Active Transport of Potassium Ion in Heart Mitochondria

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ABSTRACT

Several factors affecting K⁺ transport by rabbit heart mitochondria were examined, using a K⁺-sensitive electrode. The histone fractions f₂a and β-7 produced an energy-dependent efflux of K⁺. Inorganic phosphate was required for optimal activity; Kₘ for phosphate was 60 μM. Both rate and extent of K⁺ efflux decreased as K⁺ concentration in the reaction medium was increased. The direction of valinomycin-induced K⁺ movements was shown to depend on the net resultant of an active transport mechanism and increased membrane permeability. The detergent triton X-100 produced a nonspecific increase in membrane permeability that led to a rapid efflux of K⁺. Evidence is presented for competition between ion transport and ATP formation for some common energy intermediate. Possible mechanisms of action of histones and other agents affecting heart mitochondrial K⁺ transport are considered.

ADDITIONAL KEY WORDS

mitochondrial respiration histone common "high-energy" intermediate valinomycin rabbit

In recent years specific emphasis has been directed toward elucidation of mitochondrial activity in normal, hyper- and hypofunctioning heart muscle (1-3). Recognition of the importance of mitochondria has received added impetus with the discovery of functions other than oxidative phosphorylation. These include reversal of electron transport associated with reductive transphosphorylation (4), DNA and RNA metabolism (5, 6) and active cation accumulation and extrusion (7-10). The last two phenomena may be intimately associated with cell metabolism (11).

Previous studies from this laboratory have described an energy-dependent histone-induced net efflux of K⁺ from liver and heart mitochondria (12). The effect of valinomycin, a toxic antibiotic that is a specific inducer of K⁺ transport by mitochondria, has been discussed in relation to two basic actions: (1) stimulation of the carrier mechanism responsible for active translocation of K⁺ and (2) induction of increased "permeability" of mitochondria to K⁺ (9). Depending upon the energy state of the mitochondria, valinomycin can produce either a net K⁺ influx or efflux. Since both direction and rate of valinomycin-induced K⁺ transport is thought to be a function of extramitochondrial K⁺ concentration, a similar effect of K⁺ concentration upon histone-induced K⁺ transport would be of importance with respect to the energy requirement for the histone effects.

In view of the possible importance of K⁺ transport in heart mitochondria, the present study was designed to investigate, in depth, factors influencing this phenomenon.

Methods

New Zealand white rabbits (2- to 3-kg males) were killed by a sharp blow on the back of the neck. Their hearts were immediately excised and placed in ice-cold isolation medium for rapid chilling. The isolation medium contained 125
mm choline chloride, 20 mm tris (hydroxymethyl) aminomethane (tris), 10 mm ethylenediaminetetraacetate (EDTA), and 1% bovine serum albumin, fraction V, and was adjusted to pH 7.4. Choline chloride was chosen because it produces no known alteration of mitochondrial function and is not detected by the K\(^+\) electrode. EDTA was included to chelate Ca\(^2+\), which would otherwise produce uncoupling of oxidative phosphorylation and swelling (13). Bovine serum albumin also prevents uncoupling that would be induced by free fatty acids (13) in the tissue homogenate. The isolation medium described is a modification of that used by Von Korff (14).

The hearts were examined for any signs of pathologic changes such as necrotic areas or excessive fat. If none was noted, both atria and the adipose tissue sometimes located along the coronary arteries were removed and discarded. The ventricles were then cut into small pieces, rinsed free of blood, and weighed. The tissue was finely minced, with sharp scissors, in a small amount of isolation medium. Additional medium was then added to produce a 5% tissue homogenate. The suspension of finely minced heart tissue was homogenized in small portions using a glass Potter-Elvejhem homogenizer (A. C. Thomas, type C). Only four complete passes were made with a loosely fitting Teflon pestle driven at 500 rpm to minimize mitochondrial damage. Microscopic examination of the homogenate revealed a small residue of undisrupted myofibers (<10%). In some experiments a tissue homogenate was obtained by use of the Polytron tissue processor (Brinkmann Instruments). The pooled homogenates were transferred to 50-ml Teflon tubes and centrifuged at 600 \(\times\) g for 10 min. The supernate was centrifuged at 6,000 \(\times\) g for 10 min. The mitochondrial pellet was collected and washed, using choline chloride, 20 mm tris (hydroxymethyl) aminomethane (tris), 10 mm ethylenediaminetetraacetate (EDTA), and 1% bovine serum albumin, fraction V, and was adjusted to pH 7.4. Choline chloride was chosen because it produces no known alteration of mitochondrial function and is not detected by the K\(^+\) electrode. EDTA was included to chelate Ca\(^2+\), which would otherwise produce...
conditions induces a net K⁺ efflux. However, there is no requirement for energy or inorganic phosphate.

Effect of K⁺ Concentration.—Increasing concentrations of K⁺ in the reaction medium reduces both the rate and extent of f₂a-induced K⁺ efflux (Fig. 1). When the external concentration of K⁺ is zero, the initial rate of K⁺ efflux induced by the histone is 42 μEq/g mitochondrial protein per min. Under similar conditions, valinomycin effects a K⁺ loss at a rate of 120 μEq/g per min. As the external K⁺ concentration is raised, valinomycin-in-

![Figure 1](image1)

**Figure 1**

Effect of external K⁺ concentration on K⁺ transport by rabbit heart mitochondria. Each experiment was carried out in 8 ml of medium containing 125 mM choline chloride, 20 mM tris-chloride (pH 7.4), 2.5 mM tris-glutamate, 2.5 mM tris-phosphate, and varying concentrations of added KCl as indicated (from 0 to 1 mM). Three groups of experiments, each containing four different KCl concentrations, contained 1.6 μg valinomycin, 40 μg f₂a, and 250 μg triton. Addition of 8 mg mitochondrial protein initiated the K⁺ movements, which were measured by means of a Beckman electrode (see text). The point on the ordinate at which each of the 12 curves begins represents the concentration of K⁺ in the medium before addition of valinomycin, histone, or triton. The rate of K⁺ efflux was obtained using the maximum slope of μM K⁺/min.

![Figure 2](image2)

**Figure 2**

Inhibition of the effects of histone by ADP. For curves A and B, the medium consisted of 0.25 M sucrose, 10 mM tris-chloride (pH 7.4), 5 mM tris-phosphate, 1 mM tris-glutamate, and 17 mg mitochondrial protein in a final volume of 10.0 ml. The additions for curve A were 500 μM tris-ADP and 250 μg β-7 histone. The additions for curve B were 500 μM tris-ADP, 250 μg β-7 histone, and 25 μg oligomycin. Curves A and B represent the efflux of K⁺ from the mitochondria. For curves C and D, the medium was the same as for curves A and B except that the final volume was 2.0 ml and 4.3 mg of mitochondrial proteins were added. The additions for curve C were 500 μM tris-ADP and 50 μg β-7 histone. The additions for curve D were 500 μM tris-ADP, 50 μg β-7 histone, and 10 μg oligomycin. Curves C and D represent the uptake of O₂ by the mitochondria.
K⁺ efflux (data not shown). Furthermore, increasing the external K⁺ concentration up to 1 mM does not retard the triton-induced K⁺ loss. Microscopic examination did not reveal any alteration of mitochondrial structure at the low triton concentration.

**ADP and K⁺ Transport.**—Mitochondria suspended in K⁺-free medium and respiring in state 3 (presence of excess substrate and ADP) maintain the same slow efflux of K⁺ observed in state 4 (presence of excess substrate and low ADP) and are unresponsive to histone. However, when ATP formation is blocked by the addition of oligomycin, the histone f₂a induces a rapid K⁺ efflux (Fig. 2). Under the identical conditions, valinomycin effects a K⁺ efflux whether the mitochondria are in state 3 or state 4. At concentrations of K⁺ greater than 200 µM, after maximal uptake of K⁺ induced by valinomycin, the addition of ADP causes an immediate loss of K⁺ (Figs. 3 and 4). The duration of the efflux corresponds to the time required to complete a transition from state 3 to state 4. When a transition is completed, K⁺ reenters to the extent present before the addition of ADP. Both NAD-linked substrates and succinate support the ADP-induced K⁺ efflux. Addition of larger amounts of ADP proportionately delays the recovery of K⁺, but the extent of K⁺ loss is not increased (Fig. 4). These cyclic K⁺ changes may be repeated numerous times upon successive additions of ADP and are prevented by atractyloside or oligomycin, antibiotics which respectively inhibit ADP transport and phosphorylation (21, 22). Other nucleoside 5'-diphosphates (GDP, UDP, IDP, and CDP) in similar concentrations have no effect on K⁺ transport.

Uncoupling of mitochondria produced by aging in 0.25 M sucrose at 0°C for 30 hours increasingly delays the reentry (both rate and extent) of K⁺. Addition of oligomycin, antimycin A, cyanide, octylguanidine or 2,4-dinitrophenol, after valinomycin-induced K⁺ uptake, causes a K⁺ loss similar to that observed when mitochondria are treated with 0.001% triton X-100 (Fig. 3). In the presence of NAD-linked substrates, addition of rotenone also yields similar results; the K⁺ reenters the mitochondria upon addition of succinate. While oligomycin appears to have little or no effect on substrate-supported ion transport, the antibiotic completely prevents ATP-supported K⁺ movements (Figs. 2, 4). Octylguanidine and 2,4-dinitrophenol do not inhibit ATP-supported valinomycin-induced K⁺ uptake (data not shown).
Discussion

Factors Affecting K⁺ Transport.—The results of these experiments are basically in agreement with recent conceptual models proposed for movement of cations across the inner mitochondrial membrane (9, 10, 23). The direction of net K⁺ movement has been shown to depend on a number of factors such as the energy state of the carrier molecule, existence of a chemical or electrical gradient or both, presence of permeable counterions, and membrane permeability. Recent studies, based on the assumption that the total K⁺ within the mitochondria is in solution, estimate minimal intramitochondrial K⁺ concentration to be about 90 mM (24). Under the present experimental conditions of low external K⁺ and the presence of a permeable anion (phosphate), net accumulation of K⁺ by mitochondria is opposed primarily by the presence of a chemical potential gradient directed from the matrix to the external medium. Under our experimental conditions, net accumulation of K⁺ induced by valinomycin in rabbit heart proceeds only at or above 200 μM K⁺. Below this concentration of K⁺, addition of valinomycin produces an energy-independent K⁺ efflux. The fact that rate and extent of efflux increased as energy source was diminished confirms the suggestion that valinomycin possesses two actions, one on "permeability" (efflux) and the other on active carrier-linked transport (influx). Rate of histone-induced K⁺ efflux also responded to external K⁺ concentration, suggesting a mechanism similar to the valinomycin-induced K⁺ influx. It is important to note that triton-induced K⁺ movements were completely unresponsive to alteration of K⁺ concentration or to a complete inhibition of energy. The primary effect of triton is, therefore, thought to be due to a nonspecific increase in mitochondrial permeability. This is distinct from the effects of histone-induced K⁺ efflux, which are strictly energy dependent.

Mechanism of Histone-Induced K⁺ Transport.—The ability of a theoretical carrier molecule of cation transport to produce counter-gradient K⁺ translocation depends on both the slope of the gradient and energy level of the carrier (23). The energy level of the carrier would in turn regulate the binding of variable amounts of specific cation to the carrier. Agents affecting ion transport could conceivably act by influencing the degree of cation binding to the carrier at a specific energy level. The ability of an agent to increase or decrease carrier binding may determine the direction of net cation movement. The relative availability of the cation for combination with the carrier molecule would also specify the direction of net cation transport. The primary action of histones and other basic proteins influencing ion transport, therefore, might be to alter the binding affinity of the carrier molecule (possibly an acidic protein) for K⁺, without producing an accompanying "detergent-like" increase in membrane permeability. However, until a net accumulation of K⁺ can be observed, such a proposed mechanism is merely speculative. A previous suggestion that histone exerts its effect via some energy-dependent conformational change of the mitochondrial membranes is consistent with the present data (12).

Another possible interpretation of the data is that histone uptake might require energy. The basic molecule may exchange for K⁺; the latter is then extruded via an energy-independent mechanism. The recent report of Chappell and Crofts (25), describing an amine-induced efflux of K⁺, is relevant.

Possible "Competition" between Ion Transport and ATP Formation.—While the mechanism of oxidative-phosphorylation is unknown, current evidence suggests the presence of some intermediate energetic state prior to the formation of ATP (13). Several recent publications further hypothesize that K⁺ transport in mitochondria and oxidative phosphorylation are linked through common intermediate energetic state(s) (9, 10, 23).
The present data support these conclusions, although no interpretation may be made concerning presence or absence of specific chemical intermediates.

Addition of ADP to substrate-supplemented mitochondria in the presence of inorganic phosphate appears to shift the flow of energy toward the formation of ATP, diminishing the amount of energy available for K⁺ transport. The latter is restored when all of the ADP is esterified (see Fig. 4). Therefore, it would seem that both ion transport processes and at least part of the ATP-producing mechanism compete for the available energy.

Acknowledgments

We are indebted to Drs. C. M. Mauritzen and W. C. Starbuck for providing all histone fractions used in this study. Valinomycin was generously provided by Dr. B. C. Pressman, Johnson Research Foundation, University of Pennsylvania. Potassium ion transport data were programmed and processed by the Computational Research Center of Baylor University of Medicine.

References


Myosine and Adenosinetriphosphatase

Ordinary aqueous or potassium chloride extracts of muscle exhibit but a slight capacity to mineralize adenosinetriphosphate. Even this slight liberation of phosphate is mainly due, not to direct hydrolysis of adenosinetriphosphate, but to a process of secondary, indirect mineralization, accompanying the transfer of phosphate from the adenylcylic to creatine, the corresponding enzymes (for which the name 'phosphopherases' is suggested) being readily soluble.

In contrast to this lack of adenosinetriphosphatase in the soluble fraction, a high adenosinetriphosphatase activity is associated with the water-insoluble proteins of muscle. This enzymatic activity is easily brought into solution by all the buffer and concentrated salt solutions usually employed for the extraction of myosine. On precipitation of myosine from such extracts, the adenosinetriphosphatase activity is always found in the myosine fraction, whichever mode of precipitation be used: dialysis, dilution, cautious acidification, salting out. On repeated precipitations of myosine, the activity per mgm. nitrogen attains a fairly constant level, unless denaturation of myosine takes place. Under the conditions of our experiments (optimal conditions have not been determined) the activity of myosine preparations ranged in different experiments from 350 to 600 microgram phosphorus liberated per mgm. nitrogen in 5 min. at 37°. Expressed as

\[ Q_p = \frac{\mu g m. P}{m g m. N \times 22.4 \times 6.25 \times h o u r} \]

this gives values of 200-350.

Acidification to pH below 4, which is known to bring about the denaturation of myosine, rapidly destroys the adenosinetriphosphatase activity. Most remarkable is the extreme thermolability of the adenosinetriphosphatase of muscle: the enzymatic activity shown by myosine solutions is completely lost after 10 min. exposure to 37°. This corresponds with the well-known thermolability of myosine1. In respect of its high thermolability adenosinetriphosphatase resembles the protein of the yellow enzyme, which when separated from its prosthetic group is also rapidly inactivated at 38° (Theorell1). Evidently in the intact tissue of the warm-blooded animal (all experiments were performed on rabbit muscles), some conditions must exist which stabilize the myosine against the action of temperature. A marked stabilizing effect on the adenosinetriphosphatase activity seems to be produced by the adenylcylic nucleotide itself. As can be seen from the accompanying graph, in the presence of adenosinetriphosphate the liberation of phosphate proceeds at 37° over a considerable period (Curves I, 1a and 1b), whereas the same myosine solution warmed alone to 37° for 10-15 min. shows on subsequent addition of adenosinetriphosphate an insignificant or no mineralization whatever.

Crude buffer extracts accomplish a quantitative hydrolysis of the labile phosphate groups of adenosinetriphosphate; myosine, reprecipitated three times, liberates but 80 per cent of the theoretical amount of phosphorus (see figure). It acts as true adenosine-triphosphatase and yields adenosinediphosphatase, which is not further dephosphorylated and has been isolated in substance. This may serve as a convenient way of preparing adenosinediphosphatase, instead of using crayfish muscle. The adenosinediphosphatase is thus associated with the more soluble proteins, occupying an intermediate position between adenosinetriphosphatase and the most readily soluble phosphopherases.

Under no conditions tested could we obtain a separation of adenosinetriphosphatase from myosine. Either the activity was found in the myosine precipitate or else it was absent from the precipitates and from the remaining solution. This disappearance of the enzymatic activity we regard as the result of the start of denaturation of the very unstable myosine.

We are led to conclude that the adenosinetriphosphatase activity is to be ascribed to myosine or, at least, to a protein very closely related to and present not distinguishable from myosine. Thus the mineralization of adenosinetriphosphate, often regarded as the primary exothermic reaction in muscle

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Myosine and Adenosinetriphosphatase


Demonstration that the adenosinetriphosphatase (ATPase) activity of muscle is mainly associated with, and not separable from, myosin itself.
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doi: 10.1161/01.RES.21.1.25

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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