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SODIUM-DEPENDENT CALCIUM INFLUX IN DIALYZED BARNACLE MUSCLE FIBERS*

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SUMMARY

Cannulated single muscle fibers were dialyzed with a porous glass capillary. The membrane potential was clamped at the normal resting potential value by means of a voltage clamp technique. The resting Ca^{2+} influx in Na^+-containing external medium ranged from 0.11 to 0.38 pmole·cm^{-2}·s^{-1}. When Na^+ was substituted by Li^+ the resting influx ranged from 0.5 to 0.8 pmole·cm^{-2}·s^{-1} Ca^{2+} influx was found to be directly dependent on the internal Na^+ concentration of the dialyzing fluid. Increasing the internal Na^+ from 0 to 15 mM caused a small increment in the Ca^{2+} influx. The increase was much greater when the internal Na^+ was increased to 30, 60 or 90 mM. The increment in the Ca^{2+} influx in high internal Na^+ was found to be much greater when the external medium contained Li^+ instead of Na^+.

INTRODUCTION

There are a number of observations which suggest the presence of Na^+–Ca^{2+} exchange in a variety of tissues. Baker *et al.*[1] have clearly shown that in squid giant axons the Ca^{2+} fluxes depend on external and internal Na^+ concentrations. There is evidence for Na^+-dependent transport of Ca^{2+} in heart muscle fibers[2], crab peripherical nerve[3] and in synaptosomes from mammalian brain[4].

Some evidence on the effect of external Na^+ on Ca^{2+} accumulation in frog skeletal muscle has been obtained by Cosmos and Harris[5]. Recently Blaustein *et al.*[6] has reported that Ca^{2+} accumulates into giant barnacle muscle fibers when the concentration gradient for Na^+ is made smaller than normal. The large size of barnacle muscle fibers and the possibility of using a combined dialysis and voltage clamp technique prompted us to carry out a systematic analysis of the effect of internal Na^+ on the Ca^{2+} influx in these fibers. The present communication shows that Ca^{2+} entry in barnacle muscle fibers is strongly dependent on both external and internal Na^+ concentration, increasing when the internal to external Na^+ concentration ratio is increased under conditions of constant membrane potential.

Abbreviation: EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N*, *N'*-tetraacetic acid.

* A preliminary report of this work was presented at the 4th International Biophysics Congress, Moscow.

MATERIALS AND METHODS

Single muscle fibers from the *Balanus aquila* were carefully dissected in artificial sea water of composition (mM): NaCl, 465; KCl, 10; $CaCl_2$, 25; $MgCl_2$, 8; sodium *N*-tris(hydroxymethyl)methyl-2-aminoethane sulphonate, 2 (pH 7.4). The dialysis technique used in this work and the experimental chamber have been described in detail by DiPolo and LaTorre[7]. A voltage clamp system similar to that used by Cole and Moore[8] serves to hold the membrane potential at its normal level (69 mV), as well as to monitor the membrane resistance during the course of the experiment. Holding the membrane potential at its normal level throughout the experiment seems necessary to avoid any effect of membrane depolarization on Ca^{2+} influx. The mean membrane resistance was found to be $1150 \pm 200\ \Omega \cdot cm^2$ ($n=18$).

The internal potential electrode consisted of a 90-μm glass capillary filled with 0.5 M KCl and a bare platinum wire inside it, down its length. A 75-μm platinized-platinum wire was coiled around the porous glass capillary to space clamp the fibers. Internal dialysis solutions were delivered to the porous capillary at a rate of 2–2.5 μl/min. The basic internal medium contained (mM): potassium isothionate, 170; KCl, 20; sodium isothionate, 0,15, 30, 60, 90 or 105; ethyleneglycol-bis-(β-amino ethyl ether)-*N,N'*-tetraacetic acid (EGTA)–Ca^{2+} buffer, 7.5 in the ratio 7:1 to obtain a final internal Ca^{2+} concentration of $5 \cdot 10^{-8}$ M; Tris–HCl, 10. Sucrose was used to adjust the internal osmolarity. $^{45}CaCl_2$ (20 Ci/g Ca) was obtained from International Chemical and Nuclear Corporation. All experiments were performed at 22 °C. Ca^{2+} influx experiments were carried out simply by placing the radioactive tracer in the solution bathing the fiber and collecting the perfusate at regular periods. The time required for the Ca^{2+} influx to reach a steady state ranged from 50 to 70 min. Therefore at least 60 min of perfusion were allowed prior to any change of solutions. In computing the Ca^{2+} influxes the area was assumed to be that of a cylinder.

RESULTS AND DISCUSSION

The mean Ca^{2+} influx from sea water containing 25 mM Ca^{2+} into fibers clamped at their normal resting potential was 0.25 ± 0.05 pmole$\cdot cm^{-2} \cdot s^{-1}$ ($n=15$). This value, although apparently very much similar to that found in nerve fibers[9], has not been corrected for the extra area due to membrane invaginations present in these fibers[10]. This might indicate that barnacle muscle fibers have a lower permeability for Ca^{2+} than nerve fibers. However, the difference might not be large since Ca^{2+} influx appears to be dependent on internal Na^+ concentration which is lower in barnacle muscle fibers.

The influence of external Na^+ on the Ca^{2+} influx has been examined in several experiments using different internal Na^+ concentrations. Table I shows that replacing the external Na^+ by Li^+ in the presence of a normal internal Na^+ concentration (15 mM) causes an increase in the Ca^{2+} influx. The increase is larger when the internal Na^+ concentration has been previously raised (90 mM) by dialysis. Fig. 1 shows a typical experiment in which replacement of all the external Na^+ by Li^+ in the presence of a high internal Na^+ (105 mM) causes a reversible

increase in the Ca^{2+} influx. A similar effect was observed when Na^+ was replaced by Tris or dextrose. If the Ca^{2+}-buffering agent EGTA was left out of the internal medium, local contractures were seen both in the absence of external Na^+ and in the presence of a high internal Na^+ concentration. The presence of these local contractions under the conditions mentioned above probably reflects the large increase in the Ca^{2+} influx. However, the question arises as to whether the shortening of the fiber might be due to a decrease in the ATP levels after prolongued dialysis. This does not seem to be the case. In fact unpublished experiments with barnacle fibers have shown that the Na^+ pump which requires ATP can operate within its normal range even after 3 h of internal perfusion with a medium containing no ATP. Probably the reason for the failure in removing the ATP in these fibers might reside in the very large concentrations of ATP precursors as well as in the relatively large amounts of poorly dialyzable ATP (mitochondria, Sarcoplasmic reticulum, *etc.*).

Since the main aim of this paper is to study the effect of internal Na^+ on the Ca^{2+} influx, several fibers were dialyzed with different internal Na^+ concentrations. One of the main advantages of the dialysis technique is that the effect of different internal Na^+ concentrations on the Ca^{2+} influx can be tested in the

TABLE I

EFFECT OF $[Na^+]_o$ AND $[Na^+]_i$ ON Ca^{2+} INFLUX IN GIANT BARNACLE MUSCLE FIBERS

Ca^{2+} influx is expressed as mean ±S.E.

$[Na^+]_o$ (*mM*)	$[Na^+]_i$ (*mM*)	$[Ca^{2+}]_o$ (*mM*)	$[Ca^{2+}]_i$ (*M*)	$-V_m$ (*mV*)	*Ca^{2+} influx* (*pmoles·cm^{-2}·s^{-1}*)
465	15	25	$5\cdot10^{-8}$	69	0.25±0.06 (15)
465	90	25	$5\cdot10^{-8}$	69	0.43±0.04 (4)
2 (Li^+)	15	25	$5\cdot10^{-8}$	69	0.62±0.12 (3)
2 (Li^+)	90	25	$5\cdot10^{-8}$	69	2.1±0.15 (4)

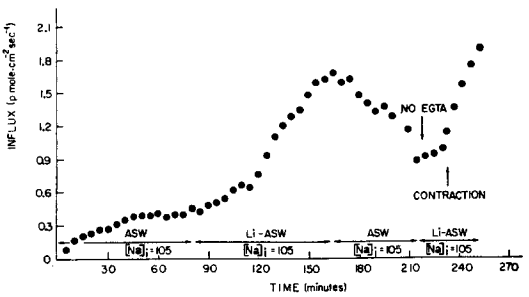

Fig. 1. Effect of replacing the external Na^+ by Li^+ on the Ca^{2+} influx in a fiber perfused with a high (105 mM) internal Na^+ concentration. Under this condition, EGTA removal from the perfusion solution lead to contracture and damage of the fiber. $[Ca^{2+}]_i \simeq 5.10^{-8}$ M (Buffer Ca-EGTA). $[Ca^{2+}]_o$, 25 mM. Temperature, 22 °C. V_m(holding membrane potential), −69 mV. R_m(membrane resistance), 1010 $\Omega\cdot cm^2$. ASW= artifical sea water.

same fiber. Table I shows that in the presence of a normal external Na^+ concentration (467 mM) the Ca^{2+} influx increases by a factor of 1.7 when the internal Na^+ concentration is artificially raised from 15 to 90 mM. Moreover, in low external Na^+ solutions (2 mM) the Ca^{2+} influx increases by a factor of 3.4 when the internal Na^+ is raised from 15 to 90 mM. Fig. 2 clearly shows that the Ca^{2+} influx in Li^+ artificial sea water increases with increasing internal Na^+. These results are qualitatively similar to those reported in nerve fiber by other authors[11] although the magnitude of this phenomenon seems to be greater in nerve fibers.

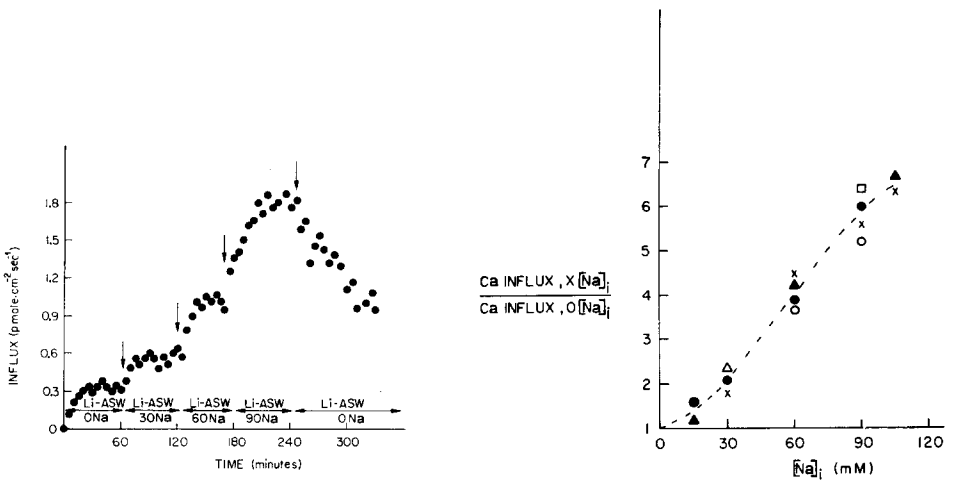

Fig. 2. Ca^{2+} influx at different internal Na concentrations in the absence of external Na^+. Note the reversibility of the effect when Na^+ is removed from the perfusion solution. $[Ca^{2+}]_i \simeq 5.10^{-8}$ M (buffer Ca-EGTA). $[Ca^{2+}]_o$, 25 mM. Temperature, 22 °C. V_m, −69 mV. R_m, 990 $\Omega \cdot cm^2$. ASW= artificial sea water.

Fig. 3. Ca^{2+} influx as a function of the internal Na^+ concentration. The abscissa represents the Ca^{2+} influx at a given internal Na^+ concentration relative to that at zero internal Na^+. The curve has been drawn to fit the experimental points. No appreciable saturation of the Ca^{2+} influx is seen. At low internal Na^+ concentrations the curve does not seem to be linear. $[Ca^{2+}]_o$, 25 mM. $[Na^+]_o$, 2 mM.

Baker[12] has reported that the Ca^{2+} buffer EGTA blocks the component of the Na^+ efflux which depends on external Ca^{2+}. No systematic study is available for the effect of EGTA on the Ca^{2+} influx. However a partial inhibition of the Na^+–Ca^{2+} exchange might have occurred in these experiments in which a substantial amount of EGTA must be used to avoid muscle contraction. Fig. 3 summarizes the dependency of Ca^{2+} influx on the internal Na^+ concentration in fibers in which the external Na^+ was replaced by Li^+. This curve which clearly shows an activation of the Ca^{2+} influx by internal Na^+ is not linear at low internal Na^+ concentrations; furthermore no signs of saturation are observed even at internal Na^+ concentrations ten times the normal value. Be it as it may, this curve predicts sizeable changes in the internal Ca^{2+} concentration for relatively small alterations in the Na^+ concentration gradients. This might be of significance in view of the known role of Ca^{2+} as contractile activator in muscle fibers.

These experiments provide strong evidence for exchange of internal Na^+ for external Ca^{2+} under conditions of constant membrane potential, and give information on the relationship between Ca^{2+} influx and internal Na^+ concentration in barnacle muscle fibers. They do not however prove that this is the only mechanism responsible for Ca^{2+} influx. Ca^{2+} influx in nerves from the walking legs of the crab *Maia squinado*[12] seems to consist of two components in the absence of external Na^+: one dependent and one independent on internal Na^+. The last component is related to the external K^+ concentration, increasing with increasing the K^+ concentration. Experiments are required in which the effects of membrane potential on the Na^+–Ca^{2+} exchange can be determined to decide whether the K^+-activated Ca^{2+} uptake is related to the K^+ *per se*, or is the consequence of an increase of Ca^{2+} permeability due to membrane depolarization.

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