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THE EFFECT OF ATP ON CALCIUM EFFLUX IN DIALYZED BARNACLE MUSCLE FIBRES

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Summary

Calcium efflux has been studied in barnacle muscle fibres under internal dialysis conditions. Prolonged dialysis of these fibres, with a medium free of ATP and containing 2 mM cyanide and 1 mM iodoacetate, causes the ATP in the perfusion effluent to fall to less than 20 μM. The mean calcium efflux from fibres dialyzed with EGTA buffered solution containing 0.3 μM ionized Ca and no ATP is 0.6 pmol $\cdot$ $cm^{-2} \cdot s^{-1}$. A two-fold stimulation of the calcium efflux is observed when ATP is added to fibres previously dialyzed with an ATP-free medium. Withdrawal of Na^+ and Ca^{2+} from the external medium causes a marked drop in the Ca^{2+} efflux in the presence of internal ATP.

Introduction

A dependence of calcium transport on external sodium and calcium has been described by Baker et al. [1] in squid axons, and by Reuter and Seitz [2] in heart muscle. Recently DiPolo [3,4], working with squid axons dialyzed free of ATP, has shown that a sizeable increase in the sodium and calcium-dependent calcium efflux can be obtained by addition of ATP to the dialysis medium.

There are several reports indicating the existence of a coupled sodium and calcium transport in barnacle muscle fibres [5—8]. However, no information is available on the role of ATP in calcium transport in muscle.

In this work we have used the intracellular dialysis technique as a means of studying the possible role of ATP in the calcium transport system in barnacle muscle fibres. Prolonged dialysis of fibres poisoned with cyanide and iodoacetate was found to be effective in reducing the intracellular levels of ATP to low values. The effectiveness of this treatment was confirmed first by analyses of the ATP content in both the dialysis effluent and in the dialyzed portion of the fibre, and secondly by the observation that sodium efflux was significantly reduced after prolonged dialysis with an ATP-free medium. The results

presented here show that the addition of ATP to fibres, previously depleted of this compound, causes a significant stimulation of calcium efflux. This ATP-dependent fraction of the calcium efflux was found to depend on the presence of external sodium and calcium. These results are qualitatively similar to those obtained in squid axons [3] suggesting a common mechanism for calcium transport in both preparations.

Materials and Methods

The experiments reported herein were performed with single fibres from the barnacle *Balanus aquila*. The specimens were supplied by the Pacific Biomarine Co., Venice, Calif. Care was taken to choose fibres of relatively small diameter to reduce the diffusional delays inherent in the dialysis technique. The mean fibre diameter was 990 ± 60 μM (n = 25). The dissection of the fibre and most of the dialysis procedure were similar to those previously described [9]. The main difference from the earlier procedure was that almost the whole fibre length was dialyzed using the experimental setup shown schematically in Fig. 1. The fibre was cannulated only at the cut end while a small hole was pierced in the tendon to allow the passage of the porous capillary. The collecting chamber was separated from the fibre ends by two air gaps. A guard flow outlet allowed the removal of the fluid bathing the regions of the fibre exposed to the water-air interface. This arrangement permitted the collection of only that isotope coming from the centre region of the dialyzed fibre. Cellulose acetate dialysis capillaries of about 200 μM diameter, kindly supplied by Dr. F.J. Brinley, were employed. They were made porous by soaking in 50 mM NaOH for 24 h. The porosity was routinely checked following the procedure of DiPolo [10]. All experiments were performed at 22°C. The basic external solution had the following composition (mM): NaCl, 465, KCl, 10; $CaCl_2$, 11; $MgCl_2$, 53; Tris · Cl,

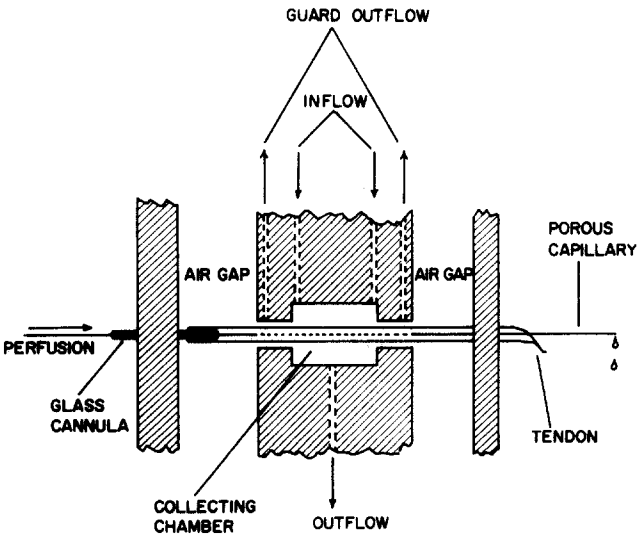

Fig. 1. Schematic drawing of the experimental chamber used to hold the fibre during dialysis.

10; pH, 8; cyanide, 2 mM; iodoacetic acid, 1 mM. When external sodium and calcium were reduced they were substituted by Tris and magnesium, respectively. The internal dialysis medium contained (mM): potassium aspartate, 150; NaCl, 32; $MgCl_2$, 10; Tris · Cl, 5, pH 7.2. The ionic calcium concentration was controlled with EGTA (ethyleneglycol-bis-(β-aminoethyl ether)-*N,N'*-tetraacetic acid) at a final concentration of 1 mM. The ionized calcium concentration was calculated using an apparent equilibrium constant [11] of $5 \cdot 10^6$ M^{-1} for the chelation of Ca^{2+} by EGTA at pH 7.0. The osmolarity was adjusted by adding sucrose to the dialysis medium. ATP was added to the internal medium as MgATP. All compounds were purchased from Sigma. Radioactive dialysis solutions were made by adding $^{22}NaCl$ (Amersham Searle Corporation, Arlington, Ill.) 33 mCi/mg or $^{45}CaCl_2$ (New England Nuclear 10 mCi/mg). In all the experiments, prior to the introduction of radioactive solution into the dialysis capillary, the fibres were incubated in artificial seawater containing CN^- + iodoacetic acid for more than 1 h.

ATP analyses were performed employing the firefly flash method. Measurements of ATP concentrations in the dialysis effluent were made immediately after collecting 5 μl of the perfusate with a glass capillary tube. Determination of ATP in nondialyzed fibres was performed after homogenization of carefully blotted and weighed fibres in 3 ml of 50% propyleneglycol solution at about −15°C. The same procedure was used to assay the ATP content of fibres previously dialyzed. To calculate the ATP concentration, an extracellular space of 7% was used [12]. In this case only the segment of the fibre which had been dialyzed was analyzed.

Results and Discussion

The ATP content of fibres bathed in normal artificial seawater was 4.3 ± 0.5 mmol ATP/kg of fibre ($n = 6$). This value was not significantly changed for fibres incubated from 2 to 5 h in artificial seawater containing 2 mM cyanide and 1 mM iodoacetate. A reduction in the intracellular concentration of ATP was achieved by combining the poisoning treatment with prolonged dialysis. Poisoned fibres, dialyzed extensively (>2 h) with an ATP-free medium and then analyzed for ATP by cutting out the dialyzed region, show values of ATP in the order of 20—30 μmol/kg of fibre ($n = 4$). In confirmation of this result, for all the ATP-free dialysis experiments reported here, the perfusate fluid, when analyzed for ATP, gave values in the order of 20 μM after prolonged dialysis (see Fig. 3).

The dependence of the sodium efflux on ATP was used as a further test for the effectiveness of dialysis in reducing the ATP concentration at the membrane. Fig. 2 shows an experiment in which dialysis was started with a solution containing ^{22}Na, no ATP, cyanide, and iodoacetate. After the sodium efflux had attained a plateau value of 31 pmol · cm^{-2} · s^{-1}, it began to fall, at first slowly, and then more rapidly to a steady level of less than 10 pmol · cm^{-2} · s^{-1}. At the end of the experiment, the dialyzed portion of the fibre was analyzed for ATP, giving a value of about 15 μmol/kg fibre.

In other experiments, after the sodium efflux was diminished in the absence of ATP, it could be restored to nearly normal values by addition of ATP to the

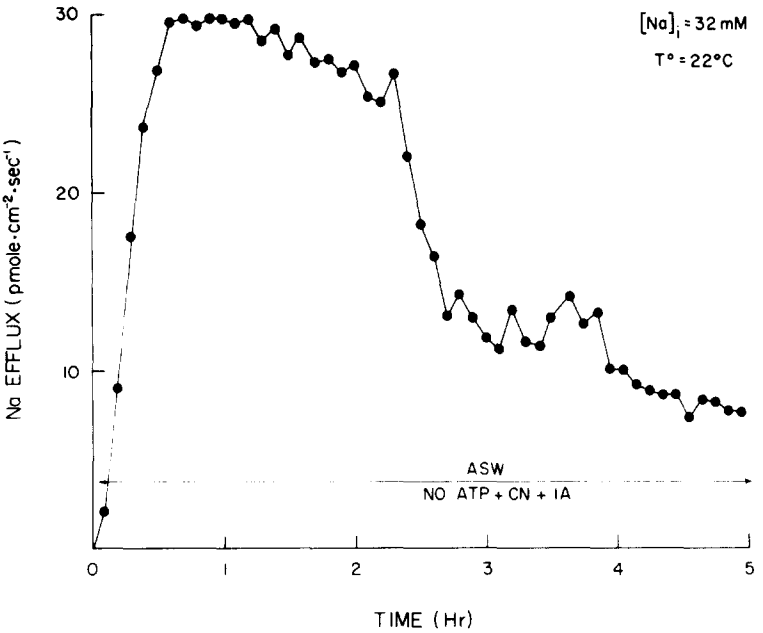

Fig. 2. The effect of washing the [ATP] on the Na efflux in a dialyzed barnacle fibre. Ordinate: Na efflux in pmol · cm^{-2} · s^{-1}. Abscissa: time in hours. The fibre was dialyzed with a medium containing no ATP, 2 mM CN$^-$ and 1 mM iodoacetic acid (IA). At the end of the experiment the dialysate fluid contained less than 10 μM ATP. About the same [ATP] was found by analysis of the dialyzed fibre segment. Abscissa: time in minutes. ASW, artificial seawater.

dialysis medium. These results confirm that the ATP concentration at the membrane level can be reduced effectively by our experimental treatment.

Recently, Brinley and Spangler [13] and Russel and Blaustein [14] have found that the magnitude of the Ca efflux in dialyzed barnacle fibres depends on both the total internal calcium (as CaEGTA) and the free, ionized Ca ($[Ca^{2+}]_i$). In the experiments to be described we have used a fixed concentra-

TABLE I

$[ATP]_i$ = 1—10 mM

Fibre	$[Ca]_i$ (μM)	Ca efflux (pmol · cm^{-2} · s^{-1})		Fractional stimulation of Ca efflux (ATP)
		No ATP	ATP	
BCaR1	0.3	0.54	0.97	1.8
BCaR2	0.3	0.66	1.12	1.7
BCaR11	0.3	0.72	1.22	1.7
BCaR12	0.3	0.48	1.49	3.1
BCaR20	0.15	0.30	0.39	1.3
BCaR21	0.15	0.35	0.63	1.8
BCaR40	0.2	0.41	0.92	2.2
BCaR41	0.2	0.43	0.86	2.0

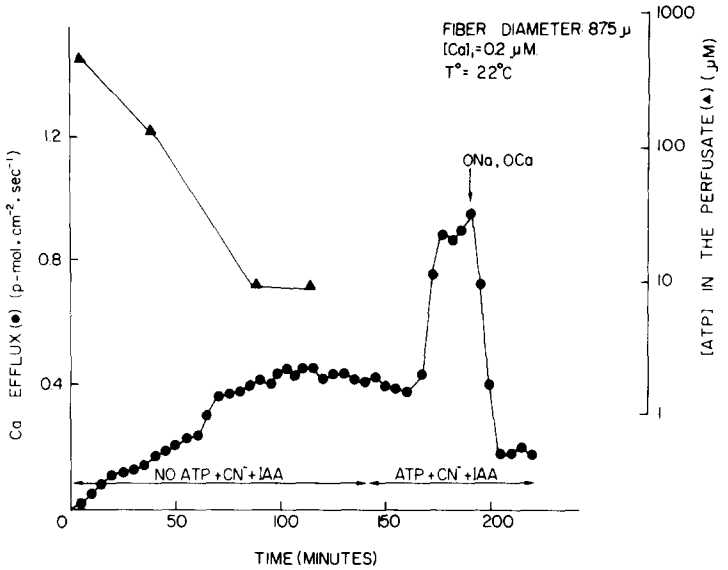

Fig. 3. Effect of ATP on the Ca efflux in a dialyzed barnacle fibre. The upper curve represents the [ATP] in the dialysate fluid. The lower curve shows the Ca efflux in pmol · cm^2 · s^{-1}.

tion of EGTA (1 mM) to obtain values of internal ionized calcium between 0.15 and 0.3 μM. Table I shows the steady state levels of Ca efflux observed in the absence and the presence of ATP. With an internal ionized calcium concentration of 0.3 μM, the mean calcium efflux in the absence of ATP was 0.6 ± 0.1 pmol · cm^{-2} · s^{-1} (n = 4). Reducing the intracellular ionized calcium concentration to 0.15 μM caused a proportional reduction of the steady state level of the Ca efflux.

Fig. 3 shows the effect of adding ATP on the Ca efflux from a fibre bathed in artificial seawater containing cyanide and iodoacetate. During the course of the experiment, the ATP concentration in the perfusate decreased significantly to a constant value near 10 μM. With this level of ATP, a steady state Ca efflux of 0.41 pmol · cm^{-2} · s^{-1} was obtained. The addition of 2 mM ATP to the dialysis medium induced an increase in the Ca efflux to a new value of 0.9 pmol · cm^{-2} . s^{-1}. Under this condition, the removal of external sodium and calcium reduced the Ca efflux to less than 0.2 pmol · cm^{-2} · s^{-1}, indicating that the ATP-dependent fraction of the Ca efflux depends on the presence of external sodium and calcium. This was confirmed in four other experiments in which addition of ATP in the absence of external sodium and calcium had no stimulating effect on the Ca efflux.

Table I summarizes the results of several experiments similar to that described in Fig. 3. The mean fractional stimulation induced by ATP in the presence of external sodium and calcium was 1.95 ± 0.18 S.E.M. (n = 8).

To summarize, the results reported here show that in barnacle muscle fibres there is a fraction of the Ca efflux which can be activated by ATP and which depends on external sodium and calcium. Similar results have been obtained in dialyzed squid giant axons by DiPolo [3] suggesting that in muscle fibre ATP

may also change the affinity of the transporting system for calcium, as has been postulated by Baker and Glitsch [15] and DiPolo [3,4] in squid axons.

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