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EFFECT OF INTERNAL AND EXTERNAL K^+ ON Na^+-Ca^{2+} EXCHANGE IN DIALYZED SQUID AXONS UNDER VOLTAGE CLAMP CONDITIONS

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The effect of external and internal K^+ on Na_o^+-dependent Ca^{2+} efflux was studied in dialyzed squid axons under constant membrane potential. With axons clamped at their resting potentials, external K^+ (up to 70 mM) has no effect on Na^+-Ca^{2+} exchange. Removal of K_i^+ causes a marked inhibition in the Na_o^+-dependent Ca^{2+} efflux component. Internal K^+ activates the Na^+-Ca^{2+} exchange with low affinity ($K_{1/2} = 90$ mM). Activation by K_i^+ is similar in the presence or in the absence of Na_i^+, thus ruling out a displacement of Na_i^+ from its inhibitory site. Axons dialyzed with ATP also show a dependency of Ca^{2+} efflux on K_i^+. The present results demonstrate that K_i^+ is an important cofactor (partially required) for the proper functioning of the forward Na^+-Ca^{2+} exchange.

Introduction

In squid axons, the outward movement of Ca^{2+} is accomplished by two separate mechanisms: the Ca^{2+} pump (high affinity-low capacity) and the Na^+-Ca^{2+} exchange (low affinity-high capacity) [1]. In injected axons, there is evidence that neither external K^+ nor membrane potential have any effect on the ATP-dependent uncoupled Ca^{2+} efflux (forward Ca^{2+} pump) [2]. However, in dialyzed squid axons, internal K^+ is an important cofactor for full activation of the Ca^{2+} pump (DiPolo, R. and Beaugé, L.A., unpublished data). This effect parallels the stimulation by K^+ of the ATP-dependent Ca^{2+} uptake in: membrane vesicles from squid nerve fibers [3], sarcoplasmic reticulum [4], human red blood cells [5], isolated cardiac plasma membranes [6] and synaptic plasma membranes [7].

For the case of the antiporter Na^+-Ca^{2+} exchange, the role of K^+ has been somewhat controversial. Blaustein [8] reported that Na_o^+-dependent Ca^{2+} efflux in squid axons is little affected by K_i^+, and Slaughter et al. [9] found no effect of K^+ (up to 10 mM) on the levels of Ca^{2+} accumulated by the Na^+-Ca^{2+} exchange in cardiac sarcolemma vesicles. Nevertheless, in these studies, K^+ appears to activate a Ca^{2+}-Ca^{2+} mode of exchange. On the other hand, it has been recently reported that K^+ strongly activated the Na^+-Ca^{2+} exchange in cardiac mitochondria [10] and synaptic plasma membranes [7]. Although an explanation for the stimulatory effect of K^+ is that K^+ combines with the carrier changing the Na^+-Ca^{2+} exchange activity, the presence of charge transfer during Na^+-Ca^{2+} exchange could be modified by the permeable K^+, thus leading to indirect K^+ effects.

The possibility of simultaneously controlling the internal medium by dialysis and the membrane potential through voltage clamp makes the squid axon an ideal preparation to study further the effect of K^+.

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

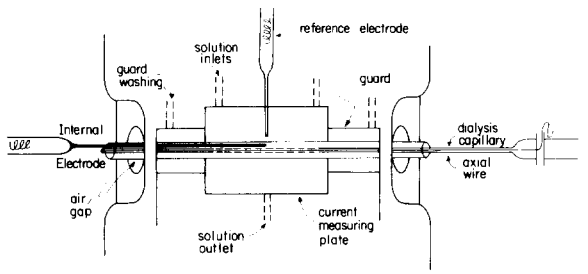

Fig. 1. Diagram of the experimental chamber. For these experiments, plastic dialysis capillaries of 150 μm outer diameter were used. A current wire (platinized platinum iridium 20% of 30 μm) was introduced into the dialysis capillary and this was stirred longitudinally through the whole length of the axon. The voltage electrode (glass cannula of about 40–60 μm containing a floating platinum wire of 25 μm and filled with 0.5 M KCl) was stirred through the other end of the axon and its tip positioned in the center of the dialysis chamber. The chamber contain all the conventional features necessary for voltage clamping and dialysis.

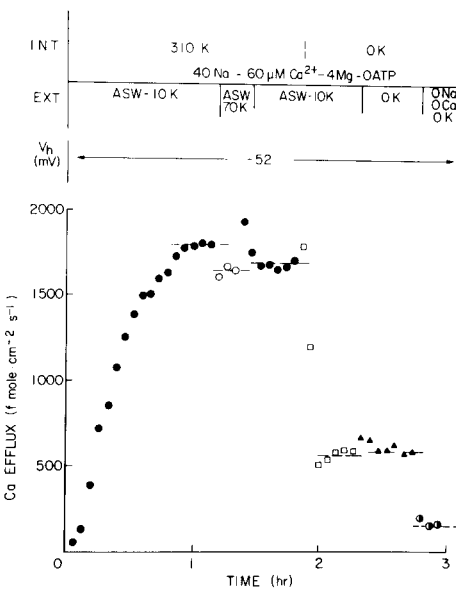

Fig. 2. The effect of external and internal K^+ on the Na_o-dependent Ca efflux. Ordinate: Ca efflux in fmol·cm^{-2}·s^{-1}. Abscissa: time in h. The composition of solutions was as follows (mM); artificial sea-water (ASW): Na^+, 440; K^+, 10; Ca^{2+}, 10; Mg^{2+}, 50; $Tris^+$, 10; Cl, 580; EDTA. 0.1 The osmolarity was 1000 mosmol/kg and the pH (17.5–18.0 °C) 7.6. When Na_o^+ and Ca_o^{2+} were removed they were replaced by $Tris^+$ and Mg^{2+}, respectively. Dialysis solutions: Na^+, 40; K^+, 310; Mg^{2+}, 4; $Tris^+$, 30; Cl^-, 82; aspartate, 310; EGTA, 1; glycine, 330; total osmolarity was 980 mosmol/kg and pH (18 °C) 7.3. All chemicals used were reagent grade. Counting was performed in a liquid scintillation counter after mixing the sea-water samples (4 ml) with 5 ml of scintillator. Unless otherwise stated, all concentrations are given in mM.

Materials and Methods

The experiments were performed with live speciments of Loligo plei, taken from Mochima Bay (Edo Sucre, Venezuela) and transported to the Instituto Venezolano de Investigaciones Cientificas in Caracas. The experimental chamber for dialyzing and voltage clamping the freshly dissected axons is described in Fig. 1. The artificial sea-water and dialysis solution compositions are given in the legend of Fig. 2. A point worth stressing here is that under the conditions used in these experiments, most (about 90%) of the Ca^{2+} efflux is Na_o^+-dependent [11].

Results

The experiment of Fig. 2 shows the effect of K_o^+ and K_i^+ in an axon dialyzed with 60 μM Ca^{2+}, 40 mM Na^+ and no ATP. The membrane potential was held constant (−52 mV) during the entire course of the experiment. Increasing the K_o^+ from 10 to 70 mM causes no effect on the Ca^{2+} efflux. On the other hand, replacing K_i^+ by $Tris^+$ drops the efflux of Ca^{2+} by about 70%. Subsequent removal of all the K_o^+ causes no further change in the efflux. Finally, removal of Na_o^+ and Ca_o^{2+} decreases the Ca^{2+} efflux to a very low value. In order to discard the possibility that the inhibition of the Ca^{2+} efflux by K_i^+ removal is not due to $Tris^+$, few experiments were performed with *N*-methyl-D-glucamine$^+$ as the major cation. No difference in the K_i^+ effect was found with $Tris^+$ or *N*-methyl-D-glucamine$^+$ substitution. Fig. 3 shows that the inhibition of the Ca^{2+} efflux by the removal of K_i^+ is also observed in the total absence of internal Na^+. It can also be observed, that the activation of the Ca^{2+} efflux upon adding 50 mM potassium to the internal medium is the same in the absence or in the presence of 40 mM internal Na^+. Fig. 4 summarizes the results of four different experiments in which the K_i^+ activation of the Na_o^+-dependent Ca^{2+} efflux was measured using an internal medium containing 60 μM Ca^{2+}, 40 mM Na^+ and no added ATP. A $K_{1/2}$ of 90 mM for the K_i^+ effect indicates that K_i^+ activates the Na_o^+-dependent Ca^{2+} efflux with low affinity.

To see whether the activation of the Ca^{2+} efflux induced by internal K^+ takes place also in the

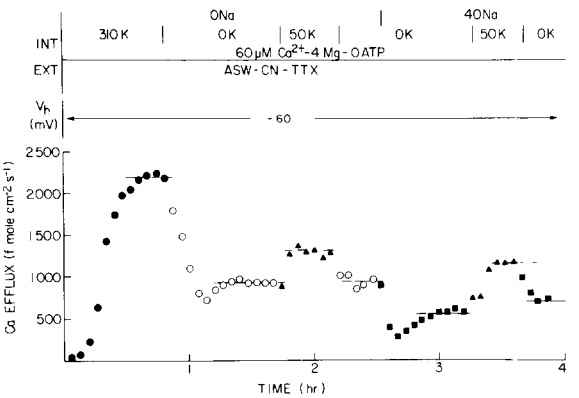

Fig. 3. The effect of internal K^+ on the Ca^{2+} efflux in an axon dialyzed without and with internal Na^+. Ordinate: Ca^{2+} efflux in fmol·cm^{-2}·s^{-1}. Abscissa: time in h. Notice that the stimulation of the Ca^{2+} efflux by the addition of 50 mM K_i^+ is the same in the presence and in the absence of Na_i^+. Axon diameter 395 μm. Unless otherwise stated, all concentrations are in mM.

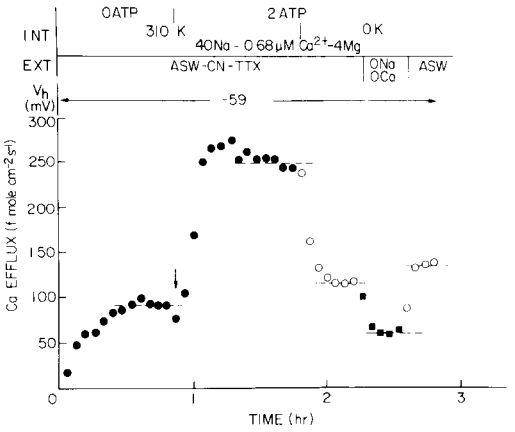

Fig. 5. The effect of removing K_i^+ on the Ca^{2+} efflux in an axon dialyzed with 2 mM ATP. Ordinate: Ca^{2+} efflux in fmol. cm^{-2}·s^{-1}. Abscissa: time in h. For details, see text and the legend to Fig. 2. Axon diameter 420 μm. Unless otherwise stated, all concentrations are given in mM.

presence of ATP, few experiments were performed with 2 mM ATP in the dialysis medium. The result of such an experiment is shown in Fig. 5. In the absence of ATP and in the presence of 40 mM Na_i^+ and 0.68 μM Ca_i^{2+}, Ca^{2+} efflux reaches a steady value of about 90 fmol·cm^{-2}·s^{-1}. Addition of ATP causes an increase in the Ca^{2+} efflux to 240 fmol·cm^{-2}·s^{-1}, and the removal of K_i^+ drops the efflux to 110 fmol·cm^{-2}·s^{-1}. Finally, the removal of Na_o^+ and Ca_o^{2+} decreases the efflux to about 60 fmol·cm^{-2}·s^{-1}. Since the magnitude of the Na_o^+-dependent Ca^{2+} efflux component in the presence of ATP and in the absence of K_i^+ is smaller than that in the absence of ATP and in the presence of K_i^+, it can be inferred than the Na_o^+-dependent Ca^{2+} efflux observed in the presence of ATP must also be activated by internal potasium.

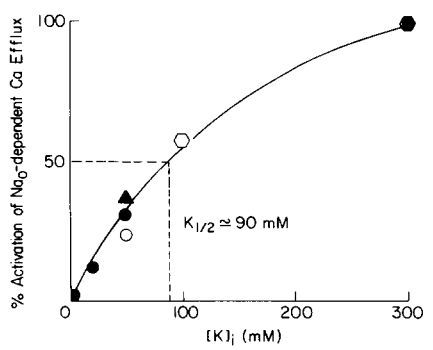

Fig. 4. Na_o^+-dependent Ca^{2+} efflux in dialyzed axons containing 40 mM Na_i^+, 60 μM Ca_i^{2+} as a function of the intracellular K^+ concentration. The symbols represent different axons. Each point correspond to the steady-state Ca^{2+} efflux value attained at a given K_i^+.

Conclusions

The results presented in this work shows that in squid axons, K^+ from the cytoplasmic side strongly activates the Na_o^+-dependent Ca^{2+} efflux. The effect of K^+ is asymmetric, since external K^+ up to 70 mM does not affect the Ca^{2+} efflux. The experiments (Figs. 2 and 3) show that the requirement for internal K^+ is only partial since, even in the complete absence of this ion, it is possible to induce a sizable Na_o^+-dependent Ca^{2+} efflux. It is clear that the activation by potassium cannot be explained as a displacement of the inhibition by internal Na^+, since a similar activation is observed in the absence of Na_i^+. Since under physiological conditions the Na^+-Ca^{2+} exchange will be fully activated by K^+, one cannot regard this ion as a true regulator. However, when studying the behavior of the carrier under physiological conditions, this effect must clearly be taken into account.

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