Adenosine Triphosphate Facilitates the Na⁺-K⁺ Pump of Frog Skeletal Muscle Fibres

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Received for publication May 12, 1981

Summary: Effects of adenosine triphosphate (ATP) and dibutyryl cyclic adenosine monophosphate (dbc AMP) on the Na⁺-K⁺ pump of cell membrane were studied by testing the effects of these nucleotides on the ²²Na⁺-efflux from frog skeletal muscle fibres. The rate coefficient of ²²Na⁺-efflux was found to be significantly increased by ATP 3 mM. Dbc AMP 3 mM showed no demonstrable effect. These results support the concept that ATP facilitates the electrogenic Na⁺ pump, but do not support the concept that the electrogenic Na⁺ pump is stimulated by cyclic AMP.

Key words: adenosine triphosphate (ATP)—Na⁺-K⁺ pump—frog skeletal muscles—dibutyryl cyclic adenosine monophosphate (dbc AMP)—²²Na⁺-efflux

Introduction

The K⁺-activated hyperpolarization (Rang and Ritchie, 1968), which is induced by an activation of the electrogenic Na⁺ pump when K⁺ is added to the extracellular K⁺-free solution, is augmented by the action of adenosine triphosphate (ATP) in many types of nerve cells (Akasu, 1976). Electrophysiological analyses of the mechanisms underlying the effect of ATP on K⁺-activated hyperpolarization suggest that K⁺-activated hyperpolarization is augmented by an increase in the electrogenic Na⁺ pump current (Akasu, 1976; Morita, 1979) but not simply by an increase in the membrane resistance. Electrophysiological analyses have not, however, provided direct evidence to support the contention that the electrogenic Na⁺ pump current itself is actually increased by the action of ATP. The present experiment clearly demonstrates that, under the influence of ATP, the Na⁺ pump current is indeed increased (whatever it is electrogenic or neutral).

On the other hand, catecholamine is known to facilitate the Na⁺-efflux from frog skeletal muscles (Hays et al., 1974; Clausen and Flatman, 1977; Kaibara et al., 1981). In sympathetic ganglia, catecholamine released from SIF (small intensely fluorescent) cells induces the slow IPSP (inhibitory postsynaptic potential) of ganglion cells, which may be partially generated by activation of electrogenic Na⁺ pump (Kuba and Koketsu, 1978; Akasu et al., 1978). The possibility that the electrogenic Na⁺ pump of ganglion cells may be stimulated by the action of cyclic AMP has

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been proposed on the basis that slow IPSP is mediated by cyclic AMP, the second messenger of catecholamine in sympathetic ganglia (McAfee and Greengard, 1972). According to electrophysiological studies of the effect of cyclic AMP on sympathetic ganglion cells, however, the electrogenic Na⁺ pump of these cells does not appear to be stimulated by cyclic AMP (Akasu and Koketsu, 1977). Further, direct experimental evidence showing that cyclic AMP does not stimulate Na⁺ pump is presented in this study.

**Materials and Methods**

Sartorius muscles of frogs (*Rana nigromaculata*) were carefully isolated and the muscle weight was measured before incubation with radioactive Ringer solution. The gamma-emitting isotope, ²²Na⁺ in chloride form, was obtained from the Japan Radioisotope Association. Radioactive, ²²Na⁺-labeled Ringer solutions were prepared with a specific activity of 0.2 mCi/ml and the same ionic compositions as the cold, standard Ringer solutions: 112 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂ and 2.4 mM NaHCO₃. Reagents used in the present study were as follows: adenosine triphosphate (Wako), dibutyryl cyclic adenosine monophosphate (SIGMA), and ouabain (Merck).

Measurements of the ²²Na⁺-efflux and estimations of the rate coefficients of ²²Na⁺-efflux were carried out by standard techniques. The preparations were incubated in ²²Na⁺-Ringer solution for 2 to 4 hours. Paired muscle measurements, commonly used in flux experiments, were not necessarily employed in the present study, since the flux and rate coefficient data were normalized with respect to those of each preparation in the standard Ringer solution. The calculated results were compared with one another for the whole series of experiments.

Each incubated muscle preparation was briefly rinsed with cold standard Ringer solution, and then transferred consecutively to a series of 4 vessels; soaking period in each vessel containing 5 ml of Ringer solution was 10 min. By this procedure for 40 min, extracellular radioactivity was substantially reduced. In the next step, each preparation was soaked with consecutive 3 ml solutions of either Ringer or test solution for 5 min. Each preparation was soaked in either ATP 3 mM or dbc AMP 3 mM for a total of 15 min. Ouabain (gastrothrin) of 10 μM was used to block Na⁺ pump activity. One series of experiments required approximately 150 min. Each muscle preparation was then soaked in 3 ml of distilled water overnight to elute any remaining radioactivity. All experiments were carried out under regulated room temperature, 22-24 °C. The radioactivity of the incubation solutions was measured by the well-type gamma scintillation counter Aloka JDC-751.

Desaturation characteristics of ²²Na⁺ for each preparation were obtained by consecutively summing up the counting data beginning at infinite time and proceeding backwards. Rate coefficients relating the Na⁺-efflux with intracellular Na⁺ concentration were estimated from plots of the logarithmic ²²Na⁺ content within muscles vs. time.

**Results**

By rearranging the counting data, desaturation curves, which represent the time change in logarithmic ²²Na⁺ content remaining in muscle, are obtained, as shown in Fig. 1 A. Since the early part of the desaturation curve is contaminated with extracellular radioactivity, the characteristics of ²²Na⁺ efflux are represented by the following, linear part of this curve, described by first-order reaction kinetics, i.e.:
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\[ A_t = A_0 \exp(-kt) \]

where \( A_0 \) and \( A_t \) are the initial intracellular radioactivity and the remaining activity at time \( t \); \( A_0 \) can be estimated by means of extrapolating the linear data to time 0, and \( k \) is the constant representing the kinetic processes. Therefore, the experimentally determined \( ^{22}\text{Na}^+\)-efflux, \( J_{\text{Na}} \), is given by:

\[ J_{\text{Na}} = k A_t \]

Under the condition where the specific activities of intracellular \( ^{22}\text{Na}^+ \) are identical to those of \( ^{22}\text{Na}^+ \) crossing cell membranes, the \( ^{22}\text{Na}^+\)-efflux, \( J_{\text{Na},\text{eff}} \), can be expressed as a function of intracellular \( \text{Na}^+ \) concentration, \( [\text{Na}^+]_i \), by the same constant \( k \) related to tracer ions:

\[ J_{\text{Na},\text{eff}} = k [\text{Na}^+]_i \]

The constant \( k \), the rate coefficient of \( \text{Na}^+\)-efflux, for a given time interval \( t_1 \) to \( t_2 \), is estimated by:

\[ k = \ln \left( \frac{A_{t_1}/A_{t_2}}{(t_2-t_1)} \right) \]

Fig. 1 shows the desaturation curves and rate coefficients of the \( \text{Na}^+\)-effluxes measured in the standard Ringer solution with and without ouabain. \( \text{Na}^+\)-efflux was significantly decreased by the addition of ouabain 10 \( \mu \text{M} \) and the rate coefficient of \( \text{Na}^+\)-efflux was depressed to almost half of the control value. In our experiments, the ouabain-sensitive part of \( \text{Na}^+\)-efflux is regarded as an efflux generated by \( \text{Na}^+\)-\( \text{K}^+ \) pump system.

Fig. 2 illustrates the effects of ATP 3 mM on the \( \text{Na}^+\)-efflux. The desaturation curves in Fig. 2 A are plotted as the rela-

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**Fig. 1.** \( \text{Na}^+\)-efflux and action of ouabain (10 \( \mu \text{M} \)). Ouabain is applied during the period indicated by the horizontal mark. A, Desaturation characteristics observed in standard Ringer solution with (○) and without (△) ouabain. B, Changes in the rate coefficient of \( \text{Na}^+\)-efflux.

**Fig. 2.** Facilitatory effects of ATP (3 mM) on \( \text{Na}^+\)-efflux. Desaturation curves (A) and rate coefficient data (B) are given as the relative quantities which are standardized against the initial intracellular radioactivity and the rate coefficient of \( \text{Na}^+\)-efflux in standard Ringer solution, respectively. The horizontal mark indicates the period of ATP application (15 min).
tive $^{22}\text{Na}^+$ content values for which the initial radioactivity, $A_0$, in each muscle preparation is taken as unity. The rate coefficients in Fig. 2B are calculated as relative values to the coefficient in standard Ringer solution. In each of 4 preparations studied, $\text{Na}^+$-effluxes were augmented by the extracellular addition of ATP. The degree to which the $\text{Na}^+$-effluxes were augmented varied. The relative rate coefficients of $\text{Na}^+$-efflux were augmented from 1.4 to 2.2 by ATP application. The maximum effect of ATP is comparable to the effects of adrenaline $30\mu\text{M}$. The facilitatory effects of ATP on $\text{Na}^+$-efflux were completely blocked by $10\mu\text{M}$ of ouabain.

In Fig. 3, the experimental results examining the effects of dibutyryl derivative of cyclic AMP $3\text{mM}$ on the $\text{Na}^+$-efflux are shown. No detectable effects were observed in the 4 preparations studied.

**Discussion**

The present experiment clearly demonstrates that the ouabain-sensitive fraction of the $\text{Na}^+$-efflux is augmented by the action of ATP added to the extracellular solution. This suggests that the $\text{Na}^+-\text{K}^+$ pump is facilitated by ATP acting upon the outer surface of the cell membrane. There are three possible explanations for the increase $\text{Na}^+$-efflux shown in the present experiment:

1) an increase in the $\text{Na}^+/\text{K}^+$ ratio of $\text{Na}^+-\text{K}^+$ pump, by maintaining a constant $\text{K}^+$-influx,
2) an increase in the total number of available pumping sites, and
3) an increase in the rate of $\text{Na}^+-\text{K}^+$ pump; in other words, an increase in the number of activated pumping sites per unit time. The present experiment provides no definite answer as to which of these three possibilities are/is responsible for the observed increase of $\text{Na}^+$-efflux.

According to the electrophysiological analyses of the mechanism underlying the augmentation of $\text{K}^+$-activated hyperpolarization by ATP, it has been suggested that ATP does not increase either the $\text{Na}^+/\text{K}^+$ ratio or the total number of available pumping sites (Morita, 1979). Whatever the mechanism underlying such a facilitatory effect of ATP on $\text{Na}^+$ pump, however, the present results support the concept that ATP is able to hyperpolarize skeletal muscle membrane, provided that the coupling ratio, $\text{Na}^+/\text{K}^+$, of $\text{Na}^+-\text{K}^+$ pump in these cells is greater than unity. Assuming the fundamental mechanism of the $\text{Na}^+-\text{K}^+$ pump is the same for all excitable cell membranes, it is reasonable to expect that molecules of ATP which are present in extracellular fluid are able to hyper-
polarize these cell membranes by stimulating the Na\(^+\) pump of its target cells. Whether such an action of ATP is a result of activation of a putative ATP-receptor, or of usage of ATP or its metabolites as energy sources, is not known at present.

Another conclusion derived from the present experiment is that cyclic AMP is not able to stimulate the Na\(^+\)-K\(^+\) pump. Dbc AMP is assumed to be capable of penetrating the cell membrane. Thus, the present experiment shows that even intracellular cyclic AMP does not stimulate the Na\(^+\)-K\(^+\) pump. It is well known that catecholamine facilitates the Na\(^+\) pump (Hays et al., 1974; Clausen and Flatman, 1977; Kaibara et al., 1981) and that cyclic AMP acts as the second messenger of catecholamine (Sutherland et al., 1965; Phillips, 1977). The present experiment, however, suggests that the facilitatory action of catecholamine on Na\(^+\)-K\(^+\) pump is not mediated by cyclic AMP.

**Acknowledgement:** This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

**References**


