Sodium/calcium exchange in smooth-muscle microsomal fractions

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1. The existence of Na⁺-dependent Ca²⁺ 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 Ca²⁺ pump previously identified in these preparations. 2. The rate of Ca²⁺ release from plasmalemmal vesicles previously loaded with Ca²⁺ through the ATP-dependent Ca²⁺ 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 Ca²⁺ when an outwardly directed Na⁺ gradient was formed across the membrane. The Ca ionophore A23187 induced a rapid release of 85% of the sequestered Ca²⁺, whereas only 15% was displaced by La³⁺. Ca²⁺ accumulated by the Na⁺-induced Ca²⁺ transport was released by the addition of NaCl, but not KCl, to the medium. 4. Ca²⁺ 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 Ca²⁺ uptake was a saturable function of Ca²⁺ concentration in the medium. Half-maximum activity was obtained with 18 μM-Ca²⁺ in intestinal-smooth-muscle microsomal fraction and with 50 μM-Ca²⁺ in aortic microsomal fraction. 6. The results suggest that in these membrane preparations a transmembrane movement of Ca²⁺ can be driven by a Na⁺ gradient. However, the Na⁺-induced Ca²⁺ transport had a lower capacity, a lower affinity and a slower rate than the ATP-dependent Ca²⁺ pump.

Participation of a Na⁺/Ca²⁺ exchange mechanism in the control of cytoplasmic free Ca²⁺ concentration in the smooth muscle cell is still a matter of controversy (Brading, 1981). On the basis of the first observations on Na⁺-linked Ca²⁺ transport in nerves (Baker et al., 1969) and in heart muscle (Reuter, 1974), the Na⁺/Ca²⁺ exchange model states that some transmembrane movement of Ca²⁺ is coupled to oppositely directed Na⁺ flux, so that the Na⁺ electrochemical gradient provides the energy for Ca²⁺ transport. In smooth muscle, evidence that Na⁺ may regulate intracellular Ca²⁺ concentration (Caᵢ) 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 Caᵢ and an increase in muscle tension (Lang & Blausstein, 1980; Ozaki et al., 1978). In some tissues, contraction induced by low extracellular [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 Ca²⁺ influx dependent on internal Na⁺, whereas Droogmans & Casteels (1979) concluded that Na⁺-free contraction was not attributable to a Na⁺/Ca²⁺ 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 Na⁺/Ca²⁺ interactions occurring at extracellular binding sites (Burton & Godfraind, 1974; Brading, 1979). A Na⁺/Ca²⁺ 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 Ca²⁺ 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 0.25 M-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 10000g 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 1h to yield a microsomal pellet, which was suspended in 5 ml of 5 M-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 24h 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-MgCl\(_2\), 0.2 mM-CaCl\(_2\), labelled with \(^{45}\)CaCl\(_2\) (1.6 μCi/ml), 3 mM-ATP and 5 mM-Na\(_2\)EGTA. 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 \(^{45}\)Ca 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-CaCl\(_2\) labelled with \(^{45}\)CaCl\(_2\) (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 \(^{45}\)Ca uptake was determined by subtracting \(^{45}\)Ca uptake measured in the presence of 150 mM-NaCl from \(^{45}\)Ca 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 \(^{45}\)Ca in order to load plasmalemmal vesicles with \(^{45}\)Ca 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 \(^{45}\)Ca content, whereas dilution in the presence of KCl or LiCl
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 150 mM-NaCl (see the Methods section) and then diluted 1:40 into a medium containing 150 mM-KCl and 50 mM-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. 150 mM, sucrose concn. 300 mM) (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 50 μM-45CaCl2 and then further incubated after addition of 5 μM-A23187 or 0.5 mM-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 57 mM-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.

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

Microsomal fractions (0.3 mg of protein/ml) were loaded with 45Ca by the ATP-dependent Ca2+ uptake system (50 μM-45CaCl2; incubation time 15 min at 37°C). After filtration of duplicate 0.1 ml samples, 0.7 ml of the medium was immediately diluted 1:50 into 20 mM-Tris/maleate buffer (pH 7.4) containing 150 mM-KCl (△), -LiCl (○) or -NaCl (●), or 300 mM-sucrose (□) (zero time). Samples (5 ml) 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 50 μM, and temperature 37°C. Each point is the mean of two determinations on two different membrane preparations.
The influence of extravascular Na⁺ (Na⁺r) concentration on the extent of Ca²⁺ uptake by Na⁺-loaded vesicles is shown in Fig. 4. In this experiment, NaCl₀ 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 Na⁺r, where v is the ⁴⁵Ca content measured at the given Na⁺r value, V is the maximal ⁴⁵Ca content and Na⁺₀ is the NaCl concentration in the medium (expressed in mM). The relation between log [v/(V−v)] and log Na⁺₀ was linear, and the Hill coefficient (k, calculated by linear regression) was 1.86. The Na⁺₀ concentration producing half-maximal depression of Ca²⁺ uptake (intersection with the abscissa) was 28 mM. This value is close to that reported by Reeves & Sutko (1979) for Na⁺/Ca²⁺ exchange in cardiac sarcolemma (half-maximal inhibition at 16 mM-NaCl₀).

The inhibitory effect of Na⁺₀ on Na⁺₀-induced Ca²⁺ uptake may be due to a decrease of the Na⁺⁺/Na⁺₀ ratio when Na⁺₀ is increased, or to the interaction of Na⁺₀ with the Ca²⁺-binding sites of the carrier. A Hill coefficient of around 2 could then suggest that one Ca²⁺ exchanged with 2 Na⁺. However, it could also reflect the fact that more than one Na⁺ ion binds to the carrier on its Ca²⁺-binding sites, thereby producing inhibition of the translocation of one Ca²⁺ ion.

To confirm that a Na⁺ gradient was a prerequisite for ATP-independent Ca²⁺ 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, ⁴⁵Ca uptake was much lower than in Na⁺-loaded vesicles diluted in Na⁺₀-free medium (Table 1). Moreover, the Ca ionophore A23187 failed to release ⁴⁵Ca taken up by KCl-, LiCl- or sucrose-loaded vesicles, suggesting that this Ca²⁺ uptake might reflect Ca²⁺ binding at superficial sites (results not shown).

Effect of Ca²⁺ concentration on Ca²⁺ uptake

Fig. 5 shows that, in intestinal and aortic Na⁺-loaded microsomal fractions, the extent of ⁴⁵Ca uptake was a saturable function of Ca²⁺ concentration, the maximum being observed at 0.1 mM-
**Table 1. ATP-independent Ca^{2+} uptake in intestinal smooth-muscle microsomal fraction**

Intestinal smooth-muscle microsomal fractions were preincubated at 0°C overnight in 5 mM-Tris/HCl buffer (pH 7.4) with different salts or with sucrose. Ca^{2+} uptake was measured 3 min after dilution of the microsomal fractions 1:40 into 20 mM-Tris/maleate buffer containing 50 μM-CaCl_2 labelled with ^{45}Ca and either KCl or NaCl (150 mM). Data are means ± S.E.M. for n determinations obtained with at least two different membrane preparations.

<table>
<thead>
<tr>
<th>Loading medium</th>
<th>n</th>
<th>Incubation medium</th>
<th>Ca^{2+} content (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (150 mM)</td>
<td>8</td>
<td>KCl</td>
<td>4.36 ± 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NaCl</td>
<td>1.14 ± 0.09</td>
</tr>
<tr>
<td>KCl (150 mM)</td>
<td>8</td>
<td></td>
<td>2.06 ± 0.15</td>
</tr>
<tr>
<td>LiCl (150 mM)</td>
<td>5</td>
<td></td>
<td>2.57 ± 0.09</td>
</tr>
<tr>
<td>Sucrose (300 mM)</td>
<td>5</td>
<td></td>
<td>2.23 ± 0.15</td>
</tr>
</tbody>
</table>

**Fig. 5. Effect of free Ca^{2+} concentration on ATP-dependent (▲) and Na^{+}-induced Ca^{2+} uptake in guinea-pig intestinal smooth-muscle (●) and rat aorta (■) microsomal fractions**

Ca^{2+} content was measured 15 min after addition of ATP (ATP-dependent Ca^{2+} uptake) or 3 min after dilution of Na^{+}-loaded microsomal fractions into Na^{+}-free medium (Na^{+}-induced Ca^{2+} uptake). For Ca^{2+} concentrations up to 0.1 mM, total Ca^{2+} concentration was 0.2 mM for ATP-dependent and 0.1 mM for Na^{+}-induced Ca^{2+} uptake. Free Ca^{2+} 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.

Pitts & Okhuysen (1980) (26 μM) and by Reeves & Sutko (1979) (18 μM), and the half-maximal value of Na^{+}-dependent Ca^{2+} uptake was obtained at 7 μM-Ca^{2+} in myometrium plasma-membrane vesicles (Grover et al., 1983).

The Na^{+}-induced Ca^{2+} uptake by both types of smooth-muscle microsomal fractions operated with a lower maximal capacity than did the ATP-driven plasmalemmal Ca^{2+} pump. Moreover, the latter was characterized by a higher apparent Ca^{2+} affinity. Indeed, its K_{0.5} value was 4 μM for intestinal smooth-muscle microsomal fraction (Fig. 5), and was about 10 μM for aortic microsomal fraction in the presence of calmodulin (Morel et al., 1981). We found that the Na^{+}-dependent Ca^{2+} 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 Ca^{2+} pump, intestinal and aortic smooth-muscle microsomal fractions contain a Na^{+-} dependent Ca^{2+}-transport activity. In the absence of ATP, Ca^{2+} 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 extravascular Na^{+} concentration or by allowing the Na^{+} gradient to dissipate by passive diffusion of Na^{+} (results not shown) inhibits this Ca^{2+} uptake. Na^{+}-dependent Ca^{2+} accumulation does not appear to be the result of Ca^{2+} binding to superficial sites, as La^{3+} is ineffective in releasing Ca^{2+} accumulated by this process. Moreover, the nearly complete release of Ca^{2+} by a Ca ionophore shows that Ca^{2+} is taken up and accumulated inside the Na^{+}-loaded vesicles. The saturable nature of the Ca^{2+}-dependence of Na^{+}-induced Ca^{2+} uptake is consistent with a

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

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

The characteristics of the Na\textsuperscript{+}-driven Ca\textsuperscript{2+}
transport identified in intestinal and vascular
smooth-muscle microsomal fractions do not ap-
ppear different from Na\textsuperscript{+}/Ca\textsuperscript{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 Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange system with the
ATP-dependent Ca\textsuperscript{2+} pump in smooth-muscle
plasmalemmal vesicles is consistent with Branding’s
(1979) suggestion that the smooth-muscle cell
might possess the two Ca-extrusion mechanisms.
The two systems also coexist in cardiac sarco-
lemma (Caroni & Carafoli, 1980). In both heart
and smooth-muscle plasmalemmal membranes,
the Na\textsuperscript{+}/Ca\textsuperscript{2+}-exchange carrier shows a lower
Ca\textsuperscript{2+} affinity than the ATP-dependent Ca\textsuperscript{2+}
pump, half-maximal Ca\textsuperscript{2+} uptake being obtained
with, respectively, 20 \textmu M and 6 \textmu M-Ca\textsuperscript{2+} in guinea-
pig heart (Morel & Godfraind, 1982), and with
18 \textmu M and 4 \textmu M-Ca\textsuperscript{2+} in guinea-pig intestinal
smooth muscle. However, cardiac and smooth
muscles are quite different as far as the Ca\textsuperscript{2+}-
transport rate and the capacity of the Na\textsuperscript{+} or
ATP-dependent Ca\textsuperscript{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 Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange is
characterized by a very high Ca\textsuperscript{2+}-transport
velocity and by a Ca\textsuperscript{2+}-accumulation capacity that
is about twice that of the ATP-dependent Ca\textsuperscript{2+}
pump. Therefore the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange mech-
anism appears to be most important for Ca\textsuperscript{2+}
regulation in cardiac cells (Caroni et al., 1980). By
contrast, the activity of smooth-muscle plasmalem-
mal Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange could be weak. In both
intestinal and aortic smooth-muscle microsomal
fractions, the maximum amount of Ca\textsuperscript{2+} accumu-
lated by this system inside plasmalemmal vesicles
is one-fifth of the maximum ATP-dependent Ca\textsuperscript{2+}
uptake. Moreover, the ATP-dependent Ca\textsuperscript{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 [\textsuperscript{3}H]ouabain binding in
untreated and saponin-treated preparations (Wibo
et al., 1982; N. Morel & T. Godfraind, unpub-
lished work). Such conclusions are consistent with
the results of Grover et al. (1981, 1983) on rat
myometrium membranes. In that preparation
Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange capacity was even lower than
in intestinal plasma-membrane vesicles, as Na\textsuperscript{+}-
driven Ca\textsuperscript{2+} transport produced an intravesicular
Ca\textsuperscript{2+} concentration lower than that obtained when
the membrane permeability barrier for Ca\textsuperscript{2+} was
abolished with the Ca ionophore A23187 (Grover
et al., 1983). Thus, low capacity of Na\textsuperscript{+}/Ca\textsuperscript{2+}
exchange could be a general property of smooth
muscle, but high membrane permeability to Na\textsuperscript{+}
or alteration of this Na\textsuperscript{+} permeability or of the
Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity during the procedure
of membrane purification cannot be ruled out at
this stage. Weak activity of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange
in smooth muscle is in agreement with some
studies in intact tissue, which indicated that an
inwardly oriented Na\textsuperscript{+} gradient is not required for
the maintenance of a low Ca\textsuperscript{2+} concentration
(Casteels & van Bremen, 1975). Interestingly,
the physiological importance of Na\textsuperscript{+}/Ca\textsuperscript{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\textsuperscript{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
Na\textsuperscript{+}/Ca\textsuperscript{2+} system operates electrogenically,
exchange three or more Na\textsuperscript{+} ions for one Ca\textsuperscript{2+}
(Pitts, 1979; Reeves & Sutko, 1980; Caroni et al.,
1980; Philipson & Nishimoto, 1980). It has
therefore been suggested that in heart Na\textsuperscript{+}/Ca\textsuperscript{2+}
exchange could contribute not only to Ca\textsuperscript{2+}
extrusion during relaxation but also to Ca\textsuperscript{2+} entry
during depolarization. In intact cells from the
taenia coli, Hirata et al. (1981) found that the Na\textsuperscript{+}-
activated Ca\textsuperscript{2+} efflux was related to the square of
Na\textsuperscript{+} concentration and was independent of mem-
brane potential. They concluded that the exchange
was electroneutral in smooth muscle. Our obser-
vation on the inhibition by NaCl\textsubscript{o} of Ca\textsuperscript{2+} uptake by
Na\textsuperscript{+}-loaded vesicles indicates that more than one
Na\textsuperscript{+} ion is required to compete with one Ca\textsuperscript{2+} ion.
However, further experiments appear to be needed
to determine if the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange process
identified in smooth muscle also operates electrogenically.

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