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# SODIUM–CALCIUM EXCHANGE IN SMALL INTESTINAL MYOCYTES

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One way of removing $Ca^{2+}$ ions from the smooth-muscle cell is Na/Ca exchange, carried out by means of membrane carriers. This process has been sufficiently well studied on plasma membranes (PM) of skeletal and heart muscles, nerve fibers, blood cells, etc. Information on this type of ion antiport in smooth muscles is very restricted, and results obtained on the myometrium [2] points to its electrical neutrality.

In this paper we describe experimental proof of the substrate saturability of the transfer, its specificity for $Na^+$ and $Ca^{2+}$, and the reversibility and electrogenicity of the antiport.

## EXPERIMENTAL METHOD

Experiments were carried out on the vesiculated fraction of PM of rabbit small intestinal myocytes [6], using $^{45}Ca^{2+}$ and an "Orion 93-20" Ca-selective electrode. The concentration of PM protein was 100 $\mu$g/ml when exchange was investigated by means of an electrode and 1-5 $\mu$g/ml when the isotope method was used.

## EXPERIMENTAL RESULTS

Investigations showed that membrane vesicles containing NaCl, when added to sodium-free medium with 30 $\mu$M $CaCl_2$, can accumulate calcium. Na-dependent $Ca^{2+}$ accumulation by vesicles depends on the intravesicular $Na^+$ concentration (Fig. 1, curve 1), and is evidence that the motive force of the antiport is the sodium gradient. With reduction of this gradient calcium accumulation by membrane vesicles falls (Fig. 1, curve 2) to the level corresponding to the quantity of cation bound by the membranes [4]. Conversely, a similar increase in $K^+$ in the medium does not affect accumulation of $Ca^{2+}$ by vesicles, but in the presence of valinomycin, accumulation is increased (Fig. 1, curve 3).

The results can be explained as follows. Under conditions when the incubation medium contained KCl and valinomycin, the membrane voltage with "+" sign was created inside the vesicles, for initially the vesicles did not contain $K^+$ ions. Thus a positive charge inside the membrane vesicles stimulates Na/Ca exchange. The maximal velocity of the process is increased under these circumstances, but affinity for the $Ca^{2+}$-system of the antiport is unchanged: $K_m$ for $Ca^{2+}$, calculated from data shown in Fig. 2, in both the presence and the absence of valinomycin, was 20 $\mu$M.

Comparison of the quantity of $Ca^{2+}$ accumulated by membrane vesicles with the quantity of $Na^+$ escaping from the vesicles into the medium containing sucrose (or KCl) and KCl + 3 $\mu$M valinomycin, showed the following relationships. Within the range of extravesicular $Ca^{2+}$ concentration up to 40-50 $\mu$M (saturation concentration of the antiport), this ratio between the cations varied from 1.9 to 2.25 in medium without valinomycin and from 2.27 to 3.12 in medium with the ionophore. In the first case the ratio of $Ca^{2+}$ entering the vesicles and sodium leaving them corresponded to the $1Ca^{2+}$:$2Na^+$ stoichometry (nonelectrogenic antiport), $1Ca^{2+}$:$3Na^+$ in the second (electrogenic mode of operation of Na/Ca exchange).

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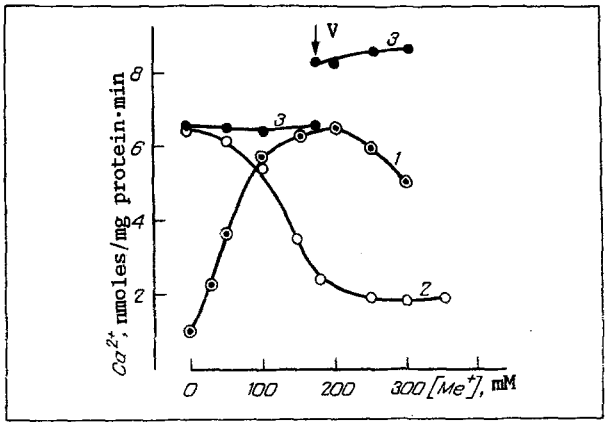


Fig. 1. Dependence of Na/Ca exchange on potassium and sodium concentrations in medium and on membrane voltage. 1, 2) Intra- and extravesicular sodium respectively; 3) extravesicular potassium; V) valinomycin, 3 $\mu$M. Incubation time 2 min.

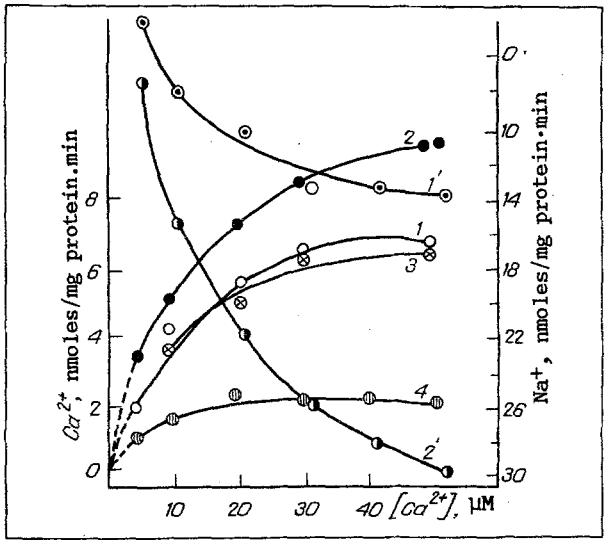


Fig. 2. Dependence of Na/Ca exchange on calcium concentration in medium. Vesicles contain 125 mM NaCl. Medium: 1) 125 mM KCl, 2) 125 mM KCl + 3 $\mu$M valinomycin, 3) 250 mM sucrose; 4) 125 mM NaCl; 1', 2') outflow of sodium into corresponding media. Incubation time 2 min.

Accumulation of $Ca^{2+}$ by vesicles increases with time during the initial period of incubation (Fig. 3, curves 1, 3) and decreases after 2 min of incubation, irrespective of the presence or absence of valinomycin in the medium. Reduction of the $Ca^{2+}$ accumulation by the vesicles after incubation for 2 min can be explained, first, by a decrease in the calcium gradient (and, consequently, dissipation of the voltage) on the membrane as a result of diffusion of $K^+$ inside the vesicles. Second, in the course of Na/Ca exchange the sodium gradient — the motive force of the antiport, also diminishes. Third, as a result of accumulation of $Ca^{2+}$ by the vesicles, generation of a "calcium" voltage, opposite in polarity to the voltage created by addition of valinomycin to the potassium medium, becomes possible.

It is also characteristic that the formation of a membrane voltage by the addition of valinomycin to medium with KCl increases both the initial velocity of exchange and the calcium capacity of the membrane vesicles (Fig. 3b). Since (Table 1) the membrane voltage increases the initial velocity of exchange from 49.2 to 61.8 nmoles $Ca^{2+}$/mg protein · min (by 25%), and the maximal velocity from 11.1 to 16.6 nmoles $Ca^{2+}$/mg protein · min (by 49%), without causing any change in affinity for $Ca^{2+}$ the increase in capacity of the vesicles from 7.8 to 10.6 nmoles $Ca^{2+}$/mg protein can be explained by acceleration of movement of

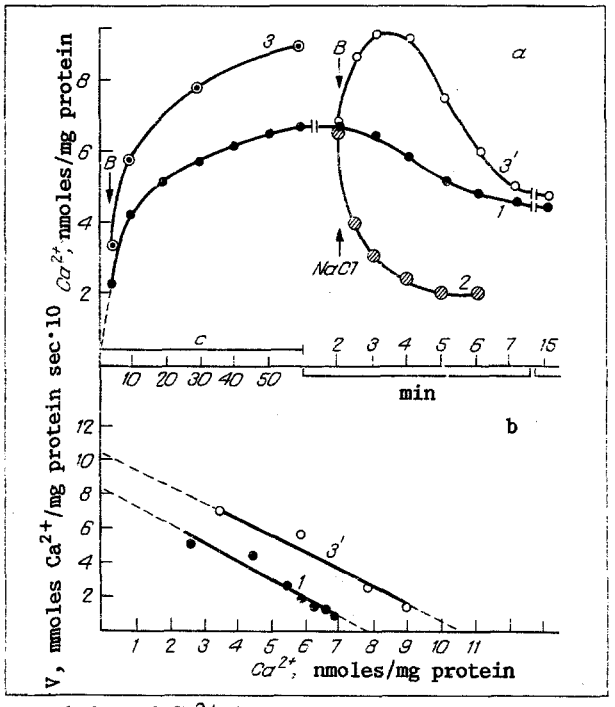


Fig. 3. Accumulation of $Ca^{2+}$ ions by membrane vesicles under conditions of potassium (125 mM) medium (1, 3) and release of cation on changing potassium medium for one containing sodium (A), and outflow of cation when potassium medium replaced by sodium (A), and calculation of initial rate of exchange (V) and calcium capacity of membrane vesicles (B). Legend as to Figs. 1 and 2.

TABLE 1. Characteristics of Sodium—Calcium Exchange

| Parameters and conditions of determination | Values of parameters |
|---|---|
| A — inflow of $Ca^{2+}$ ions into membrane vesicles Sucrose or KCl solution | |
| 1. Na-dependent accumulation of $Ca^{2+}$ ions | 6,9 nmoles $Ca^{2+}$/mg protein·min |
| 2. $K_m$ of antiport for $Ca^{2+}$ | 20 μM |
| 3. $K_m$ of antiport for $Na^+$ | 6,26 mM |
| 4. Initial velocity of antiport | 49,2 nmoles $Ca^{2+}$/mg protein·min |
| 5. Maximal velocity of antiport | 11,1 nmoles $Ca^{2+}$/mg protein·min |
| 6. Calcium capacity of membrane | 7,8 nmoles $Ca^{2+}$/mg protein |
| KCl solution + 3 μM valinomycin | |
| 1. Na-dependent accumulation of $Ca^{2+}$ ions | 9,8 nmoles $Ca^{2+}$/mg protein·min |
| 2. $K_m$ of antiport for $Ca^{2+}$ | 20 μM |
| 3. $K_m$ of antiport for $Na^+$ | 6,45 mmoles |
| 4. Initial velocity of antiport | 61,8 nmoles $Ca^{2+}$/mg protein·min |
| 5. Maximal velocity of transport | 16,6 nmoles $Ca^{2+}$/mg protein·min |
| 6. Calcium capacity of membrane vesicles | 10,6 nmoles $Ca^{2+}$/mg protein |
| B — outflow of $Ca^{2+}$ ions from membrane vesicle into medium containing 125 mM NaCl | |
| 1. Na-dependent outflow of $Ca^{2+}$ ions | 6,4 nmoles $Ca^{2+}$/mg protein·min |
| 2. $K_m$ of antiport for $Ca^{2+}$ | 19,6 μM |
| 3. Maximal velocity of antiport | 11,5 nmoles $Ca^{2+}$/mg protein·min |
| 4. Outflow of $Ca^{2+}$ ions into medium containing 125 mM KCl or LiCl, CsCl | 0,7—0,9 nmoles $Ca^{2+}$/mg protein·min |

the carrier in the membrane. This may be the result of the presence of ionogenic groups in the carrier. If this is true, then allowing for the general rules of carrier function [1, 7], the inflow and outflow of calcium in membrane vesicles against the sodium concentration gradient in our concrete case must possess closely similar kinetic parameters. In fact, as Table 1 shows, the release of $Ca^{2+}$ ions from vesicles preincubated in medium with 0.5 mM $CaCl_2$ has values of $K_m$ and $V_{max}$ closely similar to the release of calcium into membrane vesicles.

The results are evidence that PM of the small intestinal myocytes possess a Na/Ca exchange system that is independent of the electrochemical gradient of $Na^+$ ions on the membrane. Accumulation of $Ca^{2+}$ ions by membrane vesicles reaches a maximum when the cation concentration is (4-5) $\cdot$ $10^{-5}$ M, i.e., at a calcium concentration activating contraction of the myocyte maximally [3, 5]. Since Na/Ca exchange is activated by membrane voltage, it can be postulated that the antiport in the resting period of the myocyte is electrogenic, but on membrane depolarization its function is shifted toward electrical neutrality, and in the period of excitation, due to its reversibility, it makes a definite contribution to the Ca currents through PM. This conclusion is confirmed by the latent Ca inward currents, generated by Na/Ca exchange, which we recorded [8], and the change in movement of $Ca^{2+}$ ions due to the antiport to the opposite — the latent state of the carrier [9].

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