A novel 13 kDa cytoplasmic soluble protein is required for the nucleotide (MgATP) modulation of the Na/Ca exchange in squid nerve fibers

Reinaldo DiPolo[a,c,*], Graciela Berberián[b,c], Daniel Delgado[a], Hector Rojas[a], Luis Beaugé[b,c]

[a]*Centro de Biofísica y Bioquímica, IVIC, Apartado 21827 Caracas 1020A, Venezuela*
[b]*División de Biofísica, Instituto M.y.M Ferreyra, Casilla de Correo 389, 5000 Córdoba, Argentina*
[c]*Marine Biological Laboratory, Woods Hole, MA 02543, USA*

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Abstract The Na/Ca exchange is a highly regulated transport mechanism in which MgATP, a powerful modulatory intracellular substrate, has important implications for its function. As occurs with some preparations, in squid axons, nucleotide regulation is lost after membrane vesicle isolation. This has been a significant obstacle in the biochemical characterization of the MgATP effect. An important clue in solving this long-standing puzzle is presented in this work by showing that prolonged intracellular dialysis of squid axons produces a complete run down of the MgATP effect. Here we report that a soluble cytoplasmic factor isolated from fresh squid axoplasm and brain reconstitutes the MgATP stimulation of the Na-gradient-dependent ^{45}Ca uptake in squid optic nerve membrane vesicles. Partial purification of this factor uncovers the presence of a novel 13 kDa soluble cytoplasmic protein (SCPr) which, when microinjected in ATP de-regulated dialyzed squid axons, completely restores the MgATP stimulation of Na_o-dependent Ca efflux. We propose that in the squid preparation this SCPr constitutes the link between the nucleotide and target effector: the Na/Ca exchanger itself, or other plasma membrane structures which may secondarily interact with the exchanger.

Key words: Na/Ca exchange regulation: MgATP; Squid axon

1. Introduction

The Na/Ca transporter is a wide-spread plasma membrane protein responsible for a large fraction of the total transmembrane calcium fluxes that normally occur in living cells [1]. This electrogenic system which is directly involved in crucial phenomena such as contractility and photoreception is highly regulated by several intracellular factors including ATP, Ca^{2+}, Na^+ protons and lipids [1]. In squid axons, and from recent reports in cardiac myocytes and smooth muscle, it is likely that a kinase-directed phosphorylation is involved in this regulation [2–4]. A long-standing puzzle in this area has been the lack of MgATP activation of Na/Ca exchange in some membrane vesicle preparations in spite of the large activation observed in living cells. This has led us to consider the attractive hypothesis that an essential factor present in the cytosol and lost during membrane isolation may account for the de-regulation of the Na/Ca exchanger in vitro.

We report herein that a soluble cytosolic factor (SCF) isolated from squid axoplasm and optic squid ganglia is required for MgATP stimulation of Na^+-dependent Ca^{2+} uptake in plasma membrane vesicles from squid optic nerve and for the MgATP stimulation of Na_o-dependent Ca efflux in internally dialyzed squid giant axons.

2. Materials and methods

2.1. Intracellular dialysis and microinjection of squid axons

As described previously [5], squid axons from the Marine Biological Laboratory, Woods Hole, MA (*Loligo pealei*), or the Instituto Venezolano de Investigaciones Cientificas (*Loligo plei*) were dialyzed with highly permeable capillaries from regenerated cellulose fibers (OD: 230 μm; ID: 220 μm; molecular weight cut of (MWCO) of 18 kDa; Spectra/por #132225, Spectrum, Houston TX). For intracellular microinjection experiments a 50–75 μm glass capillary attached to a 1 μl syringe was positioned inside the axons at the beginning of the dialysis. Injection over the entire dialyzed region was performed by the slow mechanical withdrawal of the injector while maintaining fix the syringe plunger. The standard dialysis medium had the following composition (mM): Mops-Tris, 385; NaCl, 45; $MgCl_2$, 5; Glycine, 285, Tris-EGTA, 1–3. The estimation of $[Ca^{2+}]$ was made using the computer program Maxchelator (Chris Patton, Hopkins Marine Station, Pacific Grove, CA) using the Harrinson and Bers constants at high ionic strength [6]. The pH was 7.3 and the temperature was between 17 and 18°C. The osmolarity of all solutions was adjusted to 940 mosmol. The external solution had the following composition (mM): Na^+, 440; Ca^{2+}, 0.5; Mg^{2+}, 60; Cl^-, 570. The pH was 7.6. Removal of external sodium was compensated with Li^+. In order to stop any endogenous production of ATP, and therefore control the [ATP] through internal dialysis, 1 mM NaCN was always present in the external solutions. When present, ATP was added at a constant free $[Mg^{2+}]_i$.

2.2. ^{45}Ca uptake in membrane vesicles

^{45}Ca uptake was measured at room temperature by incubating the vesicles (50–60 μg prot) for 10 s in media with high (300 mM) or low (30 mM) Na^+ (total volume: 200 μl). In addition, all extravesicular solutions contained 0.1 mM vanadate and 20 mM Mops-Tris (pH 7.3 at 20°C); in low Na^+ medium the osmolarity was compensated with NMG-Cl. The reaction was stopped with 0.8 ml of an ice-cold solution containing 20 mM Mops-Tris, 300 mM KCl and 1 mM EGTA and filtered through Whatman F/GF glass filters. The filters were washed with 5 ml of the same solution, immersed into 5 ml of scintillation fluid and counted in a liquid scintillation counter. Experiments were performed by triplicate and expressed as the mean ± SEM.

2.3. Preparation of squid optic nerve membrane vesicles and cytosolic post-microsomal supernatant

Membrane vesicles from squid optic nerve (tropical *Sepiotheuthis sepioidea* and *Loligo plei*, and Atlantic *Loligo pealei*) were prepared by differential centrifugation and loaded with 300 mM NaCl, 0.1 mM EDTA and 30 mM Mops-Tris (pH 7.4 at 20°C). Supernatant from fresh extruded squid nerve axoplasm or optic ganglia (brain) were obtained by homogenizing them (1:1, v/v ratio) in 20 mM Mops-

*Corresponding author. Centro de Biofísica y Bioquímica, IVIC, Apartado 21827, Caracas 1020A, Venezuela. Fax: (58) 2-504-1093

Abbreviations: EGTA, (ethylene glycol-bis-β(aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Mops, (3-(*N*-morpholino) propanesulphonic acid); NMG, *N*-methylglucamine; p-NPP, *p*-nitrophenylphosphate; PKA, protein kinase A; PKC, protein kinase C; PtyK, tyrosine kinase; CAM-dependent PK, calmodulin-dependent protein kinase; ATPγS, adenosine 5'-*O*-(thiotriphosphate)

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Tris (pH 7.4 at 20°C), 1 mM DDT, 0.1 mM EDTA, 0.1 mM EGTA and an antiprotease cocktail (0.5 mM PMFS plus 10 μg/ml of aprotinin, leupeptin and pestatin A) followed by centrifugation at 12000×*g* for 10 min. This supernatant was further centrifugated at 100000×*g* for 30 min (post-microsomal fraction). ^{45}Ca uptake was carried out in media (see above) with 0.6–0.8 μM Ca^{2+}, 1 mM ATP, 1 mM Mg^{2+}, 0.1 mM vanadate and 10 μl (150–180 μg total protein) of the post-microsomal fraction (PMF). Heat denaturation of both brain and axoplasm extracts was carried out at 70°C for 15 min prior to the ^{45}Ca uptake experiment. Trypsin digestion was done by incubating trypsin and PMF at 8°C at a ratio of 0.02μg trypsin/μg total protein. The reaction was stopped after 30–60 min with soybean trypsin inhibitor at an inhibitor/trypsin ratio of 4:1 (w/w). In control experiments the PMF was incubated with trypsin inhibitor during 10 min prior to the addition of trypsin.

2.4. *Fractionation of the post-microsomal supernatant*

After obtaining the 100000×*g* supernatant fraction, a series of filtrations through 100, 50, 30 and 10 kDa cut-off filters (Amicon Centricon) were carried out, and their protein content determined. ^{45}Ca uptake was carried out as above using 10 μl of each fraction. The lyophilized 30 kDa fraction remained active after more than 96 h storage at room temperature. For further purification of the 30 kDa fraction aliquots of 200 μl (≈1.2 mg of total protein) suspended in 30 mM Mops-Tris (pH 7.4 at 20°C) were passed through an FPLC system using a superdex-75 column (Pharmacia). The runs were performed with the same buffer at 0.4 ml/min flow rate. Aliquots of 0.25 ml were assayed for total protein content and stimulation of a MgATP-dependent ^{45}Ca uptake in nerve vesicles.

3. Results

3.1. *Run down of the MgATP stimulation of Na/Ca exchange by prolonged intracellular dialysis*

The initial evidence that a SCF could be involved in the MgATP stimulation of Na/Ca exchange in squid axons came from in vivo experiments on Na_o-dependent Ca efflux (forward Na/Ca exchange) at different dialysis times. We tested the two ATP-dependent components of the Ca efflux: Ca pump (Na_o-independent) and Na/Ca exchange (Na_o-dependent) at short (< 120 min) and prolonged (> 300 min) periods of intracellular dialysis (Fig. 1A). At short times, addition of ATP to the dialysis medium in the presence of external Na^+ (Fig. 1A, ●) activates a fast (Ca pump) and a slow (Na/Ca exchange) component of Ca efflux. Withdraw of external Na^+ (Fig. 1A, ○) drops the Ca efflux to the level of the Ca pump flux and removal of ATP brings Ca efflux back to its initial low level. The magnitude of the ATP-stimulated Na_o-dependent Ca efflux amounts to about 200 fmol·cm^{-2}·s^{-1} (compare with 20 fmol·cm^{-2}·s^{-1} in the absence of ATP). In the same axon and after a long dialysis time (350 min), re-addition of ATP activates only the fast component (Ca pump) with little stimulation of Na/Ca exchange. This is confirmed by the fact that vanadate which inhibits the ATP-stimulated Na_o-independent Ca efflux has no noticeable effect on the other component; this contrasts with the marked stimulation of Na/Ca exchange by vanadate in the presence of MgATP [8]. The run down of the MgATP stimulating effect cannot be due to an unspecific inhibition of the Na/Ca exchanger by the prolonged dialysis since raising the $[Ca^{2+}]_i$ to a saturating concentration (100 μM) increases the Na_o-dependent Ca efflux to its usual maximal value (Fig. 1A). The results of several experiments in which V_{max} of the total and ATP-stimulated Na_o–Ca_i exchange and the Ca pump were measured at different dialysis times are shown in Fig. 1B. Only the MgATP stimulation of Na–Ca exchange is markedly diminished.

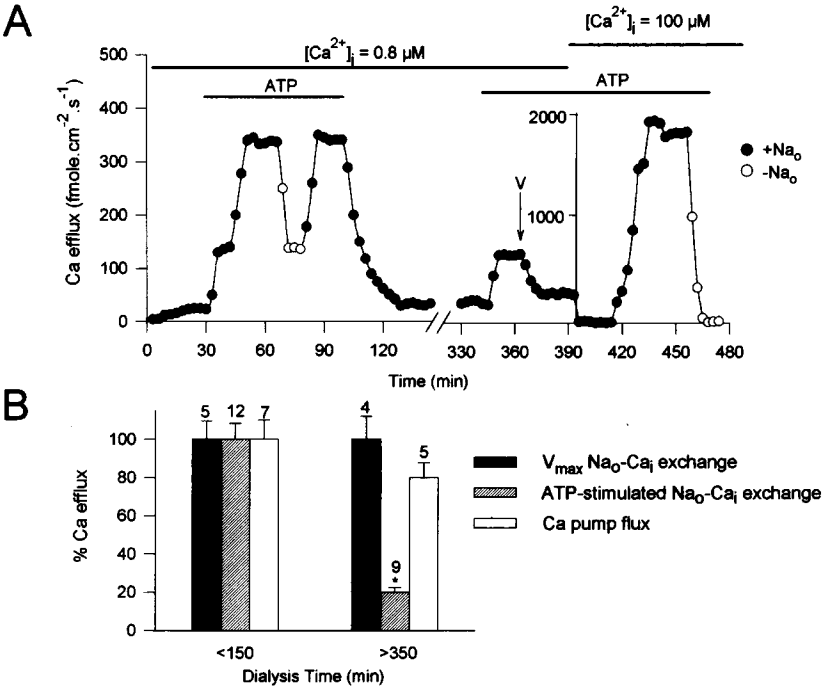

Fig. 1. Run down of the ATP-stimulated Na_o-dependent Ca efflux (Na/Ca exchange) after prolonged intracellular dialysis. A: Experiment in a single dialyzed squid axon. Axon diameter: 550 μm. Ordinate: Ca efflux in fmol·cm^{-2}·s^{-1}. Abscissa: time in minutes. ●, Ca efflux in the presence of external Na^+. ○, Ca efflux in the absence of Na^+ ions. The arrow indicates the addition of 100 μM vanadate to the dialysis medium. B: Summary of different experiments in which: (%) V_{max} of Na_o-dependent Ca efflux ($[Ca^{2+}]_i$ = 100 μM); ATP-stimulated Na_o-dependent Ca efflux ($[Ca^{2+}]_i$ = 0.8 μM) and Na_o-independent Ca efflux (Ca pump; $[Ca^{2+}]_i$ = 0.8 μM) were measured at short (< 150 min) and long (> 330 min) internal dialysis times. The asterisk (*) denotes a statistically significant difference with the other bars ($P < 0.001$). The number of experiments are indicated over the bars. The statistical errors are expressed as SEM.

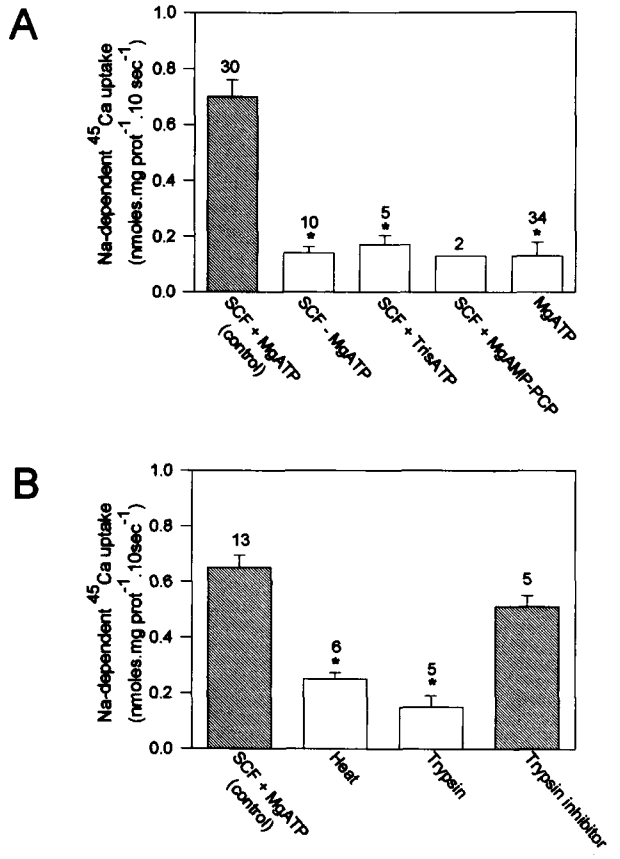

Fig. 2. A: Reconstitution of the MgATP stimulation of Na-gradient-dependent ^{45}Ca uptake in membrane vesicles from squid optic nerves in the presence of supernatant of the post-microsomal (100 000×*g*) fraction from squid optic ganglia (brain) Ordinate: Na-dependent ^{45}Ca uptake in nmol·mg prot^{-1}·10 s^{-1}. The asterisk (*) denotes a statistically significant difference ($P<0.05$) with the control condition (SCF+MgATP). The number of experiments is indicated over the bars. The statistical errors are expressed as SEM. B: The effect of heat and trypsin treatment of SCF (from axoplasm) on the reconstitution of the ATP stimulation of Na-dependent ^{45}Ca uptake. The asterisk (*) denotes a statistically significant difference ($P<0.001$) with the control condition (SCF+MgATP). The number of experiments is indicated over the bars. The statistical errors are expressed as SEM.

3.2. *Reconstitution of the MgATP effect in vitro by a soluble cytoplasmic factor (SCF)*

If the run down of the MgATP activation of Na/Ca exchange follows the washout of a SCF, and the same mechanisms applies to the lack of MgATP stimulation of the exchanger in nerve vesicles, it should be possible to recover the nucleotide effect in vitro by adding back the missing compound. Therefore, we decide to look for this unknown factor in the extruded axoplasm of giant axons by investigating its effects on the Na-dependent ^{45}Ca uptake in squid optic nerve membrane vesicles incubated with MgATP. Initially we tested the supernatant (100 000×*g*; post-microsomal fraction) under conditions resembling those of an in vivo experiment (low intracellar $[Ca^{2+}]_i$ and $[Na^+]_i$) Fig. 2A shows that, under these conditions, the supernatant of the post-microsomal axoplasm fraction induced a marked activation of a Na-dependent ^{45}Ca uptake. Three additional important findings are also shown in Fig. 2A: (1) the absence of effect of the supernatant in the absence of MgATP, (2) the absolute requirement for Mg^{2+} (Tris-ATP is ineffective), and (3) the lack of effect of the non-hydrolyzable ATP analog AMP-PCP. Confirming previous results, note that MgATP alone does not stimulate a Na-dependent ^{45}Ca uptake in these vesicles (Fig. 2A, Box 5). Unspecific effects of proteins (there was 150–180 μg of supernatant protein in the uptake media) were ruled out by the lack of effect of 300 μg albumin added to the assay solutions in the absence of the axoplasmic fraction (results not shown).

We then examined the possible nature of the SCF by treating the supernatant of the post-microsomal fraction with heat and trypsin. Both treatments completely abolished the MgATP stimulation of Na-dependent ^{45}Ca uptake (Fig. 2B). Trypsin inactivation by prior addition of the soybean trypsin inhibitor largely restored the MgATP effect (Fig. 2B). Taken together these results indicate that the SCF is a protein.

It is well known that the MgATP stimulation of the Na/Ca exchange in squid axons is the consequence of an increase in the affinity of both transport and regulatory Ca_i sites without changes in the V_{max} of the exchanger [2,7]. Thus we explored whether the SCF activated the Na-dependent ^{45}Ca uptake following a similar kinetic pattern by carrying out vesicles experiments at low (0.6 μM) and high (100 μM) Ca^{2+} in the uptake medium. At low Ca^{2+} the SCF increased Na-dependent ^{45}Ca uptake by 5.3-fold in marked contrast with only 1.1 at high Ca (Fig. 3); this suggests that the mechanism of MgATP activation of the Na/Ca exchange induced by the SCF in nerve membrane vesicles is similar to that seen in dialyzed squid giant axons.

3.3. *Partial purification of the SCF*

An initial partial purification of the SCF was carried out by filtration of the crude 12 000×*g* supernatant through 100, 50, 30 and 10 kDa (MWCO) filters. Total protein content and effect on Na/Ca exchange activity were measured in each filtrate. (Fig. 4A). Filtrations up to 30 kDa reduce the total protein content in the filtrate almost 50-fold (23.5 mg/ml in

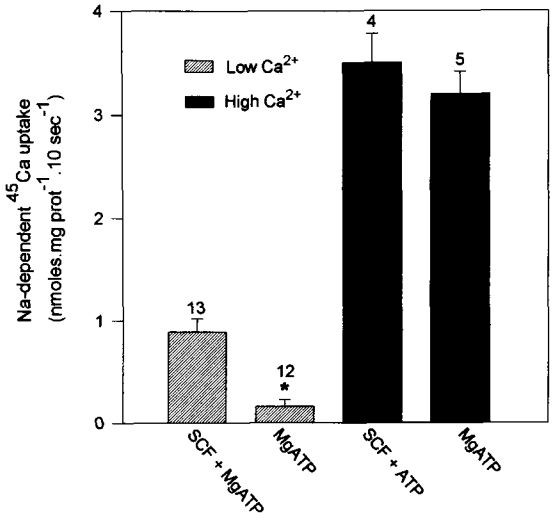

Fig. 3. Comparison of the MgATP stimulation of Na/Ca exchange in squid optic membrane vesicles induced by the SCF at low and high $[Ca^{2+}]$. Notice that at low $[Ca^{2+}]$ (0.6 μM) the MgATP stimulation of Na-gradient-dependent ^{45}Ca effect is more than 5 times larger than that at high $[Ca^{2+}]$ (100 μM). The asterisk (*) denotes a statistically significant difference ($P<0.001$) with the control condition (SCF+MgATP). The number of experiments is indicated over the bars. The statistical errors are expressed as SEM.

the crude 12000×*g* supernatant to 0.51 mg/ml in the 30 kDa filtrate) while maintaining full activity. On the other hand, activity was absent in the 10 kDa filtrate, indicating that the SCF is a protein in the 10–30 kDa molecular weight range. To further purify SCF, the 30 kDa filtrate was lyophilized, resuspended in buffer and then passed through an FPLC column (Fig. 4B). Full restoration of the MgATP effect was only observed in the #65 collection fraction, corresponding to a molecular weight close to 13 kDa (12.4 kDa molecular weight of cytochrome *c* as marker). Considering that the total protein content of fraction #65 was about 0.1–0.2 mg/ml, a purification of almost 250 times was achieved.

3.4. *In vivo reconstitution of the MgATP effect in de-regulated dialyzed squid axons*

The fact that the molecular weight of the SCPr is low enough to pass through the dialysis capillaries opens up the possibility that the run down of the MgATP effect after prolonged intracellular dialysis (Fig. 1) is actually due to the washout of this compound. To test for this hypothesis, which in turn would evaluate the physiological significance of the SCPr, we investigated the possibility of restoring the MgATP stimulation of the Na/Ca exchanger by injecting the 13 kDa protein into axons subjected to long dialysis times. The protocol for these experiments was as follows: we estimated the

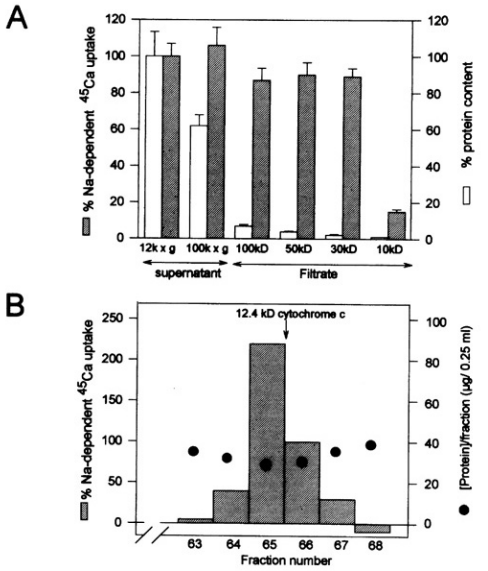

Fig. 4. Partial purification data of the SCF from squid optic ganglia that reconstitutes the MgATP stimulation of Na/Ca exchange in membrane vesicles from squid optic nerves. A: Ordinates: MgATP stimulation of Na-gradient-dependent ^{45}Ca uptake in membrane vesicles of squid optic nerves due to the addition of several filtrates (100, 50, 30 and 10 kDa) of supernatant from optic ganglia. The ^{45}Ca uptake is expressed as % taking as 100% that observed in the presence of the 12000×*g* supernatant (0.668 ± 0.04 nmol·mg^{-1}·10 s^{-1}). Protein content is expressed as percentage taking as 100 the value corresponding to the 12000×*g* supernatant (23.5 ± 3.8 mg/ml). Notice that activity is maintain up to the 30 kDa filtrate and lost in the 10 kDa filtrate. B: Ordinate: MgATP stimulation of Na-gradient-dependent ^{45}Ca uptake in the presence of FPLC fractions of the 30 kDa filtrate from squid optic ganglia. The abscissa represents the fraction numbers. The arrow shows the fraction collection at which the cytocrome *c* eludes from the columm (MW: 12.4 kDa). The values are the mean of duplicated determinations with a range of no more than 8%. ^{45}Ca uptake is expressed as percentage: (100*(with ATP−without ATP)/without ATP).

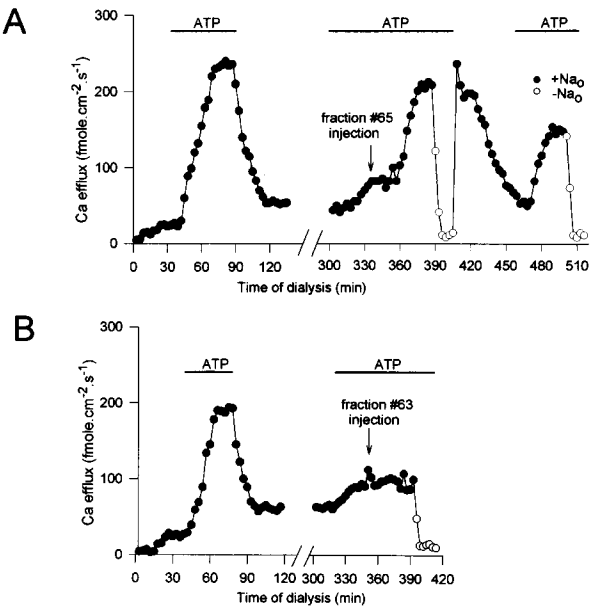

Fig. 5. Reconstitution of the MgATP stimulation of Na/Ca exchange by microinjection of the 13 kDa protein fraction in a de-regulated dialyzed squid axon. Ordinate: Ca efflux in fmol·cm^{-2}·s^{-1}. Abscissa: time in minutes. ●, Ca efflux in the presence of external Na^+. ○, Ca efflux in the absence of external Na^+ (Li^+ substitution). A: Microinjection of the active FPLC (#65) fraction containing the putative 13 kDa protein restores the MgATP stimulation of the Na_o-dependent Ca efflux. B: Microinjection of an inactive FPLC fraction (#63). Notice the absence of reconstitution of the MgATP stimulation of Na_o-dependent Ca efflux.

ATP-dependent Na_o-stimulated component of the Ca efflux at the beginning of the dialysis (< 120 min) and after an extensive dialysis period (> 330 min) when that component was almost completely lost. Thereafter, an aliquot (0.16 µl) of fraction #65 was microinjected in the presence of MgATP. As shown in Fig. 5A, microinjection of the active fraction into the axon restored (70–80%) the ATP stimulation of Na_o-dependent Ca efflux. Reactivation of Na/Ca exchange cannot be due to an unspecific effect of the microinjection or an increase in leak since stimulation was totally Na_o-dependent and completely abolished by removal of ATP from the dialysis medium. A further test for the validity of the reconstitution experiment is shown in Fig. 5B. In this experiment an aliquot of a negative fraction from the FPLC (#63; see Fig. 4B) was injected in a de-regulated squid axon. In marked contrast to the above result microinjection of the inactive fraction fails to reconstitute the MgATP effect. The average recovery of the MgATP stimulation by microinjection of the SCPr was 75 ± 9 ($n = 5$). The lack of recovery by microinjecting the inactive FPLC fraction was confirmed in three different axons.

4. Discussion

In this work we demonstrate that MgATP regulation of the squid Na/Ca exchanger requires a soluble cytosolic factor which is a protein of low molecular weight. For this reason we propose the name of SCPr. A clear evidence that the reconstitution of the MgATP effect is a genuine phenomena is that it occurs neither in the absence of ATP nor in the presence of non-hydrolizable ATP analogs and, most impor-

tant, it requires Mg^{2+} ions. All of these in vitro features are similar to those found in dialyzed squid axons. Moreover, reconstitution of the MgATP stimulation of Na/Ca exchange by the SCF preserves the kinetic modification of the exchanger induced by MgATP in vivo, that is, an increase in the affinity for the transport (and regulatory) Ca^{2+} site without significant changes in V_{max}.

In squid axons, experimental evidence points to a phosphorylation process involved in the MgATP modulation of Na/Ca exchange. ATP, hydrolizable ATP analogs (AMP–CPP) and the slowly hydrolyzable analog ATPγS activate the exchanger in the presence of Mg^{2+} and Ca^{2+} ions [2]. CrATP, an end-product inhibitor of most kinases, completely blocks the MgATP effect [8] while inorganic phosphate and p-NPP, two product inhibitors of phosphatases, and vanadate, a powerful inhibitor of phosphatases enhance that stimulation [9,10]. All these experimental observations point to a process involving the interplay of kinase(s)–phosphatase(s) system(s). Nevertheless, none of the classical inhibitors of PKA, PKC, PtyK, and CAM-dependent PK affect the MgATP stimulation of the Na/Ca exchange in squid axons [5]. On the other hand, recent works on cardiac myocytes and aortic smooth muscle have shown that the Na/Ca exchange can be directly phosphorylated via PKC (but see, [11]) and dephosphorylated by an okadaic acid-sensitive system. It appears then that phosphorylation is a mechanism for regulation of Na/Ca exchange in squid, cardiac and smooth muscle. Nevertheless, the phosphorylated structures and enzymes involved do not have, and actually do not seem, to be the same in these preparations. In this regard regulation of the cardiac exchanger is interesting. For instance, isolated sarcolemma vesicles of beef heart show MgATP and MgATPγS stimulation of Na/Ca exchange in the absence of any added cytosolic component. With ATP that stimulation is seen only in the presence of vanadate, but with ATPγS vanadate is not required. The effect is the consequence of an increase in the intracellular affinity of the carrier for Ca^{2+} [12]. On the other hand, giant membrane patches of cardiac myocytes also display MgATP stimulation of the Na/Ca exchanger but that stimulation is not mimicked by ATPγS and it is not affected by vanadate [13]. A recent important contribution indicates that, at least in myocytes under giant patch, ATP acts by generating phosphatidylinositol-4,5-biphosphate (PIP_2) from phosphoatidylinositol (PIP) [14].

From the above, it is plausible that more than one mechanism is responsible for MgATP modulation of the exchanger in different preparations and perhaps even in the same cell. Different pathways of ATP modulation could be expected, considering the large amino acid sequence variation among the different Na/Ca exchange clones. In line with this, a new splicing variant in the frog cardiac exchanger has been recently found with an incomplete ATP-binding site of the P-loop type which is not present in mammalian clones [15]. This suggests that ATP may regulate Na/Ca exchange in frog differently from the mammalian heart. Regarding this work, the mechanism by which the SCPr is involved in the reconstitution of the MgATP effect in squid axons remains unknown. One possibility is direct phosphorylation of the Na/Ca exchanger mediated by SCPr either in a manner similar to that induced by PKC in the mammalian cardiac exchanger or by phosphorylation of other membrane structures which interact with it, including lipids. An interesting alternative we suggested before [16], particularly considering the low molecular weight of SCPr, is that this molecule behaves as a type of 'response regulator'. These regulators are well known proteins present in prokaryotes and also eukaryotes; once phosphorylated, via an histidine kinase intermediate chain, they act as messengers by binding and activating directly the target system [17]. Experiments are presently under way in our laboratories to biochemically characterize this novel SCPr.

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References

[1] Hilgemann, D., Philipson, K. and Vassort G. (1996) Ann. NY Acad. Sci., 779.
[2] DiPolo, R. and Beauge, L. (1991) Ann. NY Acad. Sci. 639, 100–111.
[3] Iwamoto, T., Wakabayashi, S. and Shigekawa, M. (1995) J. Biol. Chem. 270, 8996–9001.
[4] Iwamoto, T., Pan, Y., Wakabayashi, S., Imagawa, T., Yamanaka, H. and Shigekawa, M. (1996) J. Biol. Chem. 271, 13609–13615.
[5] DiPolo, R. and Beauge, L. (1996) Ann. NY Acad. Sci. 779, 199–207.
[6] Harrison, L. and Bers, D. (1987) Biochim. Biophys. Acta 925, 133–143.
[7] Blaustein, M. (1977) Biophys. J. 20, 79–110.
[8] DiPolo, R. and Beauge, L. (1993) J. Physiol. 462, 71–86.
[9] DiPolo, R. and Beauge, L. (1994) Am. J. Physiol. 266. C1382–C1391.
[10] DiPolo, R. and Beauge, L. (1996) Biophys. J. 70, A399.
[11] Condrescu, M., Gardner, J.P., Chernaya, G., Aceto, J.F. Kroupis, C. and Reeves, J. (1995) J. Biol. Chem. 270, 9137–9146.
[12] Berberian, G. and Beauge, L. (1996) Ann. NY Acad. Sci. 779, 282–283.
[13] Hilgeman, D. (1996) Ann. NY Acad. Sci. 779, 136–158.
[14] Hilgemann, D. and Ball, R. (1996) Science 273, 956–959.
[15] Iwata, T., Kraev, A., Guerini, D. and Carafoli, E. (1996) Ann. NY Acad. Sci. 779, 37–45.
[16] Beauge, L., Delgado, D., Rojas, H., Berberian, G. and DiPolo, R. (1996) Ann. NY Acad. Sci. 779, 208–216.
[17] Parkinson, J.S. and Kofoid (1992) Annu. Rev. Genet. 26, 71–112.