Sodium/calcium exchange in smooth-muscle microsomal fractions

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1. The existence of Na^+ -dependent Ca^{2+} transport was investigated in microsomal fractions from the longitudinal smooth muscle of the guinea-pig ileum and from the rat aorta, and its activity was compared with that of the plasmalemmal ATP-dependent Ca^{2+} pump previously identified in these preparations. 2. The rate of Ca^{2+} release from plasmalemmal vesicles previously loaded with Ca^{2+} through the ATP-dependent Ca^{2+} pump was transiently faster in the presence of 150 mM-NaCl in the medium than in the presence of 150 mM-KCl or -LiCl or 300 mM-sucrose. 3. Na^+ -loaded vesicles took up Ca^{2+} when an outwardly directed Na^+ gradient was formed across the membrane. The Ca ionophore A23187 induced a rapid release of 85% of the sequestered Ca^{2+} , whereas only 15% was displaced by La^{3+} . Ca^{2+} accumulated by the Na^+ -induced Ca^{2+} transport was released by the addition of NaCl, but not KCl, to the medium. 4. Ca^{2+} uptake in Na^+ -loaded vesicles was inhibited in the presence of increasing NaCl concentration in the medium. Half-maximum inhibition was observed with 28 mM-NaCl. Data fitted the Hill equation, with a Hill coefficient (h) of 1.9. 5. Na^+ -induced Ca^{2+} uptake was a saturable function of Ca^{2+} concentration in the medium. Half-maximum activity was obtained with $18 \mu\text{M}$ - Ca^{2+} in intestinal-smooth-muscle microsomal fraction and with $50 \mu\text{M}$ - Ca^{2+} in aortic microsomal fraction. 6. The results suggest that in these membrane preparations a transmembrane movement of Ca^{2+} can be driven by a Na^+ gradient. However, the Na^+ -induced Ca^{2+} transport had a lower capacity, a lower affinity and a slower rate than the ATP-dependent Ca^{2+} pump.

Participation of a $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism in the control of cytoplasmic free Ca^{2+} concentration in the smooth muscle cell is still a matter of controversy (Brading, 1981). On the basis of the first observations on Na^+ -linked Ca^{2+} transport in nerves (Baker *et al.*, 1969) and in heart muscle (Reuter, 1974), the $\text{Na}^+/\text{Ca}^{2+}$ exchange model states that some transmembrane movement of Ca^{2+} is coupled to oppositely directed Na^+ flux, so that the Na^+ electrochemical gradient provides the energy for Ca^{2+} transport. In smooth muscle, evidence that Na^+ may regulate intracellular Ca^{2+} concentration (Ca_i) has been presented, but the significance of these results may be questioned (for review see van Breemen *et al.*, 1978). It has been reported that inhibition of the Na^+-K^+ pump leads to a rise in Ca_i and an increase in muscle tension (Lang & Blaustein, 1980; Ozaki *et al.*, 1978). In some tissues, contraction induced by low extracellular $[\text{Na}^+]$ is inhibited by α -blockade or denervation (Broeckaert & Godfraind, 1973), but

Ozaki & Urakawa (1981) and Droogmans & Casteels (1979) reported that, in some vascular smooth muscles, contractions induced by Na^+ -free medium were not blocked by phentolamine. Ozaki & Urakawa (1981) suggested that the contractile response was caused by a Ca^{2+} influx dependent on internal Na^+ , whereas Droogmans & Casteels (1979) concluded that Na^+ -free contraction was not attributable to a $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism.

Ion-flux experiments in intact tissue are difficult to interpret, owing to the high complexity of the cellular distribution of ions and to $\text{Na}^+/\text{Ca}^{2+}$ interactions occurring at extracellular binding sites (Burton & Godfraind, 1974; Brading, 1979). A $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism has been clearly demonstrated in plasma-membrane fractions from heart (Reeves & Sutko, 1979; Caroni *et al.*, 1980), brain (Gill *et al.*, 1981) and myometrium (Grover *et al.*, 1981). The purpose of the present work was to study the influence of Na^+ on Ca^{2+} movements

in plasma-membrane vesicles isolated from the longitudinal smooth muscle of guinea-pig ileum and from rat aorta. The results show that in these preparations a transmembrane movement of Ca^{2+} can be driven by an opposite Na^+ gradient, but that the Na^+ -dependent Ca^{2+} transport has a lower capacity than the ATP-dependent Ca^{2+} pump that is present in the same vesicles.

Preliminary communications of some of these results have been presented to the Belgian Physiological Society (Morel & Godfraind, 1982) and to the Physiological Society (Godfraind & Morel, 1983).

Methods

Preparation of microsomal fractions

Microsomal fractions were isolated from the longitudinal smooth muscle of the guinea-pig ileum as described previously (Wibo *et al.*, 1981). Briefly, the longitudinal muscle layer was separated from the ileum and transferred into ice-cold 0.25M-sucrose buffered at pH 7.4 with Tris (sucrose/Tris). All further steps were performed at 2°C. Tissue was homogenized with sucrose/Tris in an all-glass Potter-Elvehjem-type grinder. The homogenate was first centrifuged at 1000g for 10 min. The supernatant was spun at 20000g for 30 min in a fixed-angle rotor of an Omega II ultracentrifuge (Heraeus-Christ, Osterode am Harz, Germany). The supernatant from this was centrifuged at 100000g for 1 h to yield a microsomal pellet, which was suspended in 5 ml of 5 mM-Tris/HCl buffer (pH 7.4, 0°C). Rat aorta microsomal fractions were prepared as described previously (Morel *et al.*, 1981). Subfractionation of these microsomal preparations by density-gradient centrifugation showed that most of their membrane elements originated from the plasmalemma (see Wibo *et al.*, 1981; Morel *et al.*, 1981). Microsomal fractions were kept at 0°C until used. Ca uptake was measured within 24 h after preparation of the microsomal fractions. Protein was determined by the method of Lowry *et al.* (1951), with human serum albumin as standard.

ATP-dependent Ca^{2+} uptake

Microsomal fractions (10–40 µg of protein/ml) were incubated at 37°C with 20 mM-maleate, 100 mM-KCl, 5 mM-MgCl₂, 0.2 mM-CaCl₂ labelled with ⁴⁵CaCl₂ (1.6 µCi/ml), 3 mM-ATP and 5 mM-NaN₃. EGTA was added to control free Ca^{2+} concentration (Godfraind *et al.*, 1976). Maleate, ATP and EGTA were adjusted to pH 7.4 (37°C) with Tris. Ca^{2+} uptake was started by adding ATP. At the end of the incubation, samples were filtered through Sartorius filters (SM 11306; 0.45 µm pore size) prewashed with 1 M-KCl. The

filters were rapidly washed with 20 ml of ice-cold sucrose/Tris, and the radioactivity retained on the filters was measured as described previously (Morel *et al.*, 1981). Previous studies showed that ⁴⁵Ca uptake measured in these conditions occurred in vesicles originating from the plasmalemma (Morel *et al.*, 1981; Wibo *et al.*, 1981).

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

Microsomal fractions (1–2 mg of protein/ml) were kept overnight at 0°C in a medium containing 5 mM-Tris/HCl buffer (pH 7.4) and 150 mM-NaCl (unless otherwise stated) in order to load the vesicles with Na^+ . Ca^{2+} uptake was assayed at 37°C in a medium containing 20 mM-Tris/maleate buffer (pH 7.4), 150 mM-NaCl or -KCl and 0.1 mM-CaCl₂ labelled with ⁴⁵CaCl₂ (1.6 µCi/ml). EGTA was added to control the free Ca^{2+} concentration (Godfraind *et al.*, 1976). Ca^{2+} uptake was started by diluting Na^+ -preloaded microsomal fraction 1:40 into the incubation medium. After 3 min incubation, samples of the medium were filtered through Sartorius filters, which were then rapidly washed with 20 ml of ice-cold sucrose/Tris, and the radioactivity retained on the filters was measured by liquid-scintillation spectrometry as described previously (Morel *et al.*, 1981). Na^+ -induced ⁴⁵Ca uptake was determined by subtracting ⁴⁵Ca uptake measured in the presence of 150 mM-NaCl from ⁴⁵Ca uptake measured in Na^+ -free medium containing 150 mM-KCl instead of NaCl.

Statistical analysis

Data are expressed as means ± S.E.M. A least-squares linear-regression analysis was used to fit a straight line to data where appropriate.

Results

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

The effect of Na^+ on the rate of Ca^{2+} efflux from Ca^{2+} -loaded plasma-membrane vesicles is shown in Fig. 1. Intestinal smooth-muscle microsomal fractions were preincubated in the presence of ATP and ⁴⁵Ca in order to load plasmalemmal vesicles with ⁴⁵Ca through the ATP-dependent Ca^{2+} pump (Morel *et al.*, 1981). After 15 min incubation, membrane vesicles contained 25.5 ± 0.75 ($n = 8$) nmol of Ca^{2+} /mg of protein. They were then diluted 1:50 in 20 mM-Tris/maleate buffer (pH 7.4) containing 1 mM-EGTA (to block further Ca^{2+} uptake) and either 150 mM-KCl, -LiCl or -NaCl or 300 mM-sucrose. Fig. 1 shows that the fastest Ca^{2+} -efflux rate was observed in the presence of NaCl and the slowest in the presence of sucrose. At 1 min after dilution, NaCl-treated vesicles had lost 52% of their ⁴⁵Ca content, whereas dilution in the presence of KCl or LiCl

Fig. 1. Effect of NaCl on Ca²⁺ release from intestinal smooth-muscle microsomal fractions

Microsomal fractions (0.3mg of protein/ml) were loaded with ⁴⁵Ca by the ATP-dependent Ca²⁺-uptake system (50μM-⁴⁵CaCl₂; incubation time 15min at 37°C). After filtration of duplicate 0.1 ml samples, 0.7 ml of the medium was immediately diluted 1:50 into 20mM-Tris/maleate buffer (pH 7.4) containing 150mM-KCl (▲), -LiCl (○) or -NaCl (●), or 300mM-sucrose (■) (zero time). Samples (5ml) were then filtered at various time intervals. Microsomal Ca²⁺ content at zero time was 25.5 ± 0.75 nmol/mg of protein (n = 8). Similar results were obtained in two other experiments.

Fig. 2. Time course of ATP-dependent (▲) and Na⁺-induced (●) Ca²⁺ uptake in intestinal smooth-muscle microsomal fraction

Ca²⁺ uptake was measured in 5 ml of incubation medium; 0.6 ml samples of the medium were filtered at various time intervals. Free Ca²⁺ concentration was 50μM, and temperature 37°C. Each point is the mean of two determinations on two different membrane preparations.

produced only 30% release of the ⁴⁵Ca content. The stimulation by Na⁺ of Ca²⁺ efflux lasted only 4 or 5 min, after which Ca²⁺ loss occurred at a rate similar to that observed in KCl or sucrose solution, as indicated by the similar value of the slope that characterized the different curves after this time, when they were plotted on a semi-logarithmic scale (results not shown).

Na⁺-dependent Ca²⁺ uptake

Intestinal smooth-muscle microsomal fractions were preincubated overnight in 150mM-NaCl (see the Methods section) and then diluted 1:40 into a medium containing 150mM-KCl and 50μM-⁴⁵CaCl₂. Fig. 2 shows the time course of the Na⁺-dependent Ca²⁺ uptake, i.e. the uptake that is evoked by an outwardly directed Na⁺ gradient. This uptake reached a steady state after 1.5 min, the ⁴⁵Ca content of vesicles being then equal to about 4 nmol of Ca²⁺/mg of protein. Fig. 2 also illustrates ATP-dependent ⁴⁵Ca uptake by overnight-stored microsomal fractions. Compared with ATP-dependent uptake, the Na⁺-dependent Ca²⁺ uptake showed a slower rate and a much lower capacity. When Na⁺-loaded vesicles were diluted into incubation media containing different Na⁺ substitutes, Ca²⁺ uptake slightly increased, in the order LiCl < sucrose < KCl (salt concn. 150mM, sucrose concn. 300mM) (results not shown). KCl was routinely used in subsequent experiments.

To determine whether Na⁺-dependent Ca²⁺ accumulation resulted from Ca²⁺ uptake into the vesicles or from Ca²⁺ binding to superficial sites on the membranes, the effects of the Ca ionophore A23187 and of LaCl₃ on the ⁴⁵Ca content were examined (Fig. 3). Na⁺-loaded vesicles were incubated for 3 min in iso-osmotic KCl containing 50μM-⁴⁵CaCl₂ and then further incubated after addition of 5μM-A23187 or 0.5mM-LaCl₃. La³⁺ released only 15% of the ⁴⁵Ca content. As La³⁺ is known to displace Ca²⁺ from external binding sites (Weiss & Goodman, 1969), superficially bound Ca²⁺ might account for some 15% of the Ca²⁺ uptake. In agreement with this inference, A23187 induced a rapid release of 85% of the ⁴⁵Ca content, suggesting that indeed most of the ⁴⁵Ca had been accumulated inside the vesicles against a concentration gradient. We observed that, of the ⁴⁵Ca taken up by Na⁺-loaded vesicles after dilution in Na⁺-containing medium, i.e. in the absence of a Na⁺ gradient, 65% was displaced by LaCl₃, but the amount was unmodified by A23187.

Fig. 3 shows that addition of 57mM-NaCl to the incubation medium induced the release of the ⁴⁵Ca accumulated by the Na⁺-dependent system, whereas the addition of the same concentration of KCl had no effect on the ⁴⁵Ca content.

Fig. 3. Time course of Ca^{2+} release

Na^+ -loaded microsomal fractions were diluted 1:30 into Na^+ -free medium containing $50 \mu\text{M}$ - CaCl_2 labelled with $^{45}\text{CaCl}_2$. After 3 min incubation, A23187 (final concn. $5 \mu\text{M}$; $\square$), LaCl_3 (final concn. 0.5 mM ; $\blacksquare$), NaCl (final concn. 57 mM ; $\blacktriangle$) or KCl (final concn. 57 mM ; $\bullet$) was added to the medium. All values were corrected for Ca^{2+} content of microsomal fractions incubated for 3 min with ^{45}Ca in the presence of 150 mM - NaCl and then treated as described above. Each value is the mean of three determinations on a single preparation. Similar results were obtained in a second experiment.

The influence of extravesicular Na^+ (Na_o^+) concentration on the extent of Ca^{2+} uptake by Na^+ -loaded vesicles is shown in Fig. 4. In this experiment, NaCl_o was varied from 5 to 155 mM and the iso-osmoticity was maintained by replacing NaCl by KCl . The inset shows the same data analysed according to the Hill equation, by plotting $\log [v/(V-v)]$ versus $\log \text{Na}_o^+$, where v is the ^{45}Ca content measured at the given Na_o^+ value, V is the maximal ^{45}Ca content and Na_o^+ is the NaCl concentration in the medium (expressed in mM). The relation between $\log [v/(V-v)]$ and $\log \text{Na}_o^+$ was linear, and the Hill coefficient (h , calculated by linear regression) was 1.86. The Na_o^+ concentration producing half-maximal depression of Ca^{2+} uptake (intersection with the abscissa) was 28 mM . This value is close to that reported by Reeves & Sutko (1979) for $\text{Na}^+/\text{Ca}^{2+}$ exchange in cardiac sarcolemma (half-maximal inhibition at 16 mM - NaCl_o).

The inhibitory effect of Na_o^+ on Na_i^+ -induced Ca^{2+} uptake may be due to a decrease of the

Fig. 4. Effect of the NaCl concentration in the incubation medium on the Na^+ -induced Ca^{2+} uptake by intestinal smooth-muscle microsomal fraction

Na^+ -induced Ca^{2+} uptake was measured 3 min after dilution of Na^+ -loaded microsomal fractions into 20 mM -Tris/maleate buffer ($\text{pH } 7.4$, 37°C) containing $50 \mu\text{M}$ - $^{45}\text{CaCl}_2$ and various concentrations of NaCl . KCl was added to maintain the salt concentration in the medium at 150 mM . NaCl concentrations indicated on the abscissa are corrected for NaCl in the microsomal fraction. Values are means $\pm$ S.E.M. for three determinations. The inset shows the same data plotted by the Hill equation {abscissa, $\log$ of NaCl concentration in the medium (mM); ordinate, $\log [v/(V-v)]$, where v is the ^{45}Ca content at a given NaCl concentration in the medium and V the maximal Ca content}.

$\text{Na}_i^+/\text{Na}_o^+$ ratio when Na_o^+ is increased, or to the interaction of Na_o^+ with the Ca^{2+} -binding sites of the carrier. A Hill coefficient of around 2 could then suggest that one Ca^{2+} exchanged with 2 Na^+ . However, it could also reflect the fact that more than one Na^+ ion binds to the carrier on its Ca^{2+} -binding sites, thereby producing inhibition of the translocation of one Ca^{2+} ion.

To confirm that a Na^+ gradient was a prerequisite for ATP-independent Ca^{2+} accumulation, microsomal fractions were exposed to KCl , LiCl or sucrose instead of NaCl during the overnight preincubation, and then diluted in Na^+ or K^+ medium. Under those conditions, ^{45}Ca uptake was much lower than in Na^+ -loaded vesicles diluted in Na^+ -free medium (Table 1). Moreover, the Ca ionophore A23187 failed to release ^{45}Ca taken up by KCl -, LiCl - or sucrose-loaded vesicles, suggesting that this Ca^{2+} uptake might reflect Ca^{2+} binding at superficial sites (results not shown).

Effect of Ca^{2+} concentration on Ca^{2+} uptake

Fig. 5 shows that, in intestinal and aortic Na^+ -loaded microsomal fractions, the extent of ^{45}Ca uptake was a saturable function of Ca^{2+} concentration, the maximum being observed at 0.1 mM -

Table 1. ATP-independent Ca²⁺ uptake in intestinal smooth-muscle microsomal fraction

Intestinal smooth-muscle microsomal fractions were preincubated at 0°C overnight in 5mM-Tris/HCl buffer (pH7.4) with different salts or with sucrose. Ca²⁺ uptake was measured 3min after dilution of the microsomal fractions 1:40 into 20mM-Tris/maleate buffer containing 50μM-CaCl₂ labelled with ⁴⁵Ca and either KCl or NaCl (150mM). Data are means ± s.e.m. for *n* determinations obtained with at least two different membrane preparations.

Loading medium	<i>n</i>	Incubation medium ...	Ca ²⁺ content (nmol/mg of protein)	
			KCl	NaCl
NaCl (150mM)	8		4.36 ± 0.09	1.14 ± 0.09
KCl (150mM)	8		2.06 ± 0.15	1.04 ± 0.05
LiCl (150mM)	5		2.57 ± 0.09	1.24 ± 0.10
Sucrose (300mM)	5		2.23 ± 0.15	1.06 ± 0.08

Pitts & Okhuysen (1980) (26μM) and by Reeves & Sutko (1979) (18μM), and the half-maximal value of Na⁺-dependent Ca²⁺ uptake was obtained at 7μM-Ca²⁺ in myometrium plasma-membrane vesicles (Grover *et al.*, 1983).

The Na⁺-induced Ca²⁺ uptake by both types of smooth-muscle microsomal fractions operated with a lower maximal capacity than did the ATP-driven plasmalemmal Ca²⁺ pump. Moreover, the latter was characterized by a higher apparent Ca²⁺ affinity. Indeed, its *K*_{0.5} value was 4μM for intestinal smooth-muscle microsomal fraction (Fig. 5), and was about 10μM for aortic microsomal fraction in the presence of calmodulin (Morel *et al.*, 1981). We found that the Na⁺-dependent Ca²⁺ uptake by both types of smooth-muscle microsomal fractions was unaffected by calmodulin (results not shown), in agreement with the results reported by Caroni & Carafoli (1981) for cardiac sarcolemma.

Discussion

This paper shows that, in addition to an ATP-dependent Ca²⁺ pump, intestinal and aortic smooth-muscle microsomal fractions contain a Na⁺-dependent Ca²⁺-transport activity. In the absence of ATP, Ca²⁺ can be taken up by membrane vesicles when an outwardly directed Na⁺ gradient is generated across the membrane. Suppression of the Na⁺ gradient, either by increasing the extravesicular Na⁺ concentration or by allowing the Na⁺ gradient to dissipate by passive diffusion of Na⁺ (results not shown) inhibits this Ca²⁺ uptake. Na⁺-dependent Ca²⁺ accumulation does not appear to be the result of Ca²⁺ binding to superficial sites, as La³⁺ is ineffective in releasing Ca²⁺ accumulated by this process. Moreover, the nearly complete release of Ca²⁺ by a Ca ionophore shows that Ca²⁺ is taken up and accumulated inside the Na⁺-loaded vesicles. The saturable nature of the Ca²⁺-dependence of Na⁺-induced Ca²⁺ uptake is consistent with a

Fig. 5. Effect of free Ca²⁺ concentration on ATP-dependent (Δ) and Na⁺-induced Ca²⁺ uptake in guinea-pig intestinal smooth-muscle (●) and rat aorta (■) microsomal fractions

Ca²⁺ content was measured 15 min after addition of ATP (ATP-dependent Ca²⁺ uptake) or 3 min after dilution of Na⁺-loaded microsomal fractions into Na⁺-free medium (Na⁺-induced Ca²⁺ uptake). For Ca²⁺ concentrations up to 0.1mM, total Ca²⁺ concentration was 0.2mM for ATP-dependent and 0.1mM for Na⁺-induced Ca²⁺ uptake. Free Ca²⁺ was controlled by adding various concentrations of EGTA. Experiments were performed with three different membrane preparations; each point is the mean ± s.e.m. for 3–12 determinations.

Ca²⁺. The Ca²⁺ concentration at which half-maximal Ca²⁺ accumulation was observed (*K*_{0.5}) was 50μM for aortic microsomal fraction and 18μM for intestinal smooth-muscle microsomal fraction. Similar *K*_{0.5} values have been reported for the Na⁺/Ca²⁺ exchange system in cardiac vesicles by

carrier-mediated process. This Ca^{2+} carrier seems to operate in both directions as an inwardly directed Na^{+} -gradient-induced Ca^{2+} efflux from Ca^{2+} -loaded vesicles. A gradient of other cations, e.g. K^{+} or Li^{+} , was ineffective in driving Ca^{2+} transport.

Since Ca^{2+} sequestered by the ATP-dependent Ca^{2+} pump can be released by Na^{+} , it is likely that both ATP-dependent and Na^{+} -dependent Ca^{2+} -transport systems are located in the same membrane vesicles. In previous reports it has been shown that, when measured in the absence of oxalate, the microsomal ATP-dependent Ca^{2+} uptake reflects the activity of plasma-membrane vesicles (Wibo *et al.*, 1981; Morel *et al.*, 1981). It is therefore likely that Na^{+} -dependent Ca^{2+} transport also occurs in the plasma membrane. Although it has been reported that Na^{+} could release Ca^{2+} from mitochondria in some tissues, this system is almost undetectable in smooth-muscle mitochondria (Crompton *et al.*, 1978).

The characteristics of the Na^{+} -driven Ca^{2+} transport identified in intestinal and vascular smooth-muscle microsomal fractions do not appear different from $\text{Na}^{+}/\text{Ca}^{2+}$ exchange systems described in brain, cardiac and uterine plasma-membrane vesicles (Gill *et al.*, 1981; Reeves & Sutko, 1979; Grover *et al.*, 1981, 1983). The co-existence of a $\text{Na}^{+}/\text{Ca}^{2+}$ exchange system with the ATP-dependent Ca^{2+} pump in smooth-muscle plasmalemmal vesicles is consistent with Brading's (1979) suggestion that the smooth-muscle cell might possess the two Ca -extrusion mechanisms. The two systems also coexist in cardiac sarcolemma (Caroni & Carafoli, 1980). In both heart and smooth-muscle plasmalemmal membranes, the $\text{Na}^{+}/\text{Ca}^{2+}$ -exchange carrier shows a lower Ca^{2+} affinity than the ATP-dependent Ca^{2+} pump, half-maximal Ca^{2+} uptake being obtained with, respectively, $20\text{ }\mu\text{M}$ - and $6\text{ }\mu\text{M}$ - Ca^{2+} in guinea-pig heart (Morel & Godfraind, 1982), and with $18\text{ }\mu\text{M}$ - and $4\text{ }\mu\text{M}$ - Ca^{2+} in guinea-pig intestinal smooth muscle. However, cardiac and smooth muscles are quite different as far as the Ca^{2+} -transport rate and the capacity of the Na^{+} - or ATP-dependent Ca^{2+} -transport mechanism are concerned. In the heart, as reported by Caroni & Carafoli (1981) in dog membranes, and confirmed by ourselves in guinea-pig membranes (Morel & Godfraind, 1982), the $\text{Na}^{+}/\text{Ca}^{2+}$ exchange is characterized by a very high Ca^{2+} -transport velocity and by a Ca^{2+} -accumulation capacity that is about twice that of the ATP-dependent Ca^{2+} pump. Therefore the $\text{Na}^{+}/\text{Ca}^{2+}$ exchange mechanism appears to be most important for Ca^{2+} regulation in cardiac cells (Caroni *et al.*, 1980). By contrast, the activity of smooth-muscle plasmalemmal $\text{Na}^{+}/\text{Ca}^{2+}$ exchange could be weak. In both

intestinal and aortic smooth-muscle microsomal fractions, the maximum amount of Ca^{2+} accumulated by this system inside plasmalemmal vesicles is one-fifth of the maximum ATP-dependent Ca^{2+} uptake. Moreover, the ATP-dependent Ca^{2+} uptake operates only in inside-out vesicles, which represented about 50% of the plasma membrane in the intestinal smooth-muscle microsomal fraction, as estimated by measuring [^3H]ouabain binding in untreated and saponin-treated preparations (Wibo *et al.*, 1982; N. Morel & T. Godfraind, unpublished work). Such conclusions are consistent with the results of Grover *et al.* (1981, 1983) on rat myometrium membranes. In that preparation $\text{Na}^{+}/\text{Ca}^{2+}$ exchange capacity was even lower than in intestinal plasma-membrane vesicles, as Na^{+} -driven Ca^{2+} transport produced an intravesicular Ca^{2+} concentration lower than that obtained when the membrane permeability barrier for Ca^{2+} was abolished with the Ca ionophore A23187 (Grover *et al.*, 1983). Thus, low capacity of $\text{Na}^{+}/\text{Ca}^{2+}$ exchange could be a general property of smooth muscle, but high membrane permeability to Na^{+} or alteration of this Na^{+} permeability or of the $\text{Na}^{+}/\text{Ca}^{2+}$ exchange activity during the procedure of membrane purification cannot be ruled out at this stage. Weak activity of $\text{Na}^{+}/\text{Ca}^{2+}$ exchange in smooth muscle is in agreement with some studies in intact tissue, which indicated that an inwardly oriented Na^{+} gradient is not required for the maintenance of a low Ca_i^{2+} concentration (Casteels & van Breemen, 1975). Interestingly, the physiological importance of $\text{Na}^{+}/\text{Ca}^{2+}$ exchange may vary according to the type of smooth muscle, as illustrated by the findings by Hirata *et al.* (1981). They showed that this Ca^{2+} -extrusion system was absent from single cells prepared from the pig coronary artery, whereas it was operative in cells from the guinea-pig taenia coli.

In heart sarcolemmal preparations, the $\text{Na}^{+}/\text{Ca}^{2+}$ system operates electrogenically, exchanging three or more Na^{+} ions for one Ca^{2+} (Pitts, 1979; Reeves & Sutko, 1980; Caroni *et al.*, 1980; Philipson & Nishimoto, 1980). It has therefore been suggested that in heart $\text{Na}^{+}/\text{Ca}^{2+}$ exchange could contribute not only to Ca^{2+} extrusion during relaxation but also to Ca^{2+} entry during depolarization. In intact cells from the taenia coli, Hirata *et al.* (1981) found that the Na_o^{+} -activated Ca^{2+} efflux was related to the square of Na_o^{+} concentration and was independent of membrane potential. They concluded that the exchange was electroneutral in smooth muscle. Our observation on the inhibition by NaCl_o of Ca^{2+} uptake by Na^{+} -loaded vesicles indicates that more than one Na^{+} ion is required to compete with one Ca^{2+} ion. However, further experiments appear to be needed to determine if the $\text{Na}^{+}/\text{Ca}^{2+}$ exchange process

identified in smooth muscle also operates electrogenically.

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