomach

Phospholipid composition	% of total phospholipids
Phosphatidylethanolamin	26.1 $\pm$ 0.8
Phosphatidylcholine	19.5 $\pm$ 0.2
Phosphatidylserine + Phosphatidylinositol	15.2 $\pm$ 0.2
Sphingomyelin	13.4 $\pm$ 0.3
Lysophosphatidylcholine	13.9 $\pm$ 0.5
Unknown	11.8 $\pm$ 0.4

Data are mean $\pm$ SE in the five separate preparations.

Table 2. Effects of saponin and phospholipase C on Ca²⁺ uptake

Treatment	Ca ²⁺ uptake (μ mol/g protein/10 min)	
	ATP-dependent	ATP-independent
Control	42.7 $\pm$ 0.3	1.7 $\pm$ 0.1
Saponin		
25 μ g/ml	32.1 $\pm$ 0.5	1.6 $\pm$ 0.1
50 μ g/ml	19.4 $\pm$ 1.2	1.7 $\pm$ 0.1
Phospholipase C		
50 μ g/ml	45.1 $\pm$ 0.2	1.6 $\pm$ 0.1
100 μ g/ml	34.7 $\pm$ 0.7	1.7 $\pm$ 0.1

Plasma membrane were treated by saponin or phospholipase C for 10 min. Data are mean $\pm$ SE (n = 4).

separate preparations. The cholesterol/phospholipid ratio in the plasma membrane isolated from canine stomach smooth muscle was 0.64. The composition of phospholipids was as shown in Table 1. The phospholipids separated and identified were: phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) plus phosphatidylinositol (PI), sphingomyelin (SM), and lysophosphatidylcholine (LPC). The major phospholipid was PE. PS and PI could not be clearly separated by the method used, so we show the content of these two phospholipids together in Table 1. The LPC content was high compared to that in membrane from other tissue.

Effect of saponin and phospholipase C on Ca^{2+} uptake

Table 2 shows the effects of saponin and phospholipase C on Ca^{2+} uptake by plasma membrane isolated from canine stomach smooth muscle. Saponin inhibited ATP-dependent Ca^{2+} uptake dose dependently. 50 $\mu\text{g}/\text{ml}$ of phospholipase C increased ATP-dependent Ca^{2+} uptake slightly but significantly. Higher doses of phospholipase C inhibited ATP-dependent Ca^{2+} uptake. ATP-independent Ca^{2+} uptake was not affected by these substances after 10 min treatment.

Release of Ca^{2+} from actively loaded plasma membrane vesicles

To determine membrane permeability to Ca^{2+} , we studied the release of Ca^{2+} from plasma membrane vesicles that had been preloaded with $^{45}\text{Ca}^{2+}$ for 10 min and then diluted to 0.1 of the initial concentration in isotonic buffer in the presence of saponin or phospholipase C. As

Fig. 1. Time-dependent release of Ca^{2+} from actively loaded plasma membrane vesicles. Plasma membrane (60–100 μg protein) added to 1 ml of standard reaction mixture. After 10 min of Ca^{2+} uptake, aliquotes were either filtered directly to determine total Ca^{2+} uptake or diluted in isotonic buffer containing phospholipase C or saponin. Control, (●); phospholipase C, (△); saponin, (○).

Table 3. Effects of exogenous phospholipids on Ca^{2+} uptake

Treatment		Ca^{2+} uptake ($\mu\text{mol}/\text{g}$ protein/10 min)	
		ATP-dependent	ATP-independent
Control		46.6 ± 0.4	1.2 ± 0.2
Phosphatidylethanolamine	15 $\mu\text{g}/\text{ml}$	28.6 ± 0.6	1.6 ± 0.1
Phosphatidylserine	15 $\mu\text{g}/\text{ml}$	27.9 ± 3.3	1.4 ± 0.1
Phosphatidylcholine	45 $\mu\text{g}/\text{ml}$	30.8 ± 0.4	1.1 ± 0.2

Data are mean $\pm$ SE (n=5). Ca uptake reaction media contained phospholipid suspension (chloroform : methanol, 2 : 1).

shown in Fig. 1, saponin and phospholipase C increased the release of Ca^{2+} from vesicles. Saponin was more effective than phospholipase C and the amount of Ca^{2+} retained in vesicles in the presence of saponin was about the same as that taken up after 10 min dilution (Table 2).

Effects of exogenous phospholipids on Ca^{2+} uptake

Table 3 shows the Ca^{2+} uptake by plasma membrane isolated from canine stomach in the presence and absence of each phospholipid. All three phospholipids inhibited ATP-dependent Ca^{2+} uptake. Exogenous PC inhibited Ca^{2+} uptake with about one third the potency of PE or PS, which were nearly the same. ATP-independent Ca^{2+} uptake was small, and PE accelerated this uptake slightly but significantly.

Effects of exogenous PE, PS and PI on Ca^{2+} release from actively loaded plasma membrane

Figure 2 shows the results of experiments in which Ca^{2+} was released from actively loaded

Fig. 2. Rate of release of Ca^{2+} from actively loaded vesicles in the presence of various phospholipids. Release in control and in presence of PE and PS measured from 15 sec to 5 min, and in presence of PI from 15 sec to 2 min. Phospholipids were suspended in isotonic buffer by sonication. 100 $\mu\text{g}/\text{ml}$ final concentration was used. Data are mean $\pm$ SE ($n=3-4$).

vesicles diluted to 0.1 concentration in isotonic solution containing PE or PS or PI. These solutions were prepared by dissolving the appropriate phospholipid in chloroform/methanol (2:1, v/v) and then suspending the solution in isotonic buffer by sonication. The data in Fig. 2 were obtained from the ratio of Ca^{2+} retained in solution to the total Ca^{2+} uptake between 15 sec and 5 min for the control, PE and PS; and between 15 sec and 2 min for PI. Release of Ca^{2+} from plasma membrane vesicles was accelerated by all these phospholipids, but significantly by only PS and PI. Release of Ca^{2+} by PS was about 2 times, and by PI about 5 times that of the control. The results thus show that these two phospholipids alter plasma membrane permeability to Ca^{2+} .

Effects of exogenous PI on Ca^{2+} movement

It is well known that turnover of PI is faster than that of other phospholipids. In this study, we could not clearly separate endogenous PI from PS in isolated plasma membrane by TLC. However, exogenous PI affected the movement of Ca^{2+} relative to plasma membrane more than the other phospholipids tested. Figure 3 shows the ATP-dependent and ATP-

Fig. 3. Increase of Ca^{2+} uptake by plasma membrane in the presence of sonicated PI. ATP-dependent Ca^{2+} uptake (▨), ATP-independent Ca^{2+} uptake (□). PI was dissolved in chloroform/methanol (2:1, v/v) and the solution was suspended in the reaction mixture. Inset shows Ca^{2+} uptake by the sonicated preparations of PI in the presence of $20 \mu\text{M}$ free Ca^{2+} . Data are mean $\pm$ SE ($n=4$).

Fig. 4. Effects of PI and neomycin on Ca^{2+} uptake by plasma membrane in the presence absence of ATP. Control, (□); $15 \mu\text{g/ml}$ PI, (▨); 5 mM neomycin, (▤); $15 \mu\text{g/ml}$ PI + 5 mM neomycin, (▧). Data are mean $\pm$ SE ($n=5-9$).

independent increase of Ca^{2+} uptake in the presence of PI. Ca^{2+} uptake was increased the same by the same concentrations of PE and PS (Table 3), and increased more in both cases in the presence of higher doses (not shown). Fig. 3 inset shows the dose dependence of Ca^{2+} uptake by PI alone. Comparison of the results in Table 3 and Fig. 3 show that PI was much more effective than either PE or PS in increasing Ca^{2+} uptake by plasma membrane. The antibiotic, neomycin, binds strongly to PI and prevents both PI metabolism and PI function (e. g. Ca^{2+} uptake). Figure 4 shows the effects of neomycin on ATP-dependent and ATP-independent Ca^{2+} uptake in the presence and absence of PI. PI increased Ca^{2+} uptake in both the presence and absence of ATP as shown in Figs. 3 and 4. Neomycin inhibited Ca^{2+} uptake by plasma membrane in the presence and absence of ATP (Fig. 4). This inhibition by neomycin was greater (both absolutely and relatively) in the presence of PI, and Ca^{2+} uptake in the absence of ATP was almost undetectable.

Discussion

The plasma membrane used in this study was capable of ATP-dependent, active transport of Ca^{2+} into the vesicles. We have previously reported the K_m for Ca^{2+} this transport to be $0.85 \pm 0.01 \mu\text{M}$ which is appropriate for control of intracellular Ca^{2+} , and the Ca^{2+} gradients achievable were in the range of 500 to 2,000 ($\text{Ca}_i^{2+} : \text{Ca}_o^{2+}$), comparable to those across plasma

membrane (Grover *et al.*, 1980; Kwan *et al.*, 1982; Sakai *et al.*, 1983). We measured a cholesterol/phospholipids ratio of 0.64. Raeymaekers *et al.* (1983) reported that the cholesterol/phospholipid ratio in plasma membrane isolated from pig stomach smooth muscle was 0.52 which was 2.5 times that in the endoplasmic reticulum.

The major phospholipids were PE, PC, PS and PI which together composed more than 60% of the total lipid of the plasma membrane isolated from canine stomach smooth muscle (Table 1). There are considerable differences between our preparation and other plasma membrane preparations (Carmel *et al.*, 1985; Schick *et al.*, 1976). The content of PC in our canine stomach preparation was substantially less than the content in skeletal or cardiac plasma membrane (Hotta and Usami, 1967; Barr *et al.*, 1974) (PC 45.0%, PE 22.6%, PI+SM 22.2%, PS 8.6%, phosphatidic acid 1.5%). As far as we know, there is no report of phospholipid composition of plasma membrane isolated from smooth muscle. The high level of LPC was not an artifact but is inherent in the nature of plasma membrane that is high in cholesterol, and cholesterol and LPC associate in a 1:1 molar ratio (Ramsammy and Brockerhoff, 1982). LPC is known to lyse membranes, and cholesterol prevents such lysis (Chauhan *et al.*, 1984). Saponin inhibited ATP-dependent Ca^{2+} uptake (Table 2) and accelerated release of Ca^{2+} from preloaded vesicles (Fig. 1). Saponin binds to cholesterol to form a ring with a central hydrophilic hole in a micellar-type arrangement (Glauert *et al.*, 1962). ATP-dependent Ca^{2+} uptake by plasma membrane was inhibited about 50% by 50 $\mu\text{g/ml}$, and retained Ca^{2+} after 10 min dilution was also 50% in the presence of the same concentration of saponin (Table 2 and Fig. 1). Saponin might make the plasma membrane leaky.

Phospholipase C hydrolyzes PC, PE and SM (Rosenberg and Condrea, 1968) which comprise more than half of the total phospholipids of the plasma membrane. ATP-dependent Ca^{2+} uptake by plasma membrane was inhibited by 100 $\mu\text{g/ml}$ phospholipase C (Table 2). This effect was weaker than previously reported (Sakai *et al.*, 1982) which might be due to the difference between inside-out and outside-out vesicles. There is asymmetry of phospholipids in the plasma membrane. It is reported that PC and SM are present in the outer surface and PS and PE are in the inner surface of the platelet plasma membrane (Schick *et al.*, 1976). However, Schick *et al.* did not evaluate the inner and outer proportions of PI. ATP-dependent Ca^{2+} uptake is assumed to be due to the presence of inside-out vesicles in our preparations and presumably reflects the activity of an outwardly directed Ca^{2+} pump in plasma membrane, since this Ca^{2+} uptake is inhibited by Ca^{2+} ionophores which dissipate the Ca^{2+} gradient across the membrane vesicles (Sakai *et al.*, 1981; Kwan *et al.*, 1982). Therefore, the results of our study using inside-out vesicles might be different from a similar study using outside-out vesicles.

It has become clear that the activity of adenylate cyclase in plasma membranes of mammalian cells depends on its interactions with anionic lipids, particularly PI and PS. Hormone and neurotransmitter enhancement has shown PI turnover and increased Ca^{2+} influx in guinea pig ileum and rat vas deferens smooth muscle (Jafferji and Michell, 1976; Canessa de Scarnati and Lapetina, 1974). The mechanism of PI breakdown in the plasma membrane must be confirmed in a variety of systems and its early kinetics investigated. PI breakdown is not apparently being controlled by changes in extracellular or intracellular Ca^{2+} and therefore its role, if it lies in the main sequence of events which mediate receptor responsiveness, must be

either intracellular liberation of membrane-bound Ca^{2+} or opening of the Ca^{2+} gates (Michell, 1975). PI increased and neomycin decreased Ca^{2+} uptake by plasma membrane (Figs. 2 and 3). The results in Fig. 4 strongly suggest that PI replaced other phospholipids or fused into the membrane in our preparations. Simple increase of Ca^{2+} uptake by PI could occur if the PI was bound to the membrane surface, but the increase of neomycin inhibition of Ca^{2+} uptake by PI can only be explained by replacement or fusion. Results suggest that PI is important in for Ca^{2+} movement across the plasma membrane, and are consistent with the known high metabolism and turnover of PI.

Acknowledgements: The authors wish thank Miss A Isobe for her excellent technical assistance and Dr. A. Simpson for help in preparing the manuscript.

References

- Barr, L., Connor, J.A., Dewey, M.M., Aprille, J. and Johnston, P.V. (1974). The Isolation of plasma membrane from frog cardiac muscle cells. *Biochim. Biophys. Acta* **345**: 336-347.
- Bartlett, G.R. (1959). Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466-468.
- Birnbaumer, L. (1973). Hormone-sensitive adenyl cyclases useful models for studying hormone receptor functions in cell-free systems. *Biochim. Biophys. Acta* **300**: 129-158.
- Bray, G.A. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**: 279-285.
- Canessa, de Scarnati, O. and Lapetina, E.G. (1974). Adrenergic stimulation of phosphatidylinositol labelling in rat vas deferens. *Biochim. Biophys. Acta* **360**: 298-305.
- Carmel, G., Rodrigue, F., Carriere, S. and Le Grmellec, C. (1985). Composition and physical properties of lipids from plasma membranes of dog kidney. *Biochim. Biophys. Acta* **818**: 149-157.
- Chauhan, V.P.S., Ramsammy, L.S. and Brackerhoff, H. (1984). Molecular interactions in the hydrogen belts of membranes glucose-6-phosphatase, lysophosphatidylcholine, and cholesterol. *Biochim. Biophys. Acta* **772**: 239-243.
- Coleman, R. (1973). Membrane-bound enzymes and membrane ultrastructure. *Biochim. Biophys. Acta* **300**: 1-30.
- Cossins, A.R. and Prosser, C.L. (1978). Evolutionary adaptation of membranes to temperature. *Proc. Natl. Acad. Sci. USA* **75**: 2040-2043.
- Dominshki, J., Binaglia, L., Dreyfus, H., Massarelli, R., Mersel, M. and Ereyssy, L. (1983). A study on the topological distribution of phospholipids in microsomal membranes of chick brain using phospholipase C and trinitrobenzenesulfonic acid. *Biochim. Biophys. Acta* **743**: 257-266.
- Folch, J., Lees, M. and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497-509.
- Glauert, A.M., Dingle, J.T. and Lucy, J.A. (1962). Action of saponin on biological cell membranes. *Nature London* **196**: 953-955.
- Gordeshy, S.E. and Marinetti, G.V. (1973). The asymmetric arrangement of phospholipids in the human erythrocyte membrane. *Biochem. Biophys. Res. Commun.* **88**: 1132-1139.
- Grover, A.K., Kwan, C.Y., Crankshaw, J., Crankshaw, D.J., Garfield, R.E. and Daniel, E.E. (1980). Characteristics of calcium transport and binding by rat myometrium plasma membrane subfractions. *Am. J. Physiol.* **239** (Cell Physiol. 8): c66-c74.
- Hauser, H. and Dawson, R.M.C. (1968). The displacement of calcium ions from phospholipid monolayers by pharmacologically active and other organic bases. *Biochem. J.* **109**: 909-916.
- Hotta, K. and Usami, Y. (1967). Enzymatic activity of isolated skeletal muscle sarcolemma. *J.*

- Biochem.* **61** : 407-410.
- Jafferji, S.S. and Michell, R.H. (1976). Muscarinic cholinergic stimulation of phosphatidylinositol turnover in the longitudinal smooth muscle of guinea-pig ileum. *Biochem. J.* **154** : 653-657.
- Janis, R.A., Crankshaw, D.J. and Daniel, E.E. (1977). Control of intracellular calcium activity in rat myometrium. *Am. J. Physiol.* **232** (Cell Physiol. 1): c50-c58.
- Kwan, C.Y., Sakai, Y., Grover, A.K. and Lee, R.M.K.W. (1982). Isolation and characterization of plasma membrane fraction from gastric fundus smooth muscle of the rat. *Mol. Physiol.* **2** : 107-120.
- Levey, G.S. (1972). In: *the role of membranes in metabolic regulation*, edited by M.A. Mehlman, and R.W. Hanson, pp. 249-260, Academic Press, New York.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193** : 265-275.
- Michell, R.H. (1975). Inositol phospholipids and cell surface receptor function. *Biochim. Biophys. Acta* **415** : 81-147.
- Raeymaekers, L., Wuytack, F., Eggermont, J., Schutter, G.D. and Casteels, R. (1983). Isolation of plasma-membrane fraction from gastric smooth muscle: Comparison of the calcium uptake with that in endoplasmic reticulum. *Biochem. J.* **210** : 315-322.
- Ramsamy, L.S. and Brockerhoff, H. (1982). Lysophosphatidylcholine-cholesterol complex. *J. Biol. Chem.* **257** : 3570-3574.
- Rosenberg, P. and Condrea, E. (1968). Maintenance of axonal conduction and membrane permeability in presence of extensive phospholipid splitting. *Biochem. Pharmacol.* **17** : 2033-2044.
- Sakai, Y., McLean, J., Grover, A.K., Garfield, R.E., Fox, J.E.T. and Daniel, E.E. (1981). Isolation and characterization of subcellular membranes from canine stomach smooth muscle. *Can. J. Physiol. Pharmacol.* **59** : 1260-1267.
- Sakai, Y., Ichikawa, S., Yoshida, M., Oouchi, M. and Miyagawa, M. (1982). ATPase activity and calcium uptake of microsomes isolated from stomach smooth muscle after exposure to phospholipase C. *Jap. J. Smooth Muscle Res.* **18** : 339-345.
- Sakai, Y., Grover, A.K., Fox, J.E.T. and Daniel, E.E. (1983). Uptake and release of calcium by canine gastric corpus smooth muscle plasma membrane enriched fraction. *Can. J. Physiol. Pharmacol.* **61** : 699-704.
- Schick, P.K., Kurica, K.B. and Chacko, G.K. (1976). Location of phosphatidylethanolamine and phosphatidylserine in the human platelet plasma membrane. *J. Clin. Invest.* **57** : 1221-1226.
- Sperry, W.M. and Webb, M. (1950). A revision of schoenheimersperry method for cholesterol determination. *J. Biol. Chem.* **187** : 97-106.
- Yasuda, N. and Sakai, Y. (1984). A possible explanation for effects of Sr^{2+} on contraction-relaxation cycle in canine stomach. *Comp. Biochem. Physiol.* **78A** : 35-41.