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UNMASKING THE MITOCHONDRIAL K/H EXCHANGER: TETRAETHYLAMMONIUM-INDUCED K^+-LOSS

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Summary: Mitochondria respiring in media containing 80 mM tetraethylammonium ions lose all of their endogenous K^+ within 7 minutes. K^+-loss is associated with uptake of tetraethylammonium ions. K^+ efflux under these conditions is energy-dependent and electroneutral. It is concluded that tetraethylammonium uptake unmasks the endogenous K/H exchanger. Considered in relation to the chemiosmotic theory, these results support the existence of a "carrier-brake" mechanism which modulates K/H exchange to maintain volume homeostasis in vivo.

In a previous communication (1), swelling-induced K^+-loss was found to be rapid, energy-independent and electroneutral. It was concluded that matrix swelling unmasked the mitochondrial K/H exchanger by releasing it from an endogenous inhibitor, or "carrier brake". If this hypothesis is correct, K/H exchange should also be released by uptake of a cation which is itself incapable of cation/H exchange. To test this prediction, K^+ transport was examined in mitochondria respiring in tetraethylammonium (TEA[1]) salts.

This report describes the characteristics of TEA^+-induced K^+-loss (TIKL[1]), a second means of unmasking the endogenous K/H exchanger in rat liver mitochondria.

METHODS

Liver mitochondria were isolated from Sprague-Dawley rats by differential centrifugation (2) followed by three washes in 0.25 M sucrose. 30 sec after pretreatment with rotenone (1 μg/mg), mitochondria (5 mg/ml) were added to 10 ml of media for ion kinetic studies. Two media were used: Succinate media contained 80 mM TEA^+, 1 mM phosphate, 5 mM citrate, 10 mM succinate, 15 mM TES[1] and sufficient chloride and sucrose to provide an osmolality of 0.27 and pH 7.2 at 25° C. Malate media was identical, except that succinate was replaced by malate (10 mM). Mitochondrial K^+ (μmoles/g protein) was calculated from electrode potentials (Corning monovalent cation electrode 476220) as described previously (1). For [^{14}C]TEA uptake studies, mitochondria were incubated for various times in 2.0 ml of succinate media. Uptake and K^+-loss were stopped by the addition of 8 ml of media containing KCN (1 mM), 300 mM

[1]Abbreviations: TEA^+, tetraethylammonium; TES, N-tris (hydroxymethyl) methyl-2-aminoethanesulfonate; TIKL, TEA^+-induced K^+-loss.

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TEA, 150 mM Cl, 150 mM acetate and 1 mM [^{3}H] sucrose, followed by centrifugation and analyses of pellet and supernate. Zero-time controls were used to correct for external [^{14}C] TEA binding, which was very small under these isotope dilution conditions. Matrix TEA^+ content was estimated from [^{14}C] TEA content, corrected for sucrose space, and the specific activity of added TEA^+. K^+ content was determined by atomic absorption spectroscopy.

RESULTS AND CONCLUSIONS

1. Respiratory Control in Tetraethylammonium Salts.

Respiratory control ratios of 4.5 - 5.5 were routinely observed when fresh mitochondria were added to isosmotic media containing sucrose, 100 mM TEA^+, 2 mM succinate, 2 mM phosphate and 15 mM TES[1] buffer, pH 7.2.

2. The Phenomenon of Tetraethylammonium-Induced K^+-Loss (TIKL).

Mitochondria lose K^+ rapidly during respiration in 80 mM TEA^+ (figure 1, curve A). Following a brief lag phase, K^+ is lost at rates up to 65 μmols/g/min. K^+-loss proceeds to completion, less than 5 μmols/g remaining after 6-7 minutes of incubation. The rate of K^+-loss and the duration of the lag phase depend on the anion composition of the suspending medium, features which will be discussed in a future report.

3. TIKL is Associated with TEA^+ Uptake.

The "carrier-stop" media described in methods was found to be effective in stopping TIKL, permitting simultaneous determinations of K^+ levels and [^{14}C]TEA uptake (see figure 2). TIKL is accompanied by rapid TEA^+ uptake and matrix swelling (not shown). It is noteworthy that mitochondrial TEA^+ continues to rise after mitochondrial K^+ has dropped to negligible levels.

4. TIKL is Energy-Dependent.

Inhibition of respiration by 1 mM cyanide prevents K^+-loss in TEA^+ salts (not shown). TIKL is also prevented when rotenone-treated mitochondria are incubated in the absence of succinate (figure 1, curve B).

5. TIKL is Electroneutral.

TIKL is reversed by valinomycin in respiring mitochondria (figure 1, curve A). Since valinomycin induces electrophoretic K^+-flux (3), and since TIKL proceeds in the opposite direction, TIKL must be electroneutral. In

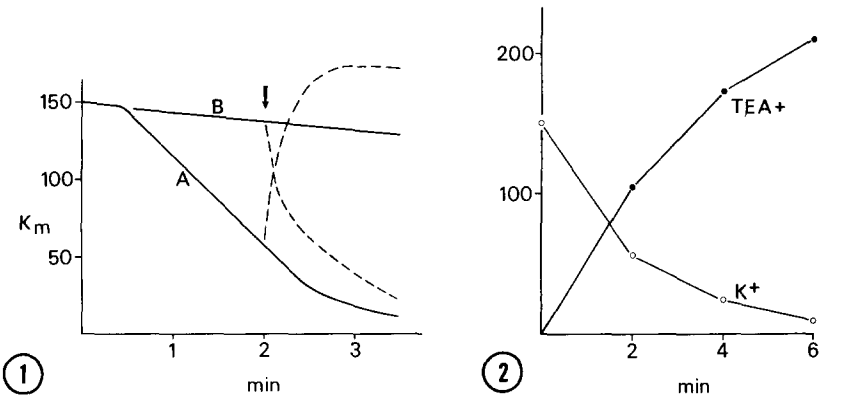

Figure 1: Mitochondrial potassium (μmoles/g protein) versus time in respiring (curve A) and non-respiring (curve B) mitochondria. Data were obtained from incubations of rotenone-treated mitochondria in succinate (A) or malate (B) media containing 80 mM TEA^+ (see Methods). At the arrow, 0.04 μg/mg valinomycin was added, and the resulting data are plotted as dashed lines, Media K^+ concentrations ranged from 0.28 mM at the start of incubation to 1.03 mM upon complete loss of matrix K^+.

Figure 2: Mitochondrial TEA^+ and K^+ (μmoles/g protein) versus time in mitochondria respiring in succinate media (see Methods).

nonrespiring mitochondria, valinomycin induces K^+-loss (figure 1, curve B). Under these conditions, the membrane potential is effectively clamped at the K^+ equilibrium potential, and electrophoretic K^+ efflux is balanced by electrophoretic TEA^+ and H^+ influx.

DISCUSSION

Two procedures for unmasking the mitochondrial K/H exchanger have now been described: osmotic swelling (1) and respiration in TEA^+ salts. Both processes are sufficiently rapid to account for estimated steady state K^+ influxes (4). Both have been shown to be electroneutral.

TIKL is energy-dependent, in agreement with ^{42}K-exchange studies in heart mitochondria (5). These authors concluded that K/H exchange itself is energy-dependent, being activated when ΔpH exceeds a limiting value. This conclusion appears not to be correct, since studies on swelling-induced K^+-loss have demonstrated the energy-independence of the exchanger (1). This apparent contradiction can readily be resolved by the carrier brake mechanism

(1) and the chemiosmotic theory (6): The negative membrane potential causes electrophoretic uptake of TEA^+ together with equivalent anions. The resulting matrix swelling releases the carrier brake, leading to K^+-efflux. Since TEA^+ uptake is irreversible, there being no endogenous TEA/H carrier, K/H exchange continues until all matrix K^+ is expelled. This K^+-loss represents osmotic compensation, and TIKL thus provides striking support for Brierley's proposal that the K/H exchanger is important in mitochondrial volume homeostasis (7).

Additional arguments in favor of the "carrier brake" model can be developed from consideration of mitochondrial homeostasis in vivo:

(a) Efflux on the K/H exchanger must precisely balance electrophoretic K^+ uptake. Matrix $(K)_i$ at equilibrium with cytosol $(K)_o$ of 100 mM and E_m of 160 mV would be 46.0 M. On the other hand, osmotic equilibrium requires that $(K)_i$ must remain below 0.2 M. K^+ influx must therefore be unceasing in respiring mitochondria; it represents the energetic cost of doing business in a high K^+ environment. An uncompensated potassium influx of 60 μmol/g/min with accompanying diavalent anions would lead to matrix swelling at the rate of about 300 μl/g/min. Regulation of the K/H exchanger must be precise indeed: Too little K/H exchange would lead rapidly to swelling and lysis, while too much would lead to contraction and anion loss. How does the K/H exchanger achieve this delicate balance in the face of such massive potassium traffic?

(b) $(K)_i$ cannot provide sensitive control of the K/H exchanger. K^+ salts are the major osmotic salts of the matrix. The uptake of K^+ with accompanying anions will occur isosmotically at 200 mM K^+, assuming the average anion valency to be -2. If matrix $(K^+)_i$ is 180 mM, a 10-fold matrix swelling (secondary to K^+ uptake) would lead to a mere 10% change in $(K^+)_i$. Thus, $(K^+)_i$ appears to be too insensitive to K^+ uptake to provide precise control of the rate of K/H exchange.

(c) ΔpH cannot provide sensitive control of the K/H exchanger. To illustrate this, consider the following simplifying approximations of matrix:cytosol relationships: (1) Cytosol osmolality ($\emptyset_o$) and pH_o are constant. (2) Organic anions, with the exception of nucleotides, are at equilibrium. That is, the anion distribution coefficient, $Q = (A)_j/(A)_o$, is solely a function of ΔpH, via the phosphate carrier (8-10). (3) Matrix concentrations of uncomplexed divalent cations are insignificant compared to those of monovalent cations. (4) Monovalent cations and organic anions comprise the major fraction, f, of matrix osmotic solutes. These assumptions lead to the following equations:

$$(C^+)_i = \Sigma z_j Q_j (A_j)_o \qquad (1)$$

$$\Sigma(1 + z_j) Q_j (A_j)_o = f\emptyset_o \qquad (2)$$

where z_j is the matrix valency of A_j and is solely a function of pH_i. Equation 2 states that ΔpH is determined solely by the concentrations and species of cytosol anions, within the range of validity of the assumptions used. In particular, Equation 2 implies that ΔpH will not change in response to K^+ influx. Once again, the primary effect of K^+ uniport is osmotic swelling, changing the amounts of anions and cations, but having little effect on their concentrations. ΔpH thus appears to be a poor candidate for controlling the K/H exchanger on the grounds that it, too, is relatively insensitive to net K^+ uptake.

(d) If neither carrier substrate is effective in regulating K/H exchange, there must exist a third substance which acts as a carrier brake to modulate K/H exchange in response to K^+ uniport. Thus, consideration of mitochondrial homeostasis in vivo leads to the same conclusion as that drawn from experiment (1).

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