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Demarcation of Ca^{2+} Transport Processes in Guinea Pig Stomach Smooth Muscle

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Summary. Microsomal fractions were isolated from gastric antrum and fundus smooth muscle of guinea pigs. Ca^{2+} uptake into and Ca^{2+} release from the membrane vesicles were studied by a rapid filtration method, and Ca^{2+} transport properties of the different regions of the stomach were compared. ATP-dependent Ca^{2+} uptake was similar in microsomes isolated from both regions. This uptake was increased by oxalate and was not affected by NaN_3. Oxalate affected Ca^{2+} permeability of both antrum and fundus microsome vesicles similarly. Fundus microsome vesicles preincubated in 100 mM NaCl and then diluted to 1/20 concentration with Na^+-free medium had significantly higher ATP-independent Ca^{2+} uptake than vesicles preincubated in 100 mM KCl and treated the same way. This was not true for antrum vesicles. Monensin abolished Na^+-dependent Ca^{2+} uptake, and NaCl enhanced Ca^{2+} efflux from fundus microsome vesicles. The halflife values of Ca^{2+} loss from fundus vesicles in the presence of NaCl were significantly smaller than those in the presence of KCl. The release of Ca^{2+} from the vesicles within the first 3 min was accelerated by NaCl to three times that by KCl. However, NaCl had no effect on Ca^{2+} release from antrum microsome vesicles.

Results suggest two distinct mechanisms of stomach membrane Ca^{2+} transport: (1) ATP-dependent Ca^{2+} uptake and (2) Na^+-Ca^{2+} exchange; the latter in the fundus only.

Key Words concentration-relaxation · Ca^{2+} transport · Na^+-Ca^{2+} exchange · microsome · smooth muscle

Introduction

Patterns of gastrointestinal motility are quite complex, regionally specific, and serve a variety of functions. The stomach has three functional regions: fundus, corpus and antrum. The fundus and corpus are known to act mainly as reservoirs, while the antrum has at least three functions: propelling, retropelling, and triturating the contents. Therefore, stomach smooth muscle might have different motility properties as well as different secretion properties in different regions. Szurszewski and his colleagues simultaneously recorded mechanical and intracellular electrical activity of canine and human gastric smooth muscle and found regional differences in the electrical signal that caused contraction (Szurszewski, 1981).

Calcium controls the contraction-relaxation cycle in smooth muscle as well as in cardiac and skeletal muscle. The level of ionized Ca^{2+} in the myoplasm of the smooth muscle cell is probably very low ($\leqq 10^{-7}$ M) at rest. Several mechanisms have been proposed for reduction of cytoplasmic ionized Ca^{2+}. Ca^{2+} sequestration by various organelles and Ca^{2+} extrusion can be accomplished by an ATP-dependent Ca^{2+} pump or by a Na^+-Ca^{2+} exchange mechanism (Raeymaekers, Wuytack, Batra & Casteels, 1977; Wibo, Morel & Godfraind, 1981; Grover, Kwan & Daniel, 1982). We have already reported sequestration of Ca^{2+} in the presence as well as in the absence of ATP by plasma membrane fraction isolated from canine gastric corpus smooth muscle (Sakai et al., 1981; Sakai, Grover, Fox & Daniel, 1983; Yasuda & Sakai, 1984) and by microsomes isolated from guinea-pig stomach smooth muscle (Sakai et al., 1982; Miyagawa & Sakai, 1985). Some studies point to the existence of a Na^+-Ca^{2+} exchange process in smooth muscle, while others contradict this (Van Breemen, Aaronson & Loutzenliser, 1979).

We observed a large difference in the resting tone of the fundus and antrum, the fundus smooth muscle tone being higher. The difference in Na/Ca permeability agrees with the difference direction of fundic and antral tone, so it could be considered to be contributory although it may not be the entire cause of the difference in tone.

We report here results of investigation of the Ca^{2+} transport properties of gastric fundus and antrum smooth muscle and discuss possible mechanisms underlying the differences between the different regions of the guinea pig stomach based on the use of microsomes isolated from smooth muscle.

Table 1. Distribution of enzyme activity and Ca^{2+} uptake in subcellular fractions

Activities / Fractions		Enzyme		Ca^{2+} uptake	
		5'-Nucleotidase	Mg^{2+}-ATPase	No ATP	5 mM ATP
Antrum	PNS	2.23 ± 0.19	2.19 ± 0.35	0.69 ± 0.10	4.06 ± 1.76
	MIT	2.35 ± 0.13	3.91 ± 0.59	0.75 ± 0.11	3.70 ± 1.00
	MIC	13.61 ± 2.79	17.08 ± 2.62	1.30 ± 0.24	21.29 ± 4.97
Fundus	PNS	3.74 ± 0.29	3.36 ± 0.59	0.45 ± 0.10	4.47 ± 1.23
	MIT	2.68 ± 0.26	3.90 ± 0.77	0.59 ± 0.06	3.44 ± 0.92
	MIC	22.89 ± 3.39	25.46 ± 4.04	1.61 ± 0.51	29.00 ± 7.51

Values are mean ± SE for 6 separate paired preparations.
5'-nucleotidase: μmol/mg protein/hr.
Mg^{2+}-ATPase: μmol/mg protein/10 min.
Ca^{2+} uptake: μmol/g protein/10 min.

Materials and Methods

Animals and Dissection

Male guinea pigs (300–400 g) were stunned, and the stomachs were immediately removed and placed in ice-cold 250 mM sucrose containing 40 mM imidazole (sucrose/IM), pH 7.0, for microsome preparation. The fundus and the antrum were cut from the stomach and opened along the greater curvature to expose the mucosal surface. The entire mucosal layer was easily separated and removed from the muscle layer.

Preparation of Subcellular Membrane Fractions

Pooled isolated smooth muscle layers (4–5 guinea pigs) were used in all experiments. Subcellular membrane fractions were prepared as described earlier (Miyagawa & Sakai, 1985). Briefly, the tissue was weighed, finely minced, and then homogenized for three 5-sec periods in sucrose/IM (1 g/10 ml), using a Braun ET-20 homogenizer. All subsequent preparation steps were carried out at 0–4°C. The homogenate was centrifuged for 10 min at 1000 × *g*. The supernatant (postnuclear supernatant, PNS) was carefully removed and centrifuged for 10 min at 12,000 × *g* to obtain mitochondrial fractions. The second supernatant was centrifuged at 140,000 × *g* for 60 min. This centrifugation yielded crude microsomal fractions. These fractions were suspended in 3–4 ml of sucrose/IM, and then centrifuged at 12,000 × *g* for 10 min to remove a substantial amount of the sediment. The refined microsomal fractions in the resulting supernatant were used for the experiments. All membrane fractions were suspended in sucrose/IM solution.

Enzyme Assays

Enzyme activity was measured as released inorganic phosphate in the medium. Mg^{2+}-ATPase activity was measured in medium containing (in mM): 100 KCl, 5 $MgCl_2$, 5 Na_2ATP, and 40 imidazole at pH 7.0, 37°C. Activity of 5'-nucleotidase was determined according to methods previously described (Matlib et al., 1979). All enzyme reactions were started by adding 0.1 ml protein (40–100 μg) to produce 1 ml final volume of reaction medium. Reaction was stopped after 10 min incubation at 37°C by addition of 1 ml cold 10% tricloroacetic acid. Following centrifugation, the inorganic phosphate present in the supernatant was determined by the method of Fiske and Subbarow (1925).

Ca^{2+} Uptake Measurement

Ca^{2+} accumulation by isolated subcellular membrane fractions was assayed using a filtration technique described earlier (Sakai et al., 1981). A typical reaction mixture contained 250 mM sucrose, 40 mM imidazole-HCl, 5 mM Na_2ATP, 5 mM $MgCl_2$ and 100 μM $CaCl_2$ labeled with $^{45}CaCl_2$ (0.2–1 μCi) at pH 7.0. This gave a calculated concentration of about 20 μM free Ca^{2+} (Janis, Crankshaw & Daniel, 1977). In the absence of ATP, only 20 μM $CaCl_2$ was added. Initial experiments were carried out in various concentrations of EGTA. The Ca^{2+} assay was started by incubating 200 μl of reaction medium plus 50 μl of membrane fraction at 37°C with constant shaking. After 10 min, 200 μl of the suspension was filtered on membrane filters (TOYO TYPE TM-2—0.45 μm pore size), which had been stored in 100 mM 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. For measurement of Na^+–Ca^{2+} exchange, the membrane fractions were preincubated with 100 mM NaCl or KCl at 37°C for 1 hr. Radioactivity on the filters and in the incubation medium was measured in Bray's solution (1960), and background radiation without membrane protein was subtracted. Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall et al. (1951) using bovine serum albumin as standard.

Since the biochemical parameters investigated depend critically upon procedures of isolation of the subcellular membranes, considerable variation from one experiment to another required that assays be performed on a paired basis. This included use of the same batch of solutions on the same day, in each experiment, for every step in preparation, and every biochemical assay of all fractions.

Electron Microscopy

Microsomal pellets were fixed, processed, sectioned, and examined as described previously by Matlib et al. (1979).

Reagents

Na_2ATP, MgATP, Na_2AMP and bovine serum albumin were purchased from Sigma Chemical Co. Analar grade sucrose was purchased from Wako Pure Chemical Industry. $^{45}CaCl_2$ was purchased from New England Nuclear. A23187 and monensin were gifts from Lilly Laboratories. All other reagents were purchased from standard commercial sources.

Results

Distribution of Enzyme Activity and Ca^{2+} Uptake

Enzyme activity and Ca^{2+} uptake by microsomes isolated from the antrum and the fundus of guinea pig stomach smooth muscle are shown in Table 1. Mean values of antrum and fundus tissue wet wt of six separate paired preparations, each pooled from 5 of 30 animals, were 2.08 ± 0.10 and 1.08 ± 0.06 g, respectively. Antrum wet wt was higher than fundus because antrum contains more circular muscle and is thicker than fundus. Protein recoveries of microsomal fractions from antrum and fundus were 1.5 ± 0.2 and 1.2 ± 0.1 mg/g wet tissue, respectively. Activities of 5′-nucleotidase and Mg^{2+}-ATPase were higher in PNS and microsomal fractions from fundus than in such fractions from antrum. There was no obvious difference in enzyme activity of mitochondria fractions from antrum and fundus. In the presence of 5 mM ATP, Ca^{2+} uptake by microsome fractions isolated from fundus was higher than that from antrum fractions. Uptake of Ca^{2+} was not significantly different when compared in PNS and mitochondrial fractions from fundus and antrum in the absence or presence of ATP. However, in all comparisons between the absence and presence of ATP, the differences were significant ($P < 0.001$, Student's t-test of paired samples). The results indicate that Ca^{2+} uptake was dependent on ATP, but independent of all other factors tested, although Ca^{2+} uptake by microsomal fractions was always significantly higher ($P < 0.001$) than uptake by either PNS or mitochondrial fractions.

Time Dependence of Ca^{2+} Uptake

Figure 1 shows time course of ATP-dependent Ca^{2+} uptake by microsomes isolated from antrum and fundus. The Ca^{2+} uptake increased throughout the first 10 min, but the slope after 20 sec was less than the slope from 0 to 20 sec. The initial (first 2 min) time course of Ca^{2+} uptake is shown in the inset of Fig. 1. The Ca^{2+} uptake in the first minute by microsomes isolated from antrum and fundus was 10.6 ± 0.6 and 12.3 ± 0.4 μmol/g protein (mean ± SEM, n = 4), respectively. The Ca^{2+} uptake (after 10 min) by microsomes isolated from fundus was usually (six of eight paired preparations, $P < 0.05$, Student's paired t test) higher than that from antrum.

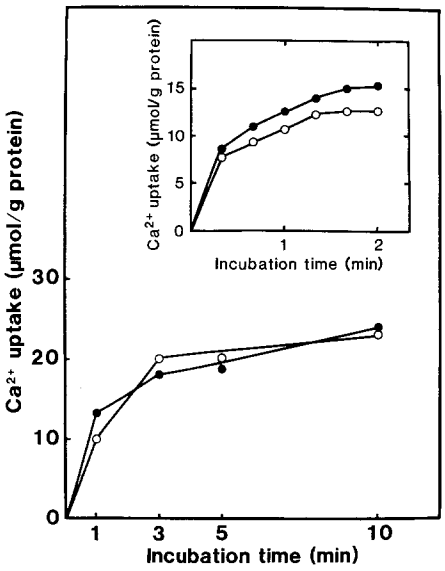

Fig. 1. Time dependence of Ca^{2+} uptake in the presence of ATP. Values are means of 3–4 determinations (○), Antrum microsome; (●), Fundus microsome.

Release of Ca^{2+} from Actively Loaded Vesicles

In order to determine membrane permeability to Ca^{2+}, we studied the release of Ca^{2+} from microsome vesicles that had been preloaded with ^{45}Ca in the presence of ATP, with or without oxalate. Microsome fractions were loaded with ^{45}Ca for 10 min, then diluted to one tenth concentration in isotonic medium containing 2 mM EGTA. Ca^{2+} was lost from vesicles loaded in the presence and absence of oxalate in two or three phases (Fig. 2). When the second phase appeared, it lasted from the first to the third minute and the third phase from the third to the 10th minute. The halflife parameters, $t_{1/2}^{1-3}$ and $t_{1/2}^{3-10}$, of microsome vesicles loaded in the absence of oxalate had mean ± SEM (n = 3) values of 3.7 ± 0.5 and 10.9 ± 1.9 min for antrum and 5.0 ± 0.4 and 10.5 ± 0.5 min for fundus. The comparative halflife values of vesicles loaded in the presence of oxalate were 15.7 ± 0.3 and 30.4 ± 1.5 min for antrum and 16.9 ± 0.4 and 26.9 ± 1.7 min for fundus. Thus, the halflife values indicated no big difference between antrum and fundus vesicles in Ca^{2+} permeability.

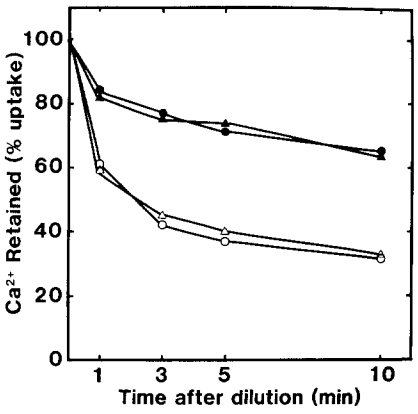

Fig. 2. Time dependence of Ca^{2+} release from actively loaded vesicles. Microsome vesicles (100–150 μg protein) were added to 1 ml of standard reaction mixture in the presence and absence of 5 mM oxalate. After 10 min of Ca^{2+} uptake reaction, aliquots were either filtered directly to determine Ca^{2+} uptake or diluted in isotonic sucrose/IM buffer containing 2 mM EGTA. Aliquots from diluted samples were drawn and filtered at various times. In the presence of 5 mM oxalate: (▲), antrum microsome; (●), fundus microsome. In the absence of 5 mM oxalate: (△), antrum microsome; (○), fundus microsome

Electron Microscopy of Isolated Microsomes

Electron micrographs of thin sections of pellets of microsome fractions are shown Fig. 3. These fractions included various vesicular shapes. The antrum and fundus fractions could not be distinguished by their appearance in the electron micrographs, and no intact mitochondria were evident.

Effects of NaN_3 and Oxalate on Ca^{2+} Uptake

As shown in Table 2, Ca^{2+} uptake in the presence of ATP was increased about two times by 5 mM oxalate, but was not inhibited by 5 mM NaN_3 in microsomes isolated from either the antrum or the fundus. Therefore, this Ca^{2+} uptake was not due to mitochondria contamination only, but was also related to sarcoplasmic reticulum contamination of unknown, but probably small, amount.

Time Dependence of ATP-Independent Ca^{2+} Uptake by Microsome Vesicles Loaded with NaCl

To study the influence of intravesicular NaCl on ATP-independent Ca^{2+} uptake, the microsome membrane vesicles were preincubated in sucrose/IM containing 100 mM NaCl or KCl at 37°C for 1 hr. Figure 4 compares the time dependence of Ca^{2+} uptake by microsomes isolated from antrum and fundus after being preloaded with NaCl or KCl. Figure 4 shows that membranes of both of these microsomes could take up Ca^{2+} and that the Ca^{2+} uptake by a NaCl-loaded vesicles isolated from fundus was greater than uptake by those loaded with KCl. The Ca^{2+} uptake increased with time but was slower after the initial 2 min. The initial (2 min) time course of the Ca^{2+} uptake is shown in Fig. 4*B*. The values of Ca^{2+} uptake by NaCl-loaded vesicles were higher than Ca^{2+} uptake by KCl-loaded vesicles for both antrum and fundus fractions. This difference was significant in the fundus but not in the antrum. The difference between initial Ca^{2+} uptake by vesicles isolated from different regions of the guinea pig stomach was obvious.

Table 2. Effects of oxalate and azide on Ca^{2+} uptake

	Ca^{2+} uptake (μmol/g protein/10 min)		
	Control	5 mM NaN_3	5 mM oxalate
Antrum	19.5 ± 0.5	19.1 ± 0.5	41.9 ± 1.6
Fundus	20.7 ± 0.4	21.2 ± 0.6	36.3 ± 3.1

Effect of NaCl on Release of Ca^{2+} from Actively Loaded Vesicles

In order to determine whether Na^+ alters the membrane permeability to Ca^{2+}, we studied the release of Ca^{2+} from membrane vesicles that had been preloaded with ^{45}Ca in the presence of ATP. Figure 5 shows the release of Ca^{2+} from actively loaded vesicles isolated from antrum and fundus smooth muscle. Actively loaded vesicles were diluted with sucrose/IM buffer containing 2 mM EGTA and 100 mM NaCl or KCl, or 1 μM A23187. Figure 5 inset shows that 1 μM A23187, a Ca^{2+} ionophore, released more than 90% of the accumulated Ca^{2+} within 15 sec. This phenomenon suggests that most of the Ca^{2+} was accumulated in vesicles. As shown in Fig. 5 inset, Ca^{2+} was lost in two or three phases from both antrum and fundus membrane vesicles. In antrum vesicles the retention of Ca^{2+} in the presence of NaCl or KCl was not significantly different, but Ca^{2+} retained by fundus vesicles in the presence of NaCl declined more rapidly than in the presence of KCl. When three phases were evident, the second phase lasted from the first to the third minute. After dilution, release of Ca^{2+} was remarkably accelerated by 100 mM NaCl in this phase in fundus. The halflives, $t_{1/2}^{-3}$, were 5.0 ± 0.2 min (n = 3) in the presence of NaCl and 14.4 ± 0.3 min (n = 3) in the presence of KCl. Therefore, the release of Ca^{2+} from vesicles within the first 3 min was accelerated

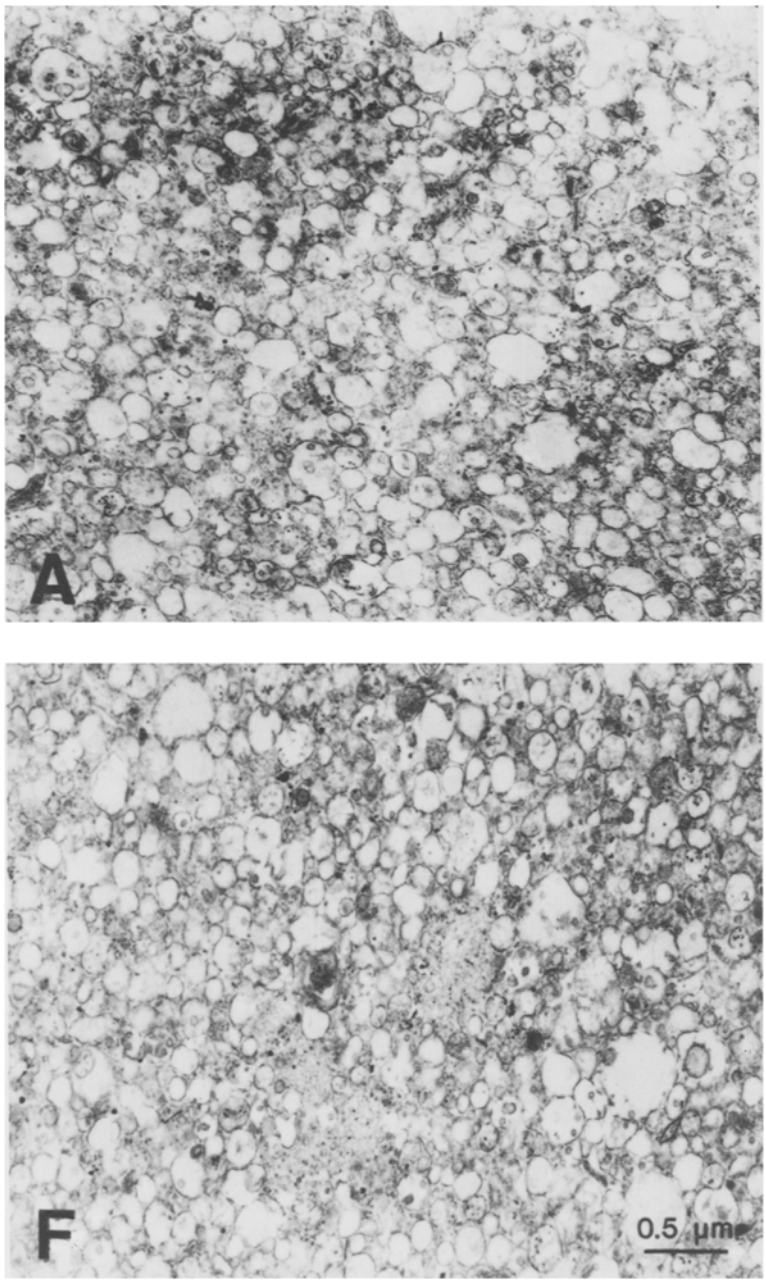

Fig. 3. Electron micrographs of microsome fractions from antrum (*A*) and fundus (*F*)

by 100 mM NaCl to three times that by 100 mM KCl.

Figure 5 shows the loss of Ca^{2+} from vesicles within 1 min. In antrum vesicles the rate of Ca^{2+} loss in the presence of NaCl or KCl was not significantly different, but the initial rate of Ca^{2+} loss in fundus vesicle in the presence of NaCl was greater than in the presence of KCl.

Effect of Monensin on ATP-Independent Ca^{2+} Uptake

Figure 6 shows the effects of monenisn on ATP-independent Ca^{2+} uptake by microsome vesicles loaded with NaCl or KCl. Monensin, a Na^+-selective ionophore, increases Na^+ permeability. There was no difference in Ca^{2+} uptake by K^+-loaded vesicles isolated from either region in the presence or absence of 10 μM monensin. The Ca^{2+} uptake by Na^+-loaded vesicles isolated from antrum was slightly higher than that by K^+-loaded vesicles, but not significantly so. This Ca^{2+} uptake was inhibited by 10 μM monensin. On the other hand, the Ca^{2+} uptake by Na^+-loaded vesicles isolated from fundus was remarkably increased, and this increase was completely blocked in the presence of 10 μM monensin.

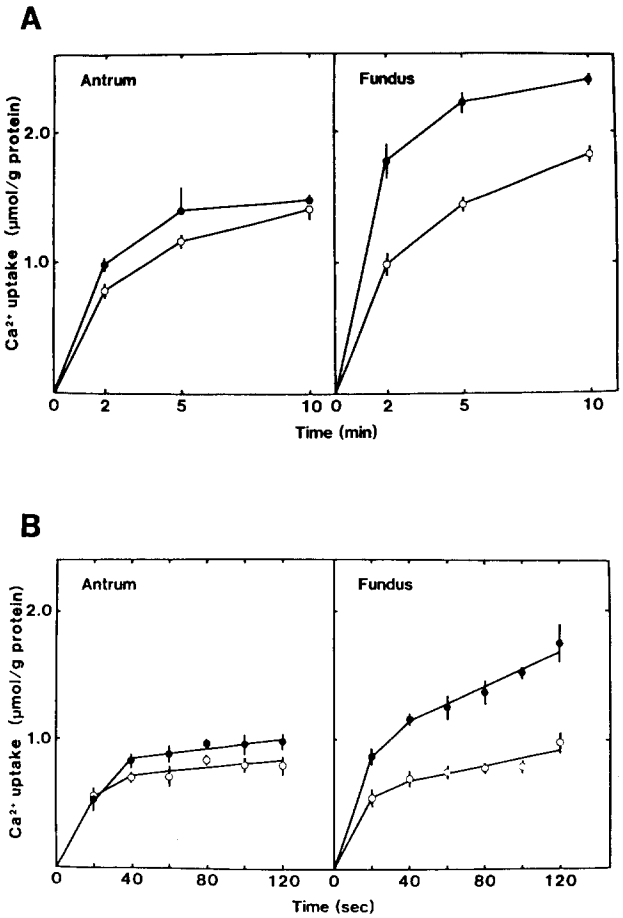

Fig. 4. (*A*) Time dependence of ATP-independent Ca^{2+} uptake. Microsome membrane vesicles were preincubated in sucrose/IM containing 100 mM KCl (○) or NaCl (●) and then diluted 20 times in Ca^{2+} uptake medium in the absence of ATP. Aliquots were filtered from the reaction medium at various times. Data expressed as mean ± SEM (n = 4). (*B*) Initial time dependence of ATP-independent Ca^{2+} uptake. Legends as in *A*

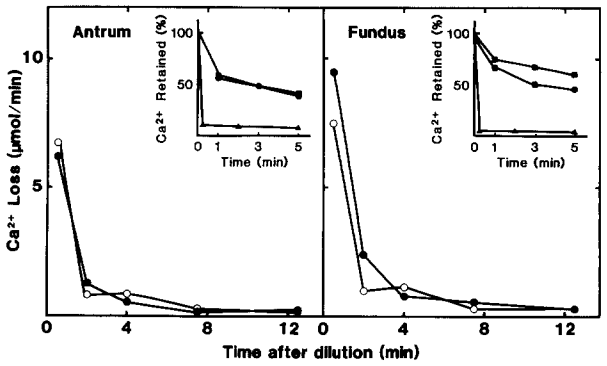

Fig. 5. Time dependence of effect of NaCl on Ca^{2+}-loaded vesicles. To load membranes with ^{45}Ca, microsome membrane vesicles (20–40 μg protein) were reacted in medium containing sucrose/IM, 5 mM MgATP, 100 μM $CaCl_2$ and trace amount of ^{45}Ca at pH 7.0, 37°C. After 10 min of Ca^{2+} uptake, aliquots were either filtered directly to total Ca^{2+} uptake or diluted in sucrose/IM buffer containing 2 mM EGTA, and 100 mM KCl (○) or NaCl (●). Aliquots of sample were filtered at various times. Insets show Ca^{2+} retained as percent of uptake by the same dilution method in the presence of KCl (■) or NaCl (●) or 1 μM A23187 (▲)

Discussion

The results suggest that microsome vesicles isolated from guinea pig stomach possess two distinct mechanisms for Ca^{2+} transport: (1) ATP-dependent Ca^{2+} uptake and (2) Na^+-Ca^{2+} exchange. The ATP-dependent Ca^{2+} uptake mechanism was found in both antrum and fundus, but the Na^+-Ca^{2+} exchange mechanism was found only in the fundus.

The specific activity of 5′-nucleotidase, which is a widely accepted plasma membrane marker enzyme, was significantly less in microsome membrane fractions isolated from the antrum than it was in the corresponding fundus fractions. Mg^{2+}-ATPase, which is also accepted as a plasma membrane marker enzyme (Kwan, 1982) had similar distributions, but the difference between fundus and antrum was not significant. In all microsomal fractions, the activity was six to seven times that of the corresponding PNS fractions (Table 1). The 5′-nucleotidase activity of mitochondrial fractions was similar for those from antrum and fundus, as was the Mg^{2+}-ATPase activity.

The Ca^{2+} uptake of subcellular membrane fractions used in this study was ATP dependent. ATP-dependent Ca^{2+} uptake increased similarly with time in microsomes isolated from antrum and fundus (Fig. 1). ATP-dependent Ca^{2+} uptake by both microsome types increased in the presence of oxalate and was not affected by NaN_3 (Table 2), so these microsome fractions had little mitochondria contamination. The microsome fractions were vesicular structures enclosed by smooth membranes, and no intact mitochondria were evident in electron micrographs (Fig. 3).

It has been previously reported that NaN_3 inhibited ATP-dependent Ca^{2+} uptake by mitochondria fractions (Sakai et al., 1981; Kwan, Sakai, Grover & Lee, 1982). The oxalate effect has been assumed to be due to endoplasmic reticulum contamination, since oxalate has been postulated to activate Ca^{2+} uptake by causing precipitation of Ca^{2+}-oxalate in the vesicles (Hasselbach, 1978). It is known that oxalate diffuses into sarcoplasmic reticulum vesicles of skeletal or cardiac muscle and increases the calcium storage capacity of these vesi-

cles up to 100 times (Hasselbach, 1978). In view of this enhancement of Ca^{2+} transport in endoplasmic reticulum vesicles by oxalate, even slight contamination of purified plasma membrane fractions by such vesicles could significantly increase Ca^{2+} uptake in the presence of oxalate. The distribution of protein, 5'-nucleotidase, NADPH-cytochrome *c* reductase and Ca^{2+} uptake in fundus microsomal suspension centrifuged on a sucrose density gradient indicated the linear correlation coefficient between Ca^{2+} uptake and 5'-nucleotidase activity of various fractions to be 0.96, and between Ca^{2+} uptake and NADPH-cytochrome *c* reductase activity of various fractions to be 0.56 (Y. Sakai, *unpublished data*). This suggests that plasma membrane content determined the extent of Ca^{2+} uptake since sarcoplasmic reticulum in most types of smooth muscle is about one third that in skeletal muscle (Devine, Somlyo & Somlyo, 1972), so most of the microsome vesicles from smooth muscle might be formed from the plasma membrane.

In the presence of ATP, Ca^{2+} uptake was inhibited by the Ca^{2+} ionophore A23187, indicating the existence of an active Ca^{2+} transport process (Wuytack, Landon, Fleisher & Hardman, 1978; Sakai et al., 1981, 1983). Intravesicular Ca^{2+} was released by A23187 (Fig. 5 inset). ATP-dependent Ca^{2+} uptake by our microsome fractions was believed to be due to the inside-out vesicles and presumably reflected the activity of an outwardly directed Ca^{2+} pump in intact cells (Sakai et al., 1981). ATP-dependent Ca^{2+} uptake and Ca^{2+} release by both types of microsome vesicles are not very different. Ca^{2+} permeability of microsome vesicles from fundus and antrum was very similar in the absence and presence of oxalate (Fig. 2). These results indicate similarities in: (1) proportion of intact vesicles, (2) orientation of the vesicles, (3) permeability to Ca^{2+}, and (4) Ca^{2+} pump activity in these two preparations.

The data presented in Figs. 4 to 6, show clear-cut evidence of a Na^+-Ca^{2+} exchange process localized in microsomal vesicles from fundus smooth muscle. The differences in Na^+-Ca^{2+} exchange by the two types of membrane vesicles must either be due to inherent differences between the two tissues or to difference in membrane leakage caused by the isolation procedures. The former is more plausible, since both were isolated by the same procedures and the latter is not indicated by permeability to Ca^{2+} in the presence and absence of oxalate (Fig. 2). Figure 5 insets show that most accumulated Ca^{2+} was intravesicular, since A23187 released most of the Ca^{2+} from the vesicles on a downhill Ca^{2+} gradient. The Na^+ gradient facilitates a downhill movement of Ca^{2+} by changing membrane permeability

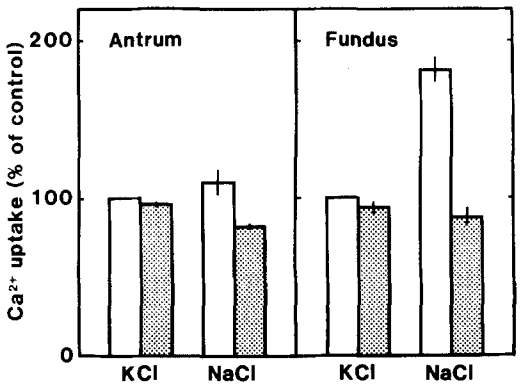

Fig. 6. Effect of monensin on ATP-independent Ca^{2+} uptake. Microsome membrane vesicles were preincubated in sucrose/IM containing 100 mM KCl or NaCl for 1 hr at 37°C. Preincubated membranes were then diluted to 1/20 concentration into Ca^{2+} uptake medium at 37°C. In the absence (□) or presence of 10 μM monensin (▨). Data expressed as mean ± SEM (n = 5) taking Ca^{2+} uptake by KCl incubated vesicles as 100% in the absence of monensin

to Ca^{2+} since Na^+ caused Ca^{2+} release from the vesicles on a downhill Ca^{2+} gradient (Fig. 5). The Ca^{2+} uptake in 2 min by Na^+-loaded vesicles was significantly greater than the uptake by K^+-loaded vesicles in fundus. This Na^+-dependent Ca^{2+} uptake was abolished in the fundus fraction by the monovalent cation (Na^+) ionophore, monensin (Fig. 6). Since monensin eliminated the Na^+ gradient by increasing the Na^+ permeability, Ca^{2+} uptake that depends on the Na^+ gradient would be depressed.

The localization of Na^+-Ca^{2+} exchange in gastric muscle might be related to membrane Na^+ permeability. The Na^+-Ca^{2+} exchange mechanism depends not only on $(Na)_o$ and $(Ca)_i$, but also on $(Na)_i$, which is modified by $(K)_o$ and by the Na^+-K^+ electrogenic pump (Taylor, Paton & Daniel, 1970; Casteels, Droogmans & Hendricks, 1973). A Na^+-Ca^{2+} exchange coupled to the Na^+ pump has also been demonstrated in isolated cardiac sarcolemma vesicles (Pitts, 1979). There are several reports of the Na^+-Ca^{2+} exchange mechanism in intact smooth muscle (Reuter, Blaustein & Haeusler, 1973; Brading, 1978; Ozaki & Urakawa, 1979). All suggest that increased intracellular Na^+ concentration, resulting from inhibition of the Na^+-K^+ pump, increases intracellular Ca^{2+} concentration by reducing Ca^{2+} efflux and/or increasing Ca^{2+} influx through a Na^+-Ca^{2+} exchange process.

Recently, Grover and coworkers (Grover, Kwan & Daniel, 1981; Grover, Kwan, Rangachari & Daniel, 1983) reported Na^+-Ca^{2+} exchange activity in rat myometrium plasma membrane vesicles.

They demonstrated that Ca^{2+} uptake or release by plasma membrane vesicles as a Na^+-Ca^{2+} exchange process depends on the direction of the Na^+ gradient. In our study we also demonstrated Na^+ gradient-dependent Ca^{2+} uptake (Figs. 4 and 6) and Ca^{2+} release (Fig. 5) by fundus microsome vesicles. However, we were unable to demonstrate a Na^+-Ca^{2+} exchange process after preincubation of microsome vesicles at 23°C, as reported for plasma membrane vesicles by Grover et al. (1981). We observed Na^+-Ca^{2+} exchange after preincubation at 37°C as reported by Pitts (1979) who used cardiac sarcolemma. We found that it was first necessary to preload the vesicles by incubation at 37°C with 100 mM NaCl to demonstrate Na^+-Ca^{2+} exchange. Thermodynamic considerations predict that, at equilibrium, the Na^+ transport. The comparison of Na^+-Ca^{2+} exchange-dependent Ca^{2+} uptake by uterine and cardiac muscle plasma membranes was reported by Grover et al. (1983). The Na^+-Ca^{2+} exchange by the cardiac sarcolemma is stimulated by KCl plus valinomycin and by H^+ uncouplers, presumably due to their ability to dissipate the electric potential generated by Na^+-Ca^{2+} exchange which allows movement of K^+ and H^+ uncouplers. However, in myometrium such stimulation was not observed, perhaps because in these membranes, even without these ionophores, the charge compensation mechanism can operate sufficiently faster than the Na^+-Ca^{2+} exchange. Thus, the low magnitude of the Na^+-Ca^{2+} exchange in the myometrium makes it difficult to study its mechanisms in detail with such low magnitude of Na^+-Ca^{2+} exchange. Our microsome fraction still had small contamination, so we want to characterize the Na^+-Ca^{2+} exchange system in detail after better isolation of plasma membrane from the microsomes.

In conclusion, the present experiments suggest that both an ATP-driven and a Na^+-Ca^{2+} exchange Ca^{2+} transport process exist in microsomes isolated from guinea pig stomach. The Ca^{2+} pump may reduce intracellular Ca^{2+} in both antrum and fundus smooth muscle, while a Na^+-Ca^{2+} exchange process is involved in the control of intracellular Ca^{2+} concentration in the fundus. However, in fundus membrane vesicles, the extent of net Ca^{2+} movement driven by the Na^+ gradient was smaller (1–2 μmol/g protein) than the ATP-dependent transport (10–40 μmol/g protein). Therefore, an active Ca^{2+} pump is capable of major control of Ca^{2+} in stomach smooth muscle and a Na^+-Ca^{2+} exchange system makes some unknown contribution to this control.

The results of the present studies provide an explanation for the report of Kuriyama et al. (1976) on the regional differences in electrical and mechanical activities of guinea pig stomach smooth muscle. It is difficult, at present, to estimate biochemical characterization on a quantitative basis because heterogeneous fractions were used in this study. Subfractions of these microsomal fractions should be characterized.

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