

Quinine Inhibition of Na⁺ and K⁺ Transport Provides Evidence for Two Cation/H⁺ Exchangers in Rat Liver Mitochondria*

(Received for publication, March 29, 1982)

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K⁺ efflux from respiring mitochondria via the K⁺/H⁺ exchanger is inhibited by quinine. In contrast, swelling in fresh mitochondria suspended in Na⁺ acetate, reflecting activity of the Na⁺/H⁺ exchanger, is unaffected by quinine. Swelling studies were also carried out in K⁺ and Na⁺ acetate in mitochondria loaded with tetraethylammonium and depleted of Mg²⁺ and K⁺. From the effects of pH and quinine on this preparation, we conclude that there are two distinct electroneutral pathways for monovalent cations. The Na⁺/H⁺ exchanger is highly specific for Na⁺ over K⁺ and is neither regulated by Mg²⁺ ions nor inhibited by quinine. The K⁺/H⁺ exchanger transports both Na⁺ and K⁺ and is regulated by Mg²⁺ ions and inhibited by quinine.

Ever since mitochondria were shown to possess high capacity for K⁺/H⁺ exchange (1-3), a singular question has been the relationship between K⁺/H⁺ and Na⁺/H⁺ exchange. Are Na⁺ and K⁺ transported by the same exchanger or by two different transport molecules? This question has been particularly difficult to resolve in view of the fact that no specific inhibitors of these processes have been found to date (see Ref. 4 for review). After numerous attempts to find inhibitors in our laboratory, we were motivated to look at quinine, which inhibits K⁺ transport in plasma membranes (5).

The data contained in Fig. 1 reveal a profound inhibitory effect of quinine on the electroneutral K⁺ efflux induced by A23187 in respiring rat liver mitochondria. K⁺ uptake follows the subsequent addition of valinomycin, which reveals the inward direction of the electrochemical potential gradient for K⁺ and establishes the electroneutrality of the K⁺ efflux (3). The combination of valinomycin and high K⁺/H⁺ exchange activity results in futile K⁺ cycling, and this energy drain will reduce the protonmotive force. Quinine increases matrix K⁺ in the presence of valinomycin (see Fig. 1), an effect consistent with inhibition of K⁺/H⁺ exchange and reduction of futile cycling. This secondary effect thus corroborates the direct observation of inhibition of K⁺ efflux and indicates that the inhibition is not due to reduction of protonmotive force.

We next addressed the question whether quinine could block Na⁺/H⁺ exchange as well. Freshly isolated mitochondria were suspended in K⁺ and Na⁺ acetates buffered at various

* These studies were supported in part by United States Public Health Service Grant GM31086. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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pH levels, and swelling secondary to ion uptake was monitored by absorbance at 520 nm. Control osmotic studies were carried out which showed that inverse absorbance was linearly related to inverse osmolality, in accord with the findings of Tedeschi and Harris (6). Initial rates of change of Abs⁻¹ were calculated and they are plotted *versus* pH in Fig. 2. In agreement with previous studies (8), mitochondria swell rapidly in Na⁺ acetate, with a peak in activity near pH 7.1, while the rate of swelling in K⁺ acetate is low throughout the pH range. Quinine does not inhibit mitochondrial swelling in this system (Fig. 2). The slight stimulation of swelling observed varies with preparation and is not considered to be significant.

Fresh mitochondria do not swell in K⁺ acetate (Fig. 2 and Ref. 8), and we wished to compare Na⁺/H⁺ and K⁺/H⁺ exchange under identical conditions. To this end, mitochondria were pre-incubated for 5 min in a medium containing succinate, tetraethylammonium, and A23187. This treatment resulted in the depletion of K⁺ and Mg²⁺ to less than 8 nmol/mg and 2 nmol/mg, respectively (9), these cations being replaced by tetraethylammonium (2). As shown in Fig. 3, these pretreated mitochondria exhibit pH profiles for swelling in acetate salts which differ substantially from those observed with normal mitochondria (Fig. 2).

Before considering the details of the results in Fig. 3, let us focus on the simple fact that rapid swelling is observed in acetate salts. The most straightforward interpretation of this phenomenon is based on the assumption that the underlying mechanism of salt uptake is cation/H⁺ exchange, coupled to nonionic diffusion of acetic acid. The K⁺/H⁺ exchanger, normally inhibited by endogenous Mg²⁺, is released by pretreatment with A23187. The gradients for Na⁺ and K⁺ uptake are virtually identical, because endogenous K⁺ has been substituted by tetraethylammonium. The finding that quinine inhibits swelling in K⁺ acetate can be attributed to inhibition of the K⁺/H⁺ exchanger, in complete agreement with the experiment reported in Fig. 1. While we believe this explanation to be correct, we must point out that the results in Fig. 3 are actually ambiguous with respect to the mechanism of quinine-inhibited swelling in acetate salts. These are altered mitochondria, and the possibility exists that pathways for K⁺, Na⁺, and Ac⁻ (or H⁺) uniport have been opened up by pretreatment and that quinine blocks one or more of these pathways. Indeed, we observe that quinine does inhibit Na⁺ and K⁺ uniport in mitochondria, a property this drug shares with other hydrophobic amine local anesthetics (10). We have examined this critical point experimentally, and the evidence against operation of a uniport pathway under the conditions of Fig. 3 may be summarized as follows. 1) Quinine blocks K⁺ transport under conditions where this transport can clearly be demonstrated to be electroneutral and not electrophoretic (see Fig. 1). It seems reasonable to assume that it is exerting the same effect in acetate, a medium in which the driving force clearly favors cation/H⁺ exchange. 2) pH profiles of swelling in Cl⁻ and NO₃⁻ salts were compared to acetate in pretreated mitochondria. Below pH 8, the rate of swelling was much higher in acetate than in Cl⁻ or NO₃⁻. We consider it very unlikely that these preparations are more permeable to the acetate anion than they are to Cl⁻ or NO₃⁻. 3) The effect of butacaine, which blocks cation uniport (10), on A23187-induced swelling in K⁺ acetate was compared to that of quinine. The highest dose studied was 0.5 mM, twice that

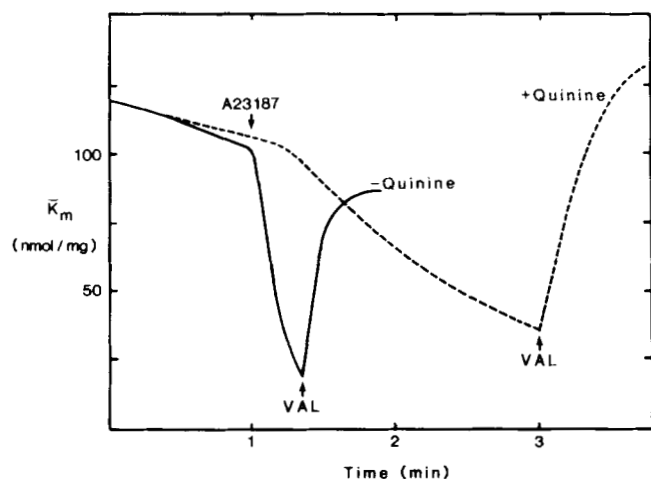


FIG. 1. Effects of quinine on A23187-induced K⁺ efflux from respiring mitochondria. The curves represent the time course of mitochondrial K⁺ content, \bar{K}_m (nmol/mg of protein), as determined from ion electrode potentials (see Ref. 9). Liver mitochondria were isolated from male Sprague-Dawley rats (9). Mitochondria pretreated with rotenone (2 nmol/mg) were suspended at 25 °C in 7.5 ml of medium containing K⁺ (0.2 mM) and the trimethylamine salts of succinate (4.3 mM), phosphate (1.1 mM), malate (1.1 mM), EDTA (0.4 mM), EGTA¹ (0.27 mM), chloride (60 mM) and TES (pH 7.4, 15 mM). A23187 (0.4 nmol/mg) was added at 1 min and valinomycin (0.5 nmol/mg) was added as indicated. The reaction was started by addition of 25 mg of mitochondrial protein to media containing either no quinine (solid line) or 0.5 mM quinine HCl (dashed line).

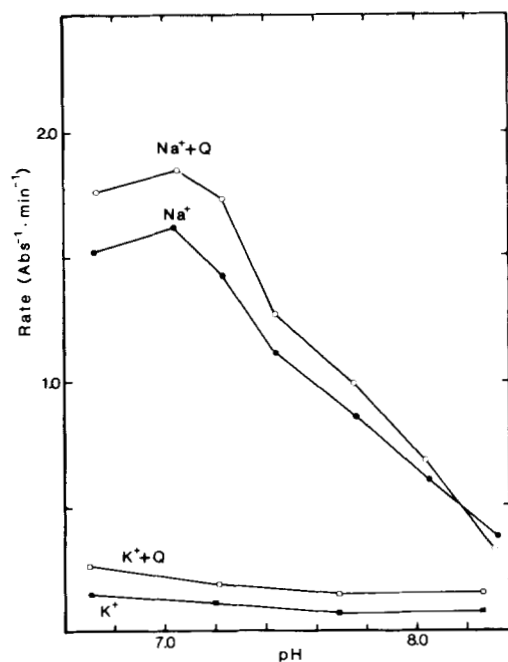


FIG. 2. Effects of pH and quinine on swelling of freshly isolated mitochondria in K⁺ and Na⁺ acetate. Mitochondria (0.1 mg/ml) were suspended at 25 °C in media containing either the K⁺ or Na⁺ salts of acetate (135 mM), TES (20 mM) and EDTA (0.1 mM) with 2 μ M rotenone, adjusted to the indicated pH. The initial rates of swelling in Na⁺ acetate (●), Na⁺ acetate plus 0.5 mM quinine (○), K⁺ acetate (■), and K⁺ acetate plus 0.5 mM quinine (□) are shown.

required to block K⁺ uniport. In contrast to quinine, butacaine had no effect on swelling in K⁺ acetate, arguing strongly against an underlying uniport mechanism. While additional studies are required to establish this point more firmly, these

¹ The abbreviations used are: EGTA, ethylene glycol bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; TES, *N*-(tris[hydroxymethyl]methyl-2-amino)ethanesulfonic acid.

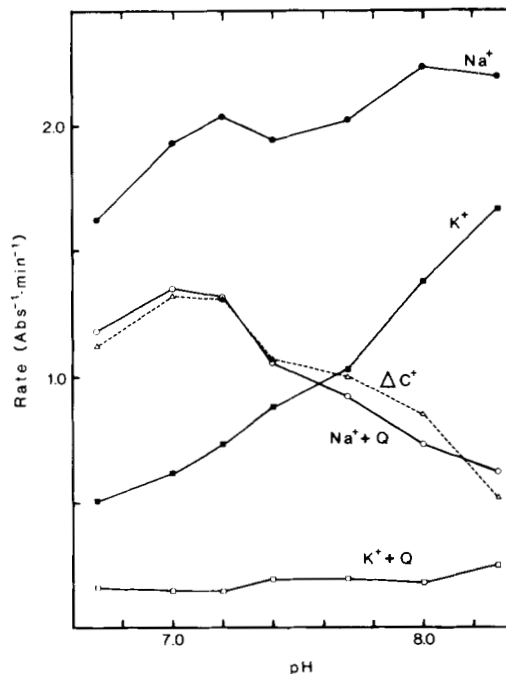


FIG. 3. Effects of pH and quinine on swelling of Mg²⁺- and K⁺-depleted mitochondria in K⁺ and Na⁺ acetate. The conditions of swelling and the symbols used are the same as in Fig. 2, except that the dashed line (Δ) represents the difference in swelling rates (Na⁺ minus K⁺) in the absence of quinine. The pH profiles in the presence and absence of quinine are a typical example of a set of three different experiments, all of which gave similar results. The following protocol was used to prepare Mg²⁺ and K⁺ depleted mitochondria. Rotenone-treated mitochondria (4 mg protein/ml) were treated with A23187 (0.4 nmol/mg) and preincubated for 5 min at 25 °C in media containing the tetraethylammonium salts of phosphate (1 mM), malate (1 mM), succinate (10 mM), citrate (5.3 mM), TES (pH 7.2, 20 mM), EGTA (0.13 mM), and EDTA (0.38 mM), made isotonic (0.272 osmol.) with sucrose. Following centrifugation, the pellet was resuspended at 0 °C in isotonic media containing sucrose, bovine serum albumin (1 mg/ml), and the tetraethylammonium salts of malate (25 mM), TES (pH 6.8, 12 mM), and EGTA (0.1 mM). After recentrifugation, mitochondria were resuspended in 0.25 M sucrose and stored on ice prior to use. These preparations are depleted of K⁺ and Mg²⁺, these cations being replaced by tetraethylammonium (2).

results provide strong evidence that the cation transport underlying the swelling reported in Fig. 3 is mediated by electro-neutral processes, that is, by Na⁺/H⁺ and K⁺/H⁺ exchange.

The data in Fig. 3 are striking in several respects, and they may now be evaluated in detail with this mechanism in mind. 1) The pH profiles for swelling in K⁺ and Na⁺ acetates are very similar to those observed for electroneutral K⁺ efflux (7). They differ sharply from the pH profile normally observed in Na⁺ acetate, which shows a broad peak at about pH 7.1–7.4 (Fig. 2 and Refs. 7 and 8). Thus, Mg²⁺ depletion either opens up a new pathway for Na⁺/H⁺ exchange or increases the velocity and changes the pH profile of the existing pathway. 2) The difference in swelling rates (Na⁺ minus K⁺) yields a pH profile which is similar to that normally observed in Na⁺ acetate in untreated mitochondria. This strongly suggests that Na⁺ is entering by two exchange pathways. That is, Na⁺ appears to share the exchanger used by K⁺ in addition to moving on its own exchanger. 3) Quinine markedly inhibited swelling in both Na⁺ and K⁺ acetate in these preparations, in contrast to its lack of effect in fresh mitochondria. 4) The pH profile obtained in the presence of quinine is flat in K⁺ acetate, and exhibits a peak at pH 7.1 in Na⁺ acetate. Thus, the pH profiles of Mg²⁺-depleted mitochondria are converted by quinine to those observed in normal mitochondria. 5) The pH

profile for Na⁺ acetate in the presence of quinine is virtually identical to the difference (Na⁺ minus K⁺) profile. The suggestion that mitochondria possess two monovalent cation/H⁺ exchangers is thus confirmed by the actions of quinine. These findings reveal, further, that the K⁺/H⁺ exchanger transports both Na⁺ and K⁺ and is inhibited by quinine and endogenous Mg²⁺. The Na⁺/H⁺ exchanger does not transport K⁺ and is not inhibited by quinine or Mg²⁺.

The properties of the cation/H⁺ exchangers can be related to their physiological function and genetic design. Mitochondria must retain at least one major cation to maintain volume and charge compensation for substrate anions. This cation is K⁺, and volume regulation is maintained by continual braking of the K⁺/H⁺ exchanger by Mg²⁺, holding matrix [K⁺] far above equilibrium with respect to K⁺/H⁺ exchange (3). Since K⁺ is retained by this mechanism, there is no need to retain Na⁺, and the Na⁺/H⁺ exchanger need not be regulated. Given the availability of an unregulated pathway for Na⁺/H⁺ exchange, it is not necessary to endow the K⁺/H⁺ exchanger with K⁺/Na⁺ selectivity. On the other hand, it is necessary to provide high Na⁺/K⁺ selectivity to the Na⁺/H⁺ exchanger since uncontrolled K⁺ efflux via this exchanger would have adverse consequences for volume homeostasis. This combination of properties provides sensitive homeostatic control, and this is apparently achieved in mitochondria by the synthesis of two carrier proteins which possess the minimal

requisite properties.

Mitchell recognized at the outset that chemiosmotic mitochondria require cation/H⁺ exchangers (11). The present findings support this hypothesis and suggest the mechanisms by which these transporters are integrated into the overall bioenergetic scheme of the mitochondrial membrane.

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