Sodium and Potassium Effects on Skeletal Muscle Microsomal Adenosine Triphosphatase and Calcium Uptake

Abstract. *The relationship between the (Na+ and K+)-activated adenosine triphosphatase enzyme system implicated in sodium-transport by cell membranes and the calcium-activated adenosine triphosphatase, which is generally associated with calcium uptake, was examined in microsomes from skeletal muscle. Whereas sodium and potassium did not modify the relatively low adenosine triphosphatase activity seen in the absence of calcium, a pattern similar to that of the sodium-transport enzyme system was seen afer the addition of $CaCl_2$. The calcium-activated adenosine triphosphatase was stimulated equally by sodium or potassium alone, but both the rate and extent of calcium uptake were enhanced more by potassium than by sodium at concentrations below 0.12 mole per liter. In the absence of either of these ions addition of calcium failed to activate adenosine triphosphatase although significant amounts of calcium were taken up by the microsomes.*

In 1957, Skou (*1*) obtained from the microsomal fraction of nerves an adenosine triphosphatase which was dependent on Mg^{++} and activated by Na^+ and K^+. He showed that this system had properties that suggested it might be derived from the sodium-transport system of the cell surface membrane. Since then, others have demonstrated a similar enzyme system in a wide variety of tissues, such as brain and kidney (*2*), and in erythrocytes (*3*). Such an enzyme system has also been identified in the deoxycholate-treated microsomal fraction of skeletal muscle (*4*). The same muscle fraction, when prepared in the absence of deoxycholate, can take up calcium (*5*, *6*), a process implicated in muscle relaxation. This calcium-accumulating muscle fraction hydrolyzes adenosine triphosphate (ATP) at a low rate (basic adenosine triphosphatase) in the absence of calcium, whereas addition of Ca^{++} in sufficient quantities ($> 10^{-5}M$) activates the enzyme (*5*, *6*). The difference between the Ca^{++}-activated adenosine triphosphatase and the basic adenosine triphosphatase activity is designated the extra adenosine triphosphatase. We have studied the possible relationship between the (Na^+ and K^+)-activated adenosine triphosphatase enzyme system in skeletal muscle microsomes, their ability to accumulate calcium, and the extra adenosine triphosphatase that has been related to this calcium uptake.

Microsomes were prepared from rabbit back muscles by the method of Seraydarian and Mommaerts (*7*). It was modified in that the tissue was initially homogenized in two volumes of solution containing a final concentration of 0.3*M* sucrose and 10 m*M* histidine (*p*H 7.45). Initial centrifugation was prolonged to 30 minutes at 15,000*g*. Microsomes recovered from the supernatant after centrifugation at 100,000*g* for 90 minutes were suspended in approximately 10 ml of the sucrose-histidine solution and gently homogenized in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. Although treatment with deoxycholate has been used to prepare skeletal muscle microsomes that exhibit activation of adenosine triphosphatase by Na^+ and K^+ (*4*), this step was omitted in our study because deoxycholate abolishes the ability of the microsomes to take up calcium. We measured adenosine triphosphatase activity by following the rate of liberation of inorganic phosphate (P_i) (*8*). The uptake of Ca^{45} was measured in the presence of 2.5 m*M* tris-(hydroxymethyl)aminomethane oxalate. The microsomes were removed by filtration through Millipore filters (*9*), and the Ca^{45} remaining in solution was determined in a liquid scintillation spectrometer.

Neither Na^+ nor K^+ had a demonstrable effect on the basic adenosine triphosphatase activity, and no enhancement of this activity was observed when both ions were present (Fig. 1). Addition of $CaCl_2$ (final concentration, 0.1 mmole per liter) enhanced the adenosine triphosphatase activity (Fig. 1), but only when the reaction mixture contained Na^+ or K^+ (Fig. 2). Sodium and potassium ions were equally effective in potentiating the effects of Ca^{++} on the microsomal adenosine triphosphatase. Additions of Ca^{++} in the absence of these alkali-metal ions did not stimulate the enzyme.

In the presence of Na^+ and K^+ together, the extra adenosine triphosphatase of the microsomes was greater than it was in the presence of either alone (Fig. 1). At a total alkali-metal ion concentration of 0.12 mole/liter maximum activity occurred in a solution having in final concentration 0.10*M* NaCl and 0.02*M* KCl.

To define further the significance of these effects of Na^+ and K^+ on the extra adenosine triphosphatase, we also examined the influence of these ions on calcium uptake. At concentrations below 0.12 mole/liter, either K^+ (*10*) or Na^+ enhanced the uptake of calcium. However, both the rate and extent of calcium uptake were greater in the presence of K^+ than they were in an equal concentration of Na^+ (Figs. 1 and 2). When both ions were present together calcium uptake was that expected from the effects of either ion alone. Thus, when K^+ was partly replaced by Na^+, the extent of calcium uptake decreased. Additional effects on calcium uptake attributable to the presence of both alkali-metal ions together could not be identified.

Although an extra adenosine triphosphatase activity was generally elicited by addition of Ca^{++}, the stoichiometry between calcium uptake and this adenosine triphosphatase was not constant (*6*, *7*) (Figs. 1 and 2). Replacements of small amounts of K^+ by Na^+ increased this adenosine triphosphatase, whereas calcium uptake decreased (Fig. 1). Most striking is the finding that, in the absence of alkali-metal ions, significant uptake of calcium occurred in the absence of an extra adenosine triphosphatase activity (Fig. 2). Other experi-

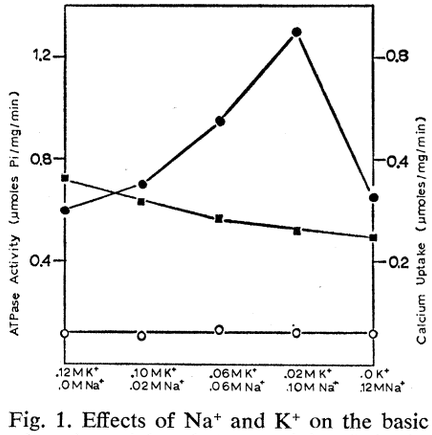

Fig. 1. Effects of Na^+ and K^+ on the basic adenosine triphosphatase activity (○), the calcium-activated adenosine triphosphatase (●), and calcium uptake (■) of skeletal microsomes. Reactions were carried out with 0.05 mg of microsomes per milliliter of solution containing in final concentration 4.0 m*M* MgATP, 2.5 m*M* tris oxalate, and 10 m*M* histidine (*p*H 7.0) at 25°C. Samples were taken 2 and 5 minutes after the addition of $CaCl_2$. Calcium-activated adenosine triphosphatase and calcium uptake were determined in the presence of 0.10 m*M* $CaCl_2$.

ments demonstrated that increasing concentrations of Na^+ or K^+ between 10 and 120 mmole/liter caused no further enhancement of the extra adenosine triphosphatase, although calcium uptake was increased.

In an earlier study of skeletal muscle microsomes, Martonosi and Feretos failed to observe differences in the adenosine triphosphatase activity in the presence of Na^+ alone, of K^+ alone, or in mixtures of Na^+ and K^+ at constant ionic strength (*11*). They presented no data to support their statement, however, and the possibility exists that the relatively small increase in the enzyme's activity seen in most mixtures of Na^+ and K^+ could have been overlooked. In the case of cardiac microsomes, several investigators have noted a (Na^+ and K^+)-activated adenosine triphosphatase enzyme system (*12–15*). This activity has been most striking in aged cardiac microsomes (*12*), and in cardiac microsomes prepared in the presence of agents such as deoxycholate (*13*) or sodium iodide (*14*).

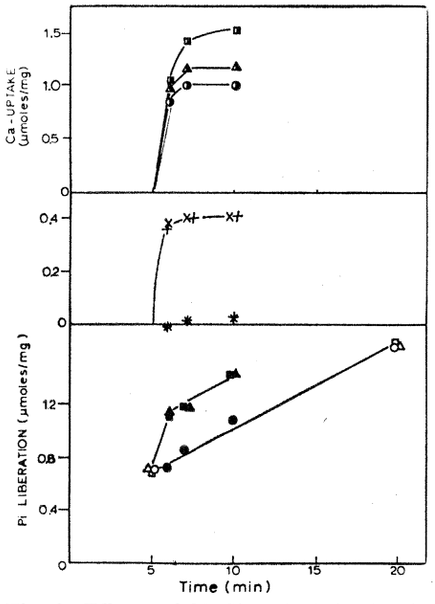

Fig. 2. Effects of NaCl and KCl on calcium uptake (top), the extra adenosine triphosphatase activity (middle), and the basic and calcium-activated activities (bottom) of skeletal muscle microsomes. (Bottom) The liberation of P_i by 0.05 mg of microsomes per milliliter of solution containing in final concentration 4.0 m*M* MgATP, 2.5 m*M* tris oxalate, and 10 m*M* histidine (*p*H 7.0) was examined in the absence (open symbols) and presence (closed symbols) of 0.10 m*M* $CaCl_2$. Samples were taken after a 5-minute period of incubation. Reactions were carried out in the absence of added alkali-metal salt (○, ●), in 0.12*M* KCl (□, ■), and in 0.12*M* NaCl (△, ▲). $CaCl_2$ was added at 5 minutes. (Middle) Extra adenosine triphosphatase activity in the absence of alkali-metal salt (*), in 0.12*M* KCl (×), and in 0.12*M* NaCl (+), calculated by subtracting the P_i liberated in the absence of added $CaCl_2$ from that liberated after the addition of $CaCl_2$ (data from lower panel). (Upper) The uptakes of Ca^{45} in the absence of alkali-metal salts (half-shaded circles), in 0.12*M* KCl (half-shaded squares), and in 0.12*M* NaCl (half-shaded triangles), determined concurrently with the measurements of adenosine triphosphatase activity. The ratios of micromoles of P_i liberated (extra adenosine triphosphatase) per micromole Ca^{++} taken up were: in no salt, 0; in KCl, 2.60; and in NaCl, 3.45. These ratios were calculated from the samples taken at 10 minutes, at which time calcium uptake had ended.

Our findings also demonstrate that the stoichiometry between calcium uptake and the calcium-activated adenosine triphosphatase of skeletal muscle microsomes can be significantly modified by low concentrations of Na^+ and K^+. This stoichiometry has been reported previously to be altered in the presence of high concentrations of Ca^{++} (*11*) or low concentrations of adenosine triphosphate (*16*). The sequestration of calcium in the absence of any discernible stimulation of the basic adenosine triphosphatase was unexpected. Although no extra adenosine triphosphatase could be detected under these conditions, significant calcium uptake was observed. It thus appears that the basic adenosine triphosphatase can, in the absence of Na^+ and K^+, provide for calcium sequestration.

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Allergic Adenohypophysitis: New Experimental Disease of the Pituitary Gland

Abstract. *The pituitary gland has been added to the roster of organs in which inflammatory disease, probably autoimmune in origin, can be induced by injection of the corresponding tissue plus adjuvants.*

Inflammatory, autoimmune diseases of several organs have been produced by injections of tissues from the corresponding organs (*1*). Organ-specific antigens occur in the pituitary gland, and they are capable of eliciting production of autoantibodies; nevertheless, no pathological lesions in the pituitary have been described (*2*). Our work concerns the experimental production, apparently for the first time, of a disease of the anterior lobe of the pituitary