Na-Ca Exchange in Sarcolemmal Vesicles Isolated from Cat Ileal Longitudinal Muscle

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ABSTRACT

Effect of a Na^+ gradient on Ca^{2+} uptake was studied in isolated sarcolemmal vesicles of cat ileal longitudinal muscle.

Ca^{2+} uptake was markedly stimulated in the presence of an outwardly directed Na^+ gradient. External Na^+, monensin and A23187 abolished the Na^+-dependent Ca^{2+} uptake. Monovalent cations such as K^+, Li^+, Rb^+, Cs^+ and choline could not substitute for Na^+ in enhancement of Ca^{2+} uptake. Divalent cations such as Ba^{2+}, Sr^{2+}, Mn^{2+} and Cd^{2+} but not Mg^{2+} inhibited the Na^+-dependent Ca^{2+} uptake. Increase in external pH in the range of 6.0 to 8.0 stimulated the Na^+-dependent Ca^{2+} uptake. Amiloride inhibited the Na^+-dependent Ca^{2+} uptake at concentrations above 0.5 mM, whereas diltiazem or vanadate did not. The apparent Km of the Na^+-depentent Ca^{2+} uptake for Ca^{2+} was 18.2 μM and apparent Vmax was 689.7 pmole/mg protein/5 sec. Kinetic analysis of the Na^+-dependent Ca^{2+} uptake showed a noncompetitive interaction between internal Na^+ and external Ca^{2+}. The dependence of Ca^{2+} uptake on internal Na^+ showed sigmoidal kinetics and Hill coefficient for internal Na^+ was 2.52. Inside positive membrane potential generated by imposing an inwardly directed K^+ gradient and valinomycin significantly stimulated the Na^+-dependent Ca^{2+} uptake.

These results indicate that a Na^+-Ca^{2+} exchange system exists in the sarcolemmal membranes isolated from cat ileal longitudinal muscle and it might operate as an electrogenic process.

Key Words: Sodium-calcium exchange; Sarcolemmal vesicle; Smooth muscle; Cat ileum

INTRODUCTION

It has been known that tension development in contractile proteins of smooth muscle is brought about primarily by a rise in the concentration of intracellular free calcium ($[Ca^{2+}]_i$). The rise in $[Ca^{2+}]_i$ may be brought about by an influx of Ca^{2+} from the extracellular compartment through the cell membrane and/or by the release from the intracellular stores. In general, influx of Ca^{2+} occurs through two types of calcium channels in the cell membrane. The one is potential-sensitive calcium channel, an ion channel population that opens as the potential across the membrane is reduced and the other is receptor-operated calcium channel, an ion channel controlled or operated by a receptor for a stimulant substance (Bolton, 1979).

The decrease in $[Ca^{2+}]_i$ in smooth muscle occurs by efflux of Ca^{2+} through the cell membrane and/or sequestration in the intracellular organelles. In striated muscle the sarcoplasmic reticulum is highly developed and functions as main source of the rise and removal of intracellular Ca^{2+}. However, intracellular organelles in smooth muscle have limited capacity to sequester Ca^{2+}. Therefore, Ca^{2+} extrusion

mechanism through the sarcolemmal membrane plays a dominant role in restoring $[Ca^{2+}]_i$ to the resting level (Huggins & England, 1985). Efflux pathways located in the sarcolemmal membrane include an ATP-dependent Ca^{2+} pump and probably a Na^+-Ca^{2+} exchange system.

The Na^+-Ca^{2+} exchange mechanism has been well documented in cardiac (Reeves, 1985) and nerve (Dipolo & Beauge, 1988) cell membranes. It has been demonstated that sarcolemmal vesicles isolated from several smooth muscles contain a Na^+-Ca^{2+} exchange system (Grover et al, 1981; Matlib et al, 1985; Slaughter, 1987). However, the existence of this system and its role in regulation of $[Ca^{2+}]_i$ in smooth muscle cells have not yet been clarified.

In intestinal smooth muscle, the existence of a Na^+-Ca^{2+} exchange system has been suggested by the observations that an outwardly directed Na^+ gradient caused contraction (Judah & Willoughby, 1964) and alteration in Na^+ concentration in bathing medium or cytoplasm affected Ca^{2+} flux, and vice versa (Brading & Widdicombe, 1975). However, Casteels and van Breemen (1975) have demonstrated that in taenia coli Ca^{2+} extrusion is by way of an ATP-dependent Ca^{2+} pump and independent of the Na^+ gradient, inconsistent result with the presence of the Na^+-Ca^{2+} exchange system. Raeymaekers et al (1974) also indicated that the apparent effects of altering Na^+ concentration on Ca^{2+} efflux are due to extracellular exchange.

In a previous study (Lee & Lee, 1983), longitudinal muscle strips of cat ileum have shown a strong contraction by either raising intracellular Na^+ concentration by Na^+ pump inhibition with ouabain, or decreasing the Na^+ concentration of the bathing medium, suggesting a probablity that sarcolemmal membrane of longitudinal muscle cells of cat ileum contain a functionally significant Na^+-Ca^{2+} exchange system. However, changes in muscle tone on altering Na^+ concentration do not provide conclusive evidence for a Na^+-Ca^{2+} exchange, since the effects may be secondary to other actions of Na^+, e.g., alterations of membrane potential and changes in electrogenic Na^+-K^+ pump activity. Direct evidence must come from flux studies in isolated sarcolemmal vesicles.

The present study was carried out to determine whether sarcolemmal vesicles from cat ileal longitudinal muscle contain a Na^+-Ca^{2+} exchange system and, if so, to determine functional characteristics of the system.

MATERIALS AND METHODS

Preparation of sarcolemmal vesicles

Cats (2~3 kg) were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg), and abdominal wall was dissected to remove the ileum. The ileum was immediately placed on an ice-cold sucrose buffer solution composed of 8% (w/w) sucrose and 20 mM Hepes/Tris (pH 7.5). All subsequent preparative steps were carried out at 4℃. Fat adhering to the pieces of the ileum was trimmed out and then the pieces were incised and placed on a filter paper previously wetted with sucrose buffer solution so that the mucosal side faced away from the paper. Mucosa was scraped off the tissue, the remaining tissue rinsed with sucrose buffer solution, and then the circular muscle was peeled off leaving behind the longitudinal muscle. The longitudinal muscle was further dissected into small pieces and cleaned from the adhering material by stirring in sucrose buffer for 30 min. And then, the ileal muscle in sucrose buffer (1:10 w/v) was finely minced with scissors and gently homogenized in a tissue grinder (Edmund Bühler, D-7400) and with a Potter-Elvehjem type homogenizer (5 strokes).

Sarcolemmal vesicles were prepared according to the method of Grover et al (1980) as depicted in Fig. 1. Differential centrifugation of the homegenate was performed to eliminate cell debris, nuclear and mitochondrial fractions in a Sorvall SS34 rotor and

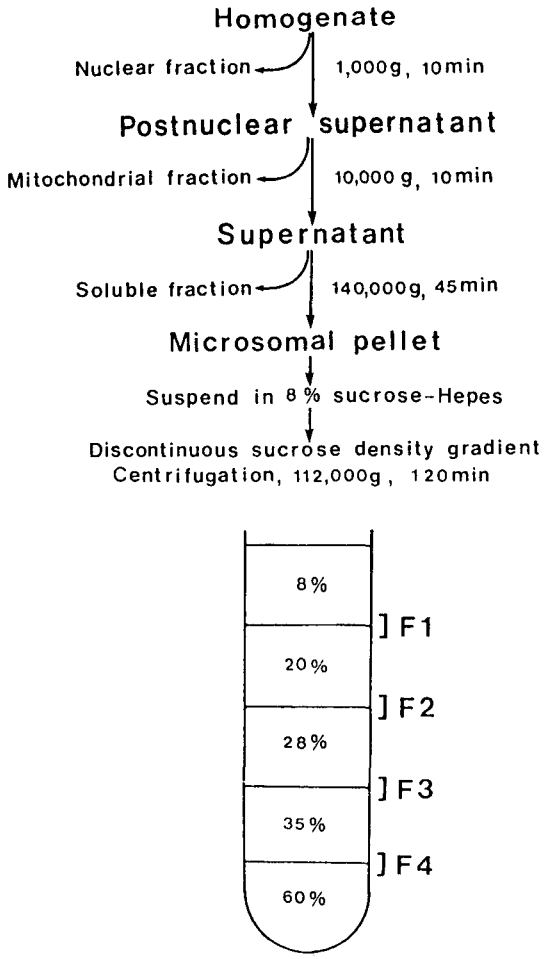

Fig. 1. Scheme for isolation of sarcolemmal vesicles from cat ileal longitudinal muscle.

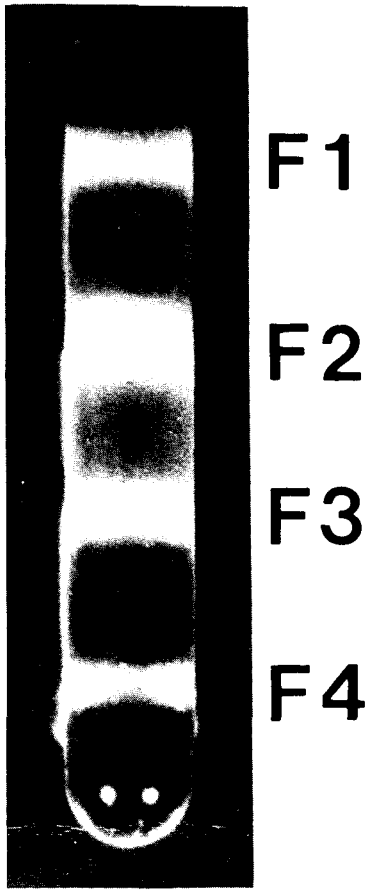

Fig. 2. Photographic illustration of the bands formed after discontinuous sucrose density gradient centrifugation.

RC 5B refrigerated centrifuge. Microsomal pellet was obtained from the supernatant after 10,000 g centrifugaton by pelleting at 140,000 g for 30 min in a Beckman 30 Ti rotor and L8-80M ultracentrifuge.

Fractionation of microsomes was carried out in a discontinuous sucrose density gradient, consisting of 4 ml of 60% sucrose (w/w) and each 3 ml of 35, 28 and 20% sucrose layered consecutively in a cellulose-nitrate tube (10.2×1.6 cm). The microsomal fraction (3 ml) was layered on top of the tube containing the discontinuous sucrose gradient. The tubes were then centrifuged at 112,000 g for 2 h in a Beckman SW 28. 1 swinging-bucket rotor and L8-80M ultracentrifuge. After centrifugation, four distinct bands were observed (Fig. 2). The bands were designated as F1; the band at the interface between 8 and 20% sucrose, F2; the band at the interface between 20 and 28% sucrose, F3; the band at the interface between 28 and 35% sucrose and F4; the band at the interface between 35 and 60% sucrose. Each individual membrane fraction was carefully removed with Pasteur pipettes, diluted with 20 mM Hepes/Tris (pH 7.5) to be a final sucrose concentration of 8% and pelleted

by centrifugation at 100,000 g for 30 min. The resultant pellets were resuspended in an appropriate loading medium. To load the vesicles with an appropriate intravesicular medium, the membrane vesicles were suspended in each medium of appropriate ligand composition, kept at 4℃ overnight and then washed twice with same medium by centrifugation at 100,000 g for 30 min. The final pellets were resuspended in a small volume of each medium. In a procedure of suspension, the membrane vesicles were sucked several times through a fine needle (26 gauge) with a plastic syringe. The final membrane vesicles were rapidly frozen and kept at −60℃. The composition of intravesicular medium was indicated in the figure legends. Before transport studies, the membrane vesicles were loaded again by incubating at 37℃ for 30 min.

Protein assay

Protein concentration was determined by the Coomasie blue dye binding method as described by Bradford (1976).

Marker enzyme assays

5'-Nucleotidase activity was assayed according to Matlib et al (1976). Membrane vesicles (20～50 μg) were preincubated in a solution containing 100 mM Tris/HCl (pH 7.5), 10 mM $MgSO_4$ and 0.1% deoxycholic acid for 10 min at 37℃ and incubation was started by adding 5 mM 5'-AMP. The final volume of incubation medium was 1 ml. After 10 min the reaction was stopped by adding 0.2 ml of ice-cold solution of 20% trichloroacetic acid. Inorganic phosphate liberated in the mdium was determined according to the method of Fiske and SubbaRow (1925).

NADPH-cytochrome-c reductase activities were determined by incubating membranes (20～50 μg protein) with 0.05 mM cytochrome-c, 1 mM KCN and 100 mM potassium phosphate (pH 7.5) at 25℃. Reactions were started by adding 0.15 mM NADPH, and the appearance of reduced cytochrome-c was monitored by recording the absorbance of the reaction mixture at 550 nm with a spectrophotometer attached to recorder as described by Sottocasa et al (1967).

Cytochrome-c oxidase activities were measured by incubating membranes (20～50 μg protein) at 25℃ in 50 mM potassium phosphate (pH 7.5) and 68 μM cytochrome-c that had been previously reduced with sodium hydrosulfite. Excess sodium hydrosulfite was oxidized by aerating the solution before use. The disappearance of reduced cytochrome-c was monitored by recording the abosorbance of the reaction mixture at 550 nm with a spectrophotometer attached to chart recorder as described by Cooperstein and Lazarow (1951).

Electron micorscopy

The membrane vesicles were fixed in 2% glutaraldhyde, postfixed in 1% osmium tetroxide, and embedded in Epon. Thin sections were made in ultramicrotome and stained with uranyl acetate and lead citrate and examined on a Jeol 100 ZX microscope.

Ca^{2+} uptake assay

The uptake of Ca^{2+} was measured by a rapid filtration technique. The reaction was initiated by adding 10 μl of membrane vesicles (15～20 μg) to 190 μl of incubation medium prewarmed to 25℃. The composition of incubation medium was indicated in the figure legends. At the stated times, 100 μl of the mixtures were taken and quickly filtered under vacuum through Millipore filters (HAWP, 0.45 μm pore size, 25 mm diameter). Filters were presoaked overnight in a solution containing 1 mM $LaCl_3$ and 2 mM Tris/HCl (pH 7.5). Filters were washed twice with 5 ml of ice-cold solution containing 300 mM mannitiol, 5 mM $LaCl_3$ and 10 mM Tris/HCl (pH 7.5). Scintillation fluid (Filter count, Packard) was added to dried filters and the amount of radioactivity remaining on the filter was determined by liquid

scintillation spectrophotometer (Packard, Tricarb 300C).

Statistical analysis

The statistical significance of difference between two experimental groups was determined using Student's t-test. P values less than 0.05 were considered significant.

RESULTS

Properties of membrance vesicles

The separation of longitudinal smooth muscle from circular muscle or other tissues depends on a good tissue preparation. The tissue preparation was effected by the careful removal of circular muscle with mucosa and connective tissues. Under visual examination through a light microscope, tissues used in this study were considered as relatively clean strips of longitudinal muscle with low levels of contaminating circular muscle or connective tissue.

The distribution of marker enzymes in various membrane fractions was described in Table 1. 5'-Nucleotidase activity, a sarcolemmal membrance marker, was highest in the F2 fraction, in which its activity was enriched over 22-fold as compared to that of the homogenate.

The sarcoplasmic reticulum marker, NADPH-cytochrome c reductase had its highest activity in the F3 fraction. The sarcolemmal membrane marker, 5'-nucleotidase activity was reduced in F3 fraction when compared to the microsome or F2 fraction, indicating reduced proportion of sarcolemma to sarcoplasmic reticulum in this fraction. Although NADPH-cytochrome c reductase activity in F2 fraction was slightly higher than that of the homogenate (1.3 fold), it was much lower than that of microsome (2.2 fold) or F3 fractions (3.1 fold) indicating a negligible contamination with sarcoplasmic reticulum.

The mitochondrial membrane marker, cytochrome c oxidase activity was reduced in F2 fraction to 0.3 fold as compared to that of the homogenate. Mitochondria are known to have a Na^+-Ca^{2+} exchange activity (Matlib & Schwartz, 1983) distinct from that found in sarcolemmal membrane, and for this reason the removal of mitochondria was of great importance. These results indicate that F2 fraction is highly enriched in sarcolemmal vesicles. Therefore, F2 fraction was used in this study.

Electron microscopic examination of the membranes from F2 fraction revealed that they were composed of heterogenous, but tightly sealed vesicles originated from sarcolemmal membranes (Fig. 3).

Table 1. Comparison of marker enzyme activities in membrane fractions

Fraction	5'-Nucleotidase		NADPH-cytochrome c reductase		Cytochrome c oxidase	
	Activity (nmol/mg/min)	Enrichement (fold)	Activity (nmol/mg/min)	Enrichment (fold)	Activity (nmol/mg/min)	Enrichment (fold)
Homogenate	66.2± 2.74	1.0	4.16±0.62	1.0	34.3±2.77	1.0
Microsome	440.6±31.68	6.7	9.24±2.05	2.2	14.5±1.76	0.4
F1	672.4±48.26	10.2	3.25±0.46	0.8	11.2±1.24	0.3
F2	1472.3±96.42	22.2	5.41±0.62	1.3	10.7±1.07	0.3
F3	320.4±30.16	4.8	12.89±0.73	3.1	16.2±1.85	0.5
F4	87.2± 7.86	1.3	7.48±0.57	1.8	37.2±4.18	1.1

Mean±S.E.M. of 6 determinations in 3 different vesicle preparations.

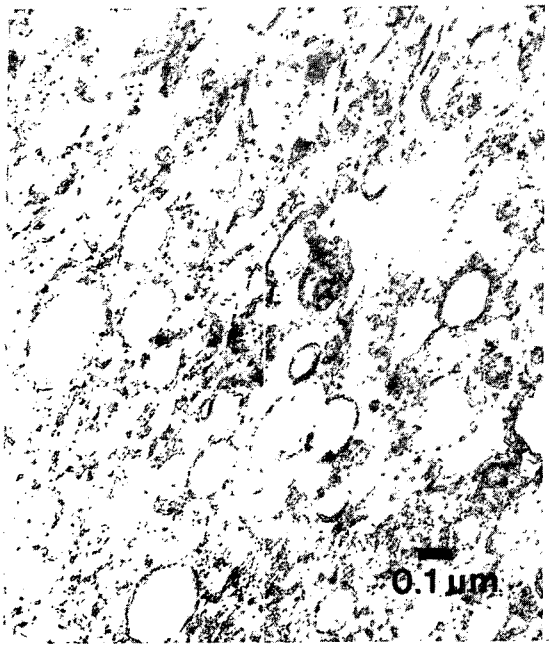

Fig. 3. Electron micrograph of membrane vesicles collected from sucrose gradient at interface between 20 and 28% sucrose (F2 fraction). Magnification, ×60,000.

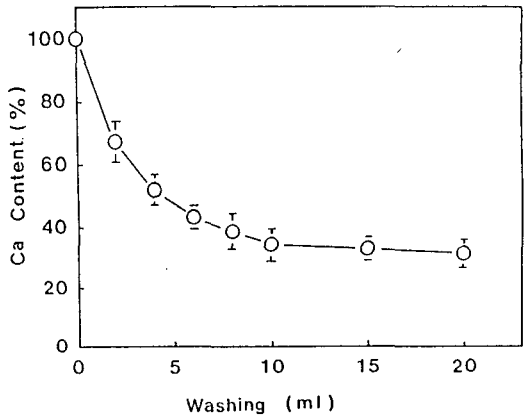

Fig. 4. The washout of ^{45}Ca from membrane vesicles by La^{3+} washing solution. The membrane vesicles were loaded with 150 mM NaCl and 20 mM Hepes/Tris (pH 7.5) and incubated in a medium containing 150 mM KCl, 20 μM $^{45}CaCl_2$ and 20 mM Hepes/Tris (pH 7.5). After 5 min of incubation, aliquots of incubation mixture were quickly filtered under vacuum through Millipore filter (HAWP, 0.45 μM pore size) and wahed sequentially with 2 ml of ice-cold La^{3+} wahing solution containing 300 mM mannitol, 5 mM $LaCl_3$ and 5 mM Tris/HCl (pH 7.5). Values were presented as % of the initial label without wahing. Each point represents the mean±S.E.M. of 4 determinations in 2 different vesicle preparations.

Na^+-dependent Ca^{2+} uptake

A major obstacle to measurement of ^{45}Ca fluxes in smooth muscle has been the presence of a relatively large amount of extracellular binding, the presence of which also complicates estimation of cellular exchangeable Ca^{2+}. This extracellular binding is particularly problematic in Na^+ substitution experiments since it exchanges differentially with various Na^+ substitutes (Aaronson & van Breemen, 1981). This is also the case with the experiments using isolated sarcolemmal vesicles. To overcome this problem, La^{3+} wash-out method was used in this study. It has been reported that La^{3+} could not only replace Ca^{2+} at superficial binding sites (Weiss & Goodman, 1969) but also block transmembrane fluxes of Ca^{2+} (Mayer et al, 1972). Fig. 4 shows the ^{45}Ca content remaining after sequential washout with 2 ml of ice cold solution containing 300 mM mannitol, 5 mM $LaCl_3$ and 5 mM Tris/HCl (pH 7.5). The ^{45}Ca content was rapidly reduced with three times of washing (total 6 ml). The size of this component and its rapid removal suggested that it was due to the washout of filter and extravesicular membrane bound ^{45}Ca. Further washing did not significantly change the content of ^{45}Ca remaining on the filter. After washing with 5 times with 2 ml of La^{3+} solution, the amount of the remaining ^{45}Ca was about 34% of initial label without washing, and this was not significantly different from that after washing twice with 5 mM of the La^{3+} solution. This La^{3+}-resistant component was taken as intravesicular uptake throughout this study.

Fig. 5 shows the effect of a Na^+-gradient on Ca^{2+} uptake. When Na^+-loaded membrane vesicles were

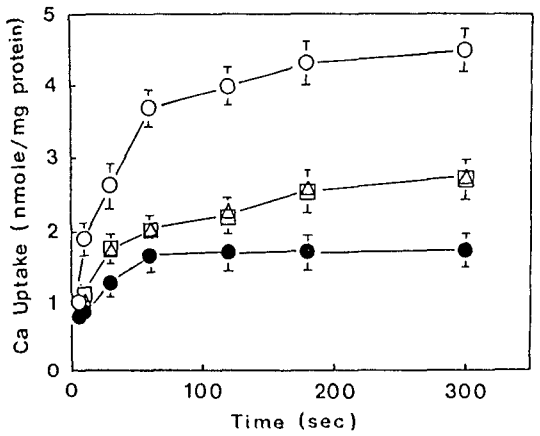

Fig. 5. Time dependence of Ca^{2+} uptake. Membrane vesicles were loaded with 20 mM Hepes/Tris (pH 7.5) and either 150 mM NaCl (○, □) or KCl (△) and incubated in 20 mM Hepes/Tris (pH 7.5), 20 μM $^{45}CaCl_2$ and either 150 mM NaCl (□) or KCl (○, △). Na^+-dependent Ca^{2+} uptake (●) was obtained by subtracting the uptake by K^+-loaded vesicles from the total uptake in the presence of a Na^+ gradient. Each point represents the mean± S.E.M. of 8 determinations in 4 different vesicle preparations.

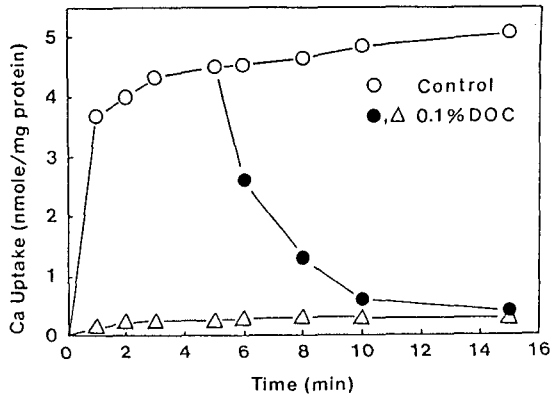

Fig. 6. Effect of deoxycholic acid (DOC) on Ca^{2+} uptake in the presence of an outwardly directed Na^+ gradient. Membrane vesicles were loaded with 150 mM NaCl and 20 mM Hepes/Tris (pH 7.5), and incubated in 150 mM KCl, 20 μM $^{45}CaCl_2$ and 20 mM Hepes/Tris. Deoxycholic acid was added to the incubation medium before (△) or 5 min after (●) the start of incubation, and Ca^{2+} uptake as a function of time was observed. Each point represents the mean of duplicate determinations.

incubated in a Na^+-free medium to create an outwardly directed Na^+ gradient, Ca^{2+} uptake was significantly stimulated as compared to that of the K^+-loaded vesicles. However, when a Na^+-gradient was eliminated by replacing the incubation medium with equal concentration of NaCl, the uptake of Ca^{2+} was markedly reduced and not significantly different from that of the K^+-loaded vesicles. The Na^+-dependent Ca^{2+} uptake was obtained by subtracting the uptake by K^+-loaded vesicles from the total uptake measured in the presence of an outwardly directed Na^+ gradient. The Na^+-dependent Ca^{2+} uptake at 1 min of incubation time was 1.66 nmole/mg protein, which was 45.1% of the total uptake. The Ca^{2+} uptake did not show so-called 'overshoot phenomenon', a transient higher uptake than the equilibrium value, at any time.

Effect of detergent, Na^+ and Ca^{2+} ionophores

To rule out that Ca^{2+} uptake resulted from the nonspecific binding of ^{45}Ca to the membrane vesicles or filters rather than trasnport into an intravesicular space, the effect of a detergent, deoxycholic acid was studied. In the presence of 0.1% deoxycholic acid in incubation medium, the La^{3+}-resistant component of Ca^{2+} uptake was negligible. When deoxycholic acid was added after incubation for 5 min to allow the vesicles to accumulate Ca^{2+}, the accumulated Ca^{2+} was rapidly and completely released (Fig. 6). The Ca^{2+} uptake in the presence of deoxycholic acid did not show a significant difference between in the presence and absence of a Na^+ gradient (data not shown).

Fig. 7 depicts the effect of monensin on Ca^{2+} uptake in the presence and absence of a Na^+ gradient. The Ca^{2+} uptake was significantly higher in

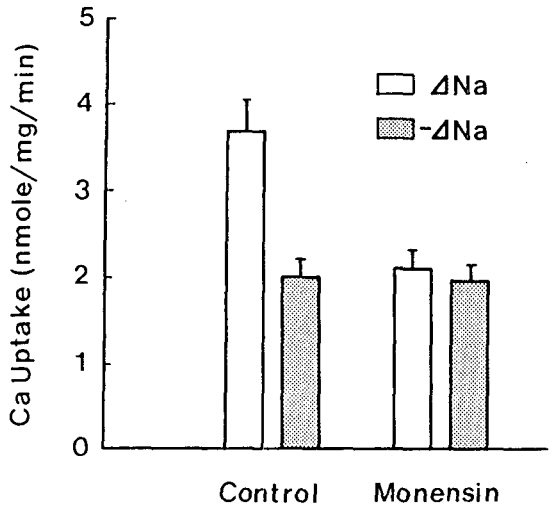

Fig. 7. Effect of monensin on Ca^{2+} uptake. Membrane vesicles were loaded with 20 mM Hepes/Tris (pH 7.5) and either 150 mM NaCl (⊿Na) or KCl (−⊿Na), and incubated in 150 mM KCl, 20 μM $^{45}CaCl_2$ and 20 mM hepes/Tris (pH 7.5) in the presence or absence of monesin (10 μM). Values are the mean±S.E.M. of 6 determination in 3 different vesicle preparations.

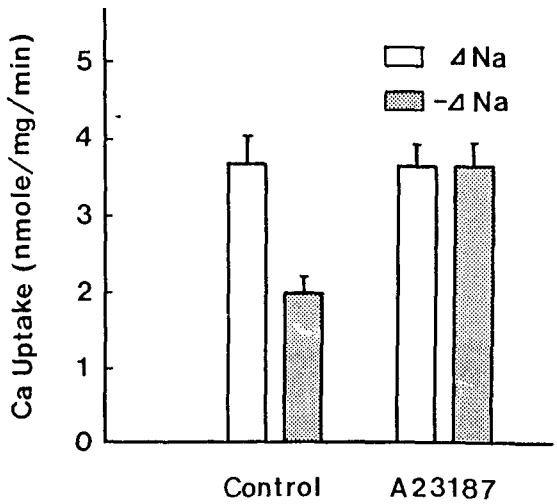

Fig. 8. Effect of A23187 on Ca^{2+} uptake. Membrane vesicles were loaded with 20 mM Hepes/Tris (pH 7.5) and either 150 mM NaCl (⊿Na) or KCl (−⊿Na), and incubated in 150 mM KCl, 20μM $^{45}CaCl_2$ and 20 mM Hepes/Tris (pH 7.5) in the presence or absence of A23187 (2 μM). Values are the mean±S.E.M. of 6 determination in 3 different vesicle preparations.

the presence than in the absence of a Na^+ gradient. However, when 10 μM monensin was added to the incubation medium, the Na^+-dependent component of the Ca^{2+} uptake was abolished.

To examine whether the membrane vesicles accumulate Ca^{2+} against its concentration gradient, the effect of a Ca^{2+} ionophore A23187 was studied. In the presence of 2 μ M A23187, the Ca^{2+} uptake measured in the presence of a Na^+ gradient was not changed, but the uptake mesasured in the absence of a Na^+ gradient was significantly increased as compared to the uptake in the absence of A23187, so that the Na^+-dependent component was abolished (Fig. 8).

Mcnovalent cation specificity

The monvalent cation specificity of the Na^+-dependent Ca^{2+} uptake was studied in vesicles loaded with 150 mM Na^+, K^+, Rb^+, Li^+, Cs^+ or choline by measuring Ca^{2+} uptake in 300 mM mannitol medium. As shown in Table 2, when Na^+ gradient was replaced by gradients of K^+, Li^+, Rb^+ or Cs^+, Ca^{2+} uptake was reduced and not significantly different from the uptake of mannitol-

Table 2. Effect of internal cations on Ca^{2+} uptake

Cation	Ca^{2+} Uptake (nmol/mg protein/min)
Control (mannitol)	1.84 ± 0.09
Na^+	3.32 ± 0.23*
K^+	1.89 ± 0.18
Li^+	1.92 ± 0.13
Rb^+	1.74 ± 0.21
Cs^+	1.78 ± 0.14
Choline	1.52 ± 0.12*

Mean±S.E.M. of 5 determinations in 3 different vesicle preparations.

*, Significantly different from control ($p<0.05$).

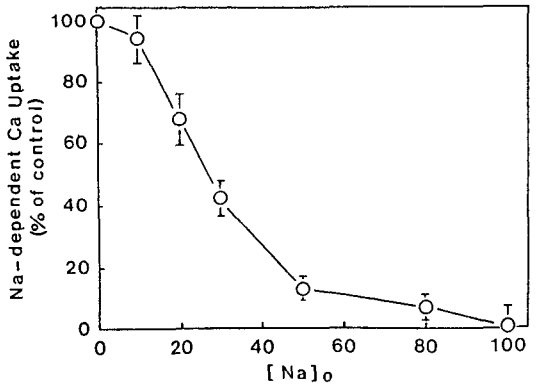

Fig. 9. Inhibition of Na^+-dependent Ca^{2+} uptake by external Na^+. Membrane vesicles were loaded with 150 mM NaCl and 20 mM Hepes/Tris (pH 7.5), and incubated in 20 mM Hepes/Tris (pH 7.5), 20 μM $^{45}CaCl_2$ and different concentrations of NaCl in which NaCl was isosmotically replaced with KCl. Uptake values were presented as % of the uptake in the absence of external Na^+. Each point represents the mean±S.E.M. of 6 determinations in 3 different vesicle preparations.

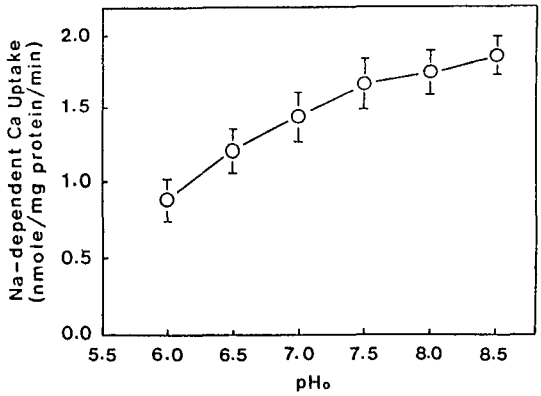

Fig. 10. Influence of external pH on Na^+-dependent Ca^{2+} uptake. Membrane vesicles were loaded with 150 mM NaCl and 20 mM Hepes/Tris (pH 7.5), and incubated in 150 mM KCl, 20 μM $^{45}CaCl_2$ and 20 mM Hepes/Tris (pH 7.5). Appropriate mixtures of Hepes and Tris buffers were used to adjust pH. Na^+-dependent Ca^{2+} uptake was obtained as described in Fig. 5. Each point represents the mean±S.E.M. of 6 determinations in 3 different vesicle preparations.

Table 3. Effect of divalent cations on Na^+-dependent Ca^{2+} uptake

Cation (0.5 mM)	Ca^{2+} Uptake (nmole/mg protein/min)	% inhibition
Control	1.66 ± 0.06	0
Ba^{2+}	0.64 ± 0.05*	61.5
Sr^{2+}	0.42 ± 0.04*	74.7
Mn^{2+}	0.76 ± 0.04*	54.2
Cd^{2+}	0.12 ± 0.02*	92.8
Mg^{2+}	1.58 ± 0.12	4.9

Mean±S.E.M. of 6 determinations in 3 different vesicle preparations.

*, Significantly different from control ($p<0.05$).

loaded vesicles. Choline slightly inhibited the uptake as compared to that of mannitol-loaded vesicles. These results indicate that these monovalent cations could not substitute for Na^+ in stimulation of Ca^{2+} uptake.

Effect of external ligands

Fig. 9 shows the effect of extenal Na^+ on Na^+-dependent Ca^{2+} uptake. Na^+-dependent Ca^{2+} uptake was decreased with increasing concentration of Na^+ in incubation medium and the uptake in the presence of 50 mM Na^+ was about 10% of that in the absence of Na^+.

Effect of certain divalent cations on Na^+-dependent Ca^{2+}uptake was studied. Ba^{2+}, Sr^{2+}, and Cd^{2+} but not Mg^{2+} inhibited Na^+-dependent Ca^{2+} uptake at concentration of 0.5 mM, and among them Cd^{2+} was the most effective one (Table 3).

Fig. 10 depicts the effect of external pH on Na^+-dependent Ca^{2+} uptake. The Ca^{2+} uptake increased slowly with increasing pH from 6.0 to 8.5.

Effect of amiloride, diltiazem and vanadate

Effects of amiloride, diltiazem and vanadate on Na^+-dependent Ca^{2+} uptake were studied. Amiloride, which has been known to inhibit Na^+-Ca^{2+} exchange process in various tissues (Kleyman & Cragoe, 1988), significantly inhibited the Na^+-

dependent Ca^{2+} uptake at concentration above 0.5 mM. However, dilitazem, an inhibitor of mitochondrial Na^+-Ca^{2+} exchange (Aaronson & van Breemen, 1981), and vanadate, an inhibitor of ATP-dependent Ca^{2+}-pump in sarcolemmal membrane (Enyedi et al, 1988) showed only a slight inhibition at 5 mM (Fig. 11).

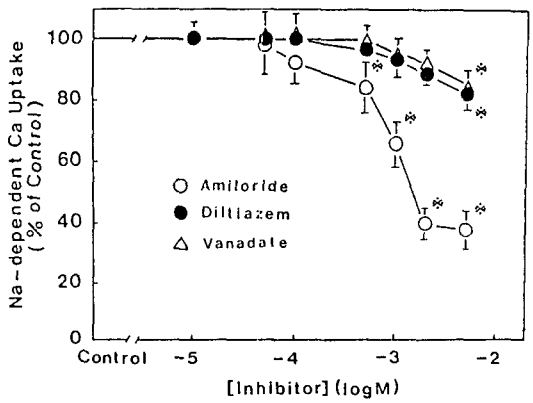

Fig. 11. Effects of amiloride, diltiazem and vanadate on Na^+-dependent Ca^{2+} uptake. Membrane vesicles were loaded with 150 mM NaCl and 20 mM Hepes/Tris (pH 7.5), and incubated in 150 mM KCl, 20 μM $^{45}CaCl_2$ and 20 mM Hepes/Tris (pH 7.5) containing various concentrations of amiloride (○), diltiazem (●) and vanadate (△). Na^+-dependent Ca^{2+} uptake was obtained as described in Fig. 5. Each point represents the mean±S.E.M. of 6 determinations in 3 different vesicle preparations.
※, Significantly different from control ($p<0.05$).

External $[Ca^{2+}]$ dependence

The rate of Na^+-dependent Ca^{2+} uptake was dependent on free Ca^{2+} concentration in incubation medium. The rate of uptake increased with increasing concentration of free Ca^{2+}. Double reciprocal plot ($1/V$ vs. $1/[Ca^{2+}]$) of the data produced a straight line ($r=0.98$) yielding Km for free Ca^{2+} of 18.2 μM and Vmax of 689.7 pmole/mg protein/5 sec (Fig. 12).

Intravesicular loaded $[Na^+]$ dependence

In Fig. 13, Na^+-dependent Ca^{2+} uptake was measured in membrane vesicles loaded with various concentration of Na^+.

The Na^+-dependent Ca^{2+} uptake was dependent on the intravesicular concentration of Na^+. The rate of Na^+-dependent Ca^{2+} uptake as a function of intravesicular Na^+ concentration was sigmoidal and Hill plot of the data produced a straight line ($r=0.98$). The apparent Hill coefficient calculated from this line was 2.52, suggesting that more than two Na^+

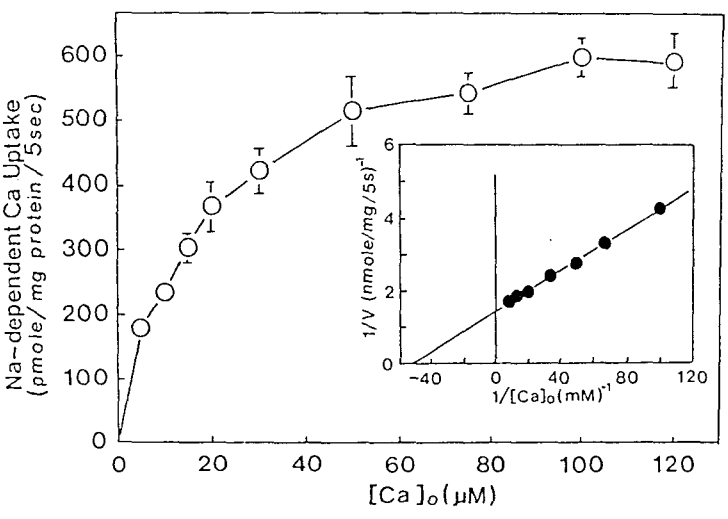

Fig. 12. Na^+-dependent Ca^{2+} uptake as a function of free Ca^{2+} concentration in incubation medium. Membrane vesicles were loaded with 150 mM NaCl and 20 mM Hepes/Tris (pH 7.5). Ca^{2+} uptake (5 sec) was determined in 150 mM KCl and 20 mM Hepes/Tris (pH 7.5) containing trace amounts of $^{45}CaCl_2$. Free Ca^{2+} concentrations were maintained with 100 μM EGTA and various concentrations of $CaCl_2$. Na^+-dependent Ca^{2+} uptake was obtained as described in Fig. 5. Inset shows a double reciprocal plot ($1/V$ versus $1/[Ca^{2+}]o$) of the data. Each point represents the mean±S.E.M. of 6 determinations in 3 different vesicle preparations.

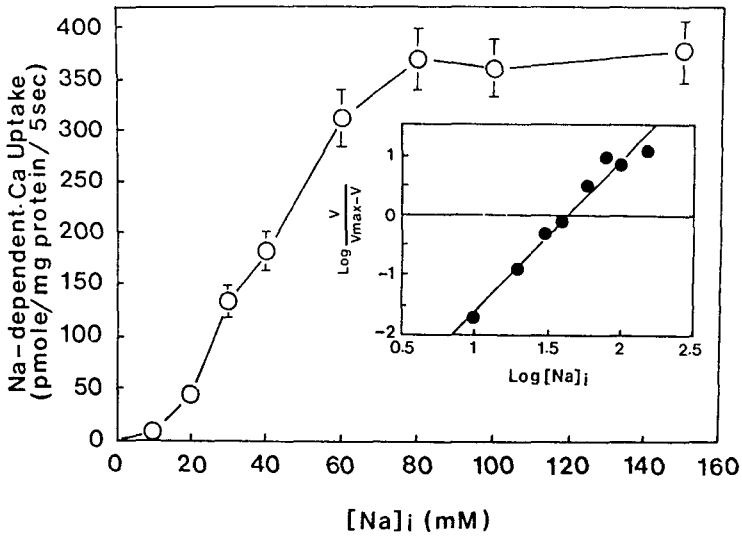

Fig. 13. Effect of internal Na^+ concentration on Na^+-dependent Ca^{2+} uptake. Membrane vesicles were loaded with 20 mM Hepes/Tris (pH 7.5) and various concentration of NaCl, in which NaCl was replaced isoosmotically with KCl. Ca^{2+} uptake (5 sec) was determined in 150 mM KCl, 20 μM $^{45}CaCl_2$ and 20 mM Hepes/Tris (pH 7.5). Na^+-dependent Ca^{2+} uptake was obtained as described in Fig. 5. Inset shows a Hill plot of the data. Each point represents the mean±S.E.M. of 6 determinations in 3 different vesicle preparations.

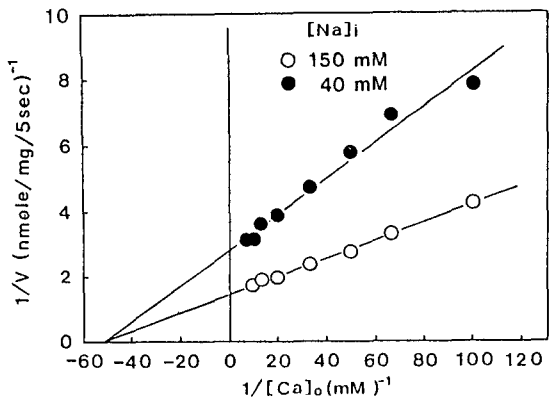

Fig. 14. Effect of internal Na^+ concentration on Na^+-dependent Ca^{2+} uptake as a function of free Ca^{2+} concentration in incubation medium. Membrane vesicles were loaded with 20 mM Hepes/Tris (pH 7.5) and either 150 (○) or 40 (●) mM NaCl, in which NaCl was isoosmotically replaced with KCl. Ca^{2+} uptake (5 sec) was determined in 150 mM KCl, and 20 mM Hepes/Tris (pH 7.5) containing trace amounts of $^{45}CaCl_2$. Free Ca^{2+} concentrations were maintained with 100 μM EGTA and various concentration of $CaCl_2$. Na^+-dependent Ca^{2+} uptake was obtained as described in Fig. 5. Data were presented as a double reciprocal plot (1/V versus $1/[Ca^{2+}]o$) of the mean of 6 determination in 3 different vesicle preparations.

may be transported for each Ca^{2+}.

To examine the nature of the stimulatory effect by intravesicular Na^+ on Ca^{2+} uptake, the Na^+-dependent Ca^{2+} uptake by vesicles loaded with 40 mM or 150 mM Na^+ was determined as a function of external Ca^{2+} concentration. Double reciprocal plot of the data was illustrated in Fig. 14. Increasing the intravesicular loaded concentration of Na^+ from 40 to 150 mM did not significantly alter the apparent Km (18.8 vs. 18.2 μM), whereas significantly increased the Vmax (363.3 to 689.7 pmole/mg protein/5 sec), consistent with a typical noncompetitive interaction.

Effect of K^+ gradient and valinomycin

Finally, the effect of membrane potential on Na^+-dependent Ca^{2+} uptake was tested, since the result in Fig. 13. suggested that the Na^+-Ca^{2+} exchange might operate as an electrogenic process. As shown in Table 4, Na^+-dependent Ca^{2+} uptake was increased by the addition of valinomycin in the presence of an outwardly directed K^+ gradient, a maneuver that rendered the inside of vesicles more positive. These results suggest that the Na^+-Ca^{2+}

Table 4. Effect of K^+ gradient and valinomycin on Na^+-dependent Ca^{2+} uptake

	Ca^{2+} Uptake (nmol/mg protein/min)
Control ($K_i = K_o = 0$)	1.38 ± 0.16
$K_i(0) < K_o(150)$	1.65 ± .14*
$K_i(0) < K_o(150)$ + Valinomycin	1.87 ± 0.19*

Mean ± S.E.M. of 6 determinations in 3 different vesicle preparations.

*, Significantly different from control ($p < 0.05$).

exchange system in sarcolemmal vesicles of cat ileal longitudinal muscle transports a net charge, in agreement with the results reported in nerve (Blaustein & Russell, 1975) and cardiac (Reeves & Hale, 1984) cell membranes. In the present study, the observation that a K^+ gradient without valinomycin stimulated Na^+-dependent Ca^{2+} uptake could resulted from a substitute for Na^+ to enhance the Ca^{2+} uptake demonstrated the specificity of the Na^+ dependence of the Ca^{2+} uptake enhancement (Table 2). A similar specificity was observed in cardiac (Bers et al, 1980) and vascular (Matlib, 1988) sarcolemmal vesicles.

The effects of divalent cations and pH were very similar to that observed in cardiac (Trosper & Phillipson, 1983) and vascular (Matlib, 1988) sarcolemmal Na^+-Ca^{2+} exchange systems. The inhibition of the Na^+-dependent Ca^{2+} uptake by divalent cations is probably caused by competition for binding at Ca^{2+} binding site of the carrier. Mg^{2+}, at 0.5 mM, did not inhibit the Na^+-dependent Ca^{2+} uptake suggesting that at physiological concentration of Mg^{2+}, 0.4 mM in smooth muscle (Kushmerick et al, 1986), the Na^+-Ca^{2+} exchange sysem might remain virtually unaffected.

Amiloride inhibited the Na^+-dependent Ca^{2+} uptake although relatively high concentrations were required, supporting the hypothesis that this component of Ca^{2+} uptake was due to a Na^+-Ca^{2+} exchange carrier activity. The inhibitory potency was similar to that reported by others (Kleyman & Cragoe, 1988).

The apparent Km for Ca^{2+} determined in this study was 18.2 μM. This value was similar to that observed in tracheal smooth muscle (Slaguhter et al, 1987), but significantly higher than that reported in uterine (Grover et al, 1981) and vascular (Matlib, 1988) smooth muscle. The apparent Vmax of 689.7 pmole/mg protein/5 sec was similar to that reported in vascular smooth muscle (Matlib, 1988), but was significantly lower or higher than that of tracheal smooth muscle (Slaughter et al, 1987) or uterine smooth uscle (Grover et al, 1981). However, the kinetic parameters observed in vitro in isolated membranes of smooth muscle should be taken with caution as they may not reflect the true ones of the system *in vivo*. As reviewed by Reeves (1985), there are a number of factors that may change the kinetic parameters of the system including isolation procedure, vesicle size, changes in intravesicular concentrations of Na^+ and Ca^{2+} during assay, membrane high permeability of the membrane vesicles to K^+.

DISCUSSION

The present study was designed to determine the existence and functional characteristics of a Na^+-Ca^{2+} exchange system in sarcolemmal vesicles isolated from cat ileal longitudinal muscle.

Based on marker enzyme study, the membrane fractions used in this study appeared to be enriched by about 22-fold with sarcolemmal membranes as compared to the homogenate. This is a higher enrichment than that reported in isolated sarcolemmal vesicles from rat myometrium (11 fold) by Grover et al (1981) and from rat mesenteric arteries (10 fold) by Matlib et al (1985). These results and electron microscopic examination showed that the membrane fractions used in this study consisted primarily of tightly sealed vesicles derived from sarcolemmal membranes.

In this study, imposition of an outwardly directed Na^+ gradient significantly stimulated the Ca^{2+} uptake as compared to the uptake in the absence of a Na^+ gradient. Such an effect of Na^+ was not replaced by other monovalent cations. These results suggest that sarcolemmal vesicles of cat ileal longitudinal muscle possess a Na^+-Ca^{2+} exchange mechanism.

The stimulation of Ca^{2+} uptake by a Na^+ gradient could result from generation of an inside-negative Na^+ diffusion potential rather than from directly coupled Na^+-Ca^{2+} exchange transport. If this were the case, the Ca^{2+} uptake should be more stimulated by the addition of a Na^+ ionophore, monensin (Pressman, 1968). However, the addition of monensin rather inhibited the Ca^{2+} uptake.

The stimulation of the Ca^{2+} uptake by internal Na^+ was abolished when the concnetration of intravesicular and extravesicular Na^+ were equilibrated. These observation indicate that the enhancement of the Ca^{2+} uptake by intravesicular Na^+ was not merely due to an effect on Ca^{2+} binding to the membranes.

The inability of K^+, Li^+, Rb^+, Cs^+ and choline to orientation and loss of endogenous activatiors or inhibitors. Furthermore, as pointed out by Slaughter et al (1987) and Matlib (1988), the uptake time is very important to estimate true kinetic parameters. Slaughter et al indicated that Na^+-Ca^{2+} exhange uptake time course was significantly curved even at early time and thus, in estimating initial velocities for characterizing kinetic parameters, it is imperative to perform assays at as short time as practicable. From this point of view, the kinetic parameters determined at 5 sec of uptake time was probably not strictly valid, since the uptake at 5 sec may not be in the linear phase. An attempt to measure the Ca^{2+} uptake at times shorter than 5 sec did not produced a consistent result. Another obstacle to extrapolate the results to the situation *in vivo* is that sarcolemmal vesicles isolated from smooth muscles are composed of a mixed population of inside-out and right-side out vesicles (Grover et al, 1980), and so, any differences in Km or Vmax for Na^+-Ca^{2+} exchange transport between these two vesicle groups could not be discerned.

The kinetic study of the enhancement of Ca^{2+} uptake by a Na^+ gradient revealed that raising the internal Na^+ increased the apparent Vmax only without affecting the apparent Km for $Ca^{2+}{}_o$, indicating a typical noncompetitive interaction of $Na^+{}_i$ and $Ca^{2+}{}_o$.

The results in Fig. 13 and Table 4 strongly suggest that the Na^+-Ca^{2+} exchange in this study would be an electrogenic process and have a stoichiometric ratio of greater than $3Na^+$:$1Ca^{2+}$. The stimulation of Na^+-dependent Ca^{2+} uptake by a K^+ gradient was observed even in the absence of valinomycin. This might be due to a chemical stimulation by K^+ *per se* as observed in nerve cells (Dipolo & Beauge, 1988). However, it would be more reasonable to ascribe it to a result of high permeability of the membrane vesicles to K^+ even in the absence of valinomycin. This phenomenon has been recently observed in synaptic plasma membrane vesicles (Barzilai & Rahamimof, 1987).

The physiological role of the Na^+-Ca^{2+} exchange system in cat ileal longitudinal muscle is unknown at this time. The effect of A23187 in this sutdy did not support uphill transport of Ca^{2+} against its concentration gradient by a Na^+-Ca^{2+} exchange system. The time course of Na^+-dependent Ca^{2+} uptake did not show an 'overshoot phenomenon', a transient higher uptake above the equilibrium value, which has been known to be a characteristic of many Na^+-dependent symport and antiport systems. However, these results should be interpreted cautiously because the isolated sarcolemmal vesicles may be abnormally leaky to Na^+. In fact, it has been reported that in sarcolemmal vesicles Na^+ gradient is dissipated in 2 min (Daniel et al, 1982). If this is the case and the time is too short to allow the vesicles to accumulate Ca^{2+} against its concentration gradient,

above results connot be an evidence against the role of the Na^+-Ca^{2+} exchange system in uphill movement of Ca^{2+}.

The apparent Km for Ca^{2+} observed in this study is too high to support the functional role of the Na^+-Ca^{2+} exchange system in regulating the intracellular Ca^{2+} concentration at the cytosolic free Ca^{2+} concentration of 0.2~0.7 μM at resting state (Williams et al, 1987). However, during a contraction or a certain pathological state, $[Ca^{2+}]_i$ may rise to significantly higher concentration range than resting level. Although, there is no precise information about the change in $[Ca^{2+}]_i$ during a contraction in smooth muscle, it has been reported that $[Ca^{2+}]_i$ may rise to between 0.5 and 10 μM at least in cardiac muscle (Huggins & England, 1985). Moreover, the observed kinetic parameters *in vitro* may not be real for various reasons discussed above. Thus, the role of this system in normal and pathological conditions remains to be elucidated. In addition, the role of the Na^+-Ca^{2+} exchange system has been focused in relation with only a Ca^{2+} extrusion mechanism. However, the direction of Ca^{2+} movement by the Na^+-Ca^{2+} exchanger will depend on the stoichiomety of exchange, the membrane potential of the cell, cytosolic Ca^{2+} and Na^+ concentrations, as well as other factors. In fact, as described by Huggins and England (1985), when the stoichiometry of the Na^+-Ca^{2+} exchange is 3:1 and other ion concentration gradients are in physiological range, the Na^+-Ca^{2+} exchange would function as a Ca^{2+} influx mechanism if the membrane potential depolarize to less than −28 mV.

In conclusion, a specific Na^+-Ca^{2+} exchange system was demonstrated in sarcolemmal vesicles isolated from cat ileal longitudinal muscle. The characteristics of the system were very similar to those observed in other tissues. More accurate and detailed knowledge concerning the regulation, and the physiological role of the system remains to be elucidated.

REFERENCES

Aaronson P & van Breemen C (1981). Effects of sodium gradient manipulation upon cellular calcium, ^{45}Ca fluxes and cellular sodium in the guinea-pig taenia coli. *J Physiol* (London) 319, 443-461

Barzilai A & Rahamimof H (1987). Stoichiometry of the sodium-calcium exchanger in nerve terminals. *Biochemistry* 26, 6113-6118

Bers DM, Philipson KD & Nishimoto AY (1980). Sodium calcium exchange and sidedness of isolated cardiac sarcolemmal vesicles. *Biochim Biophys Acta* 601, 358-371

Blaustein MP & Russell DW (1975). Sodium-calcium exchange and calcium-calcium exchange in internally dialyzed squid giant axons. *J Memb Biol* 22, 285-312

Bolton TB (1979). Mechanims of action of transmitters and other substances on smooth muscle. *Physiol Rev* 59(3), 606-718

Bradford (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-day binding. *Anal Biochem* 72, 248-254

Brading AR & Widdicombe JH (1975). Interaction between sodium and calcium movements in smooth muscle. *INSERM* 50, 235-246

Casteels R & van Breemen C (1975). Active and passive Ca^{2+} fluxes across cell membranes of the guinea-pig taenia coli. *Pflügers Arch* 359, 197-207

Cooperstein SJ & Lazarow A (1951). A micorospector-photometric method for the determination of cytochrome oxidase. *J Fiol Chem* 189, 665-670

Daniel EE, Grover AK & Kwan CY (1982). Isolation and properties of plasma membrane from smooth muscle. *Fed Proc* 41(12), 2898-2904

Dipolo R & Beauge L (1988). Ca^{2+} transport in nerve fibers. *Biochim Biophys Acta* 947, 549-569

Enyedi A, Minami J, Caride AJ & Penniston J (1988). Characteristics of the Ca^{2+} pump and Ca^{2+}-ATPase in the plasma membrane of rat myometrium. *Biochem J* 252, 215-220

Fiske CR & SubbaRow Y (1925). The colorimetric determination of phosphorous. *J Biol Chem* 66, 375-400

Grover AK, Crankshaw J, Garfield RE & Daniel EE (1980). Smooth muscle membrane vesicle orientation: a study on intactness and sidedness of rat myometrium plasma membrane vesicles. *Canad J Physiol Pharmacol* 58, 1202-1211

Grover AK, Kwan CY, Garfield RE, McLean J, Fox JET & Daniel EE (1980). Fractionation and Ca uptake studies on membranes of rabbit longitudinal and circular intestinal smooth muscle. *Canad J Physiol Pharmacol* 58, 1102-1113

Grover AK, Kwan CY, Crankshaw J & Daniel EE (1981). Na-Ca exchange in rat myometrium membrane vesicles highly enriched in plasma membranes. *Am J Physiol* 240, D175-C182

Judah JD & Willoughby DA (1964). Inhibitiors of sodium dependent relaxation of guinea-pig ileum. *J Cell Comp Physiol* 74, 363-370

Huggins J & England PJ (1985). The control of calcium pumps and channels and their role in regulating intracellular calcium In: *Molecular Mechanisms of Transmembrane Signaling* Vol 4 Cohen P & Houslay MD (eds) p 57-87 Elsevier, Amsterdam

Kleyman TR & Cragoe Jr EJ (1988). Amiloride and its analogs as tools in the study of ion transport. *J Memb Biol* 105, 1-21

Kushmerick MJ, Dillon RA, Brown TB, Krisanda JM & Sweeney HL (1986). ^{31}P NMR spectroscopy, chemical analysis, and free Mg^{2+} of rabbit bladder and uterine smooth muscle. *J Biol Chem* 261, 14420-14429

Lee HS & Lee SH (1983). Effect of Ca^{2+} and Na^{+} on contraction induced by Na-free solution in Na-loaded ileum of cat. *J Pusan Med College* 23, 179-188

Matlib MA, Crankshaw J, Garfield RE, Crankshaw DJ, Kwan CY, Branda LA & Daniel EE (1979). Characterization of membrane fractions and isolation of purified plasma membranes from rat myometrium. *J Biol Chem* 245, 1834-1840

Matlib MA & Schwartz A (1983). Selective effects of diltiazem, a benzothiazepine clacium channel blocker, diazepam, and other benzodiazepines on the Na^{+}/Ca^{2+} exchange carrier system of heart and brain mitochondria. *Life Sci* 32, 2837-2842

Matlib MA, Schwartz A & Yamori A (1985). A Na^{+}-Ca^{2+} exchange process in isolated sarcolemmal membranes of mesenteric arteries from WKY and SHR rats. *Am J Physiol* 249, C166-C172

Matlib MA (1988). Na^{+}-Ca^{2+} exchange in sarcolemmal membrane vesicles of dog mesenteric artery. *Am J Physiol* 255, C323-C330

Mayer CJ, van Breemen C & Castells R (1972). The action of lanthanum and D600 on the calcium exchange in the smooth muscle cells of the guinea-pig taenia coli. *Pflügers Arch* 337, 333-350

Pressman BC (1968). Ionophores antibiotics as models for biological transport. *Fed Proc* 27, 1283-1288

Raeymaekers L, Wuytack F & Casteels R (1974). Na-Ca exchange in taenia coli of the gunea-pig. *Pflügers Arch* 347, 329-340

Reeves JP & Hale CC (1984). The stoichiometry of the cardiac sodium-calcium exchange system. *J Biol Chem* 259, 7733-7739

Reeves JP (1985). The sarcolemmal sodium-calcium exchange system. In: *Current topics in Membranes and Transport* Vol 25 Shamoo AE (ed) p 77-127 Academic New York

Slaughter RS, Welton AR & Morgan DW (1987). Sodium-calcium exchange in sarcolemmal vesicles from tracheal smooth muscle. *Biochim Biphys Acta* 904, 92-104

Sottocasa GL, Kuylenstierna B, Ernster L & Bergstrand A (1967). An electron-transport system associated with the outer membrane of liver mitochondria. *J Cell Biol* 32, 415-438

Trosper TL & Phillipson KD (1983). Effects of divalent and trivalent cations on Na^{+}-Ca^{2+} exchange in cardiac sarcolemmal vesicles. *Biochim Biophys Acta* 731, 63-68

Weiss GB & Goodman FR (1969). Effect of lanthanum on contraction, calcium distribution and ^{45}Ca movements in intestinal smooth muscle. *J Pharmacol Exp Ther* 169, 46-55

Williams DA, Becker PL & Fey FS (1987). Regional changes in clacium underlying contraction of single smooth muscle cells. *Science* 235, 1644-1648

Abstract =

고양이 회장 종주근에서 Na-Ca 교환 기전의 특성에 관한 연구

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고양이 회장 종주근에서 세포막 소포를 분리하여 Na^+의 농도 경사에 의존하여 일어나는 Ca^{2+} 이동의 특성에 대하여 연구하였다.

막소포 내부에서 외부로 향하는 Na^+의 농도 경사 존재시 Ca^{2+}의 축적이 현저히 증가하여 Na^+ 의존성 Ca^{2+} 축적을 보였으며, 이는 외부용액에 Na^+ *ionophore*인 *monensin*을 처리시 소실되었다. 한편 이러한 Ca^{2+} 축적의 증가 작용은 Na^+에 특이적이었으며 K^+, Li^+, Rb^+, Cs^+ 및 choline 이온은 Na^+의 작용을 대치하지 못하였다. Ba^{2+}, Sr^{2+}, Mn^{2+} 및 Cd^{2+} 등의 2가 양이온들은 0.5 mM의 농도에서 Na^+ 의존성 Ca^{2+} 축적을 억제하였으나 Mg^{2+}은 이 농도에서 억제 효과를 보이지 않았다. 막소포 외부의 pH를 pH 6.0에서 8.5까지 증가시 Na^+ 의존성 Ca^{2+} 축적이 증가하였다. Amiloride는 0.5 mM 이상의 농도에서 Na^+ 의존성 Ca^{2+} 축적을 유의하게 억제하였으나 diltiazem 및 vanadate는 이 농도에서 유의한 억제효과를 보이지 않았다. 동력학적으로 분석하여 측정한 Na^+ 의존성 Ca^{2+} 축적의 Ca^{2+}에 대한 K_m 값은 18.2 μM이었으며 5초에서 측정한 V_{max}값은 689.7 pmole/mg protein이었다. Ca^{2+} 축적에 대한 Na^+ 농도 경사의 효과를 동력학적으로 분석한 결과 막소포 외부에서의 Ca^{2+}에 대한 친화도에는 변화없이 최고 이동치만 증가시켜 전형적인 비상경적 작용 양상을 보였다. Ca^{2+} 축적에 대한 Na^+ 농도 경사의 효과를 Na^+ 농도에 따라 측정하여 Hill plot을 시행한 결과 Hill coefficient가 2.52로 나타났다. 막소포 내부로 향하는 K^+ 농도 경사하에서 valinomycin을 처리하여 막소포 내부에 양전위를 발생시킨 결과 Na^+ 의존성 Ca^{2+} 축적이 증가하였다.

이와 같은 결과들은 고양이 회장 평활근에서 분리한 세포막에 Na^+-Ca^{2+} 교환기전이 존재하고 이는 다른 조직에서 밝혀진 것과 유사한 특성을 지녔으며 electrogenic한 기전으로 작용할 가능성을 시사하였다.