ENERGY-DEPENDENT EFFLUX OF K+ FROM HEART MITOCHONDRIA

Edmundo Chavez, Dennis W. Jung and Gerald P. Brierley

Department of Physiological Chemistry, Ohio State University
Columbus, Ohio 43210

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Summary - The efflux of $^{42}K^+$ from the matrix of isolated heart mitochondria under conditions of steady state K+ has the properties of an energy-linked K+/K+ exchange reaction. Efflux requires respiration and external K+, is sensitive to uncouplers and to Mg^{+2}, and is markedly decreased by oxidative phosphorylation. Efflux is stimulated by Pi and by mersalyl, but declines under conditions which promote net uptake of K+ and acetate. Acetate strongly inhibits efflux in the presence of mersalyl. These data suggest that mitochondrial K+ levels are not maintained by a balance between inward K+ pumping and a passive outward leak, but rather that a nearly constant K+ pool results from a regulated interplay between an inward K+ uniport (responsive to membrane potential) and a K+/H+ exchanger (responsive to the transmembrane pH gradient).

Several lines of evidence (1-3) suggest that net accumulation of cations by isolated mitochondria results from an electrophoretic distribution of the cation in response to an internal negative membrane potential ($\Delta\Psi$). For example, valinomycin which induces a large net uptake of K+ also increases the efflux of $^{42}K^+$ from the matrix, a result which is consistent with increased transmembrane permeability to K+ in both directions (2). However, Diwan and Tedeschi (4) have found that, in the absence of added ionophores, outward flux of K+ is decreased when respiration is blocked or uncoupled. Since these conditions would decrease a membrane potential dependent on metabolism, the results are clearly incompatible with a simple potential-dependent distribution of cations across the membrane (4). The well-known ability of isolated mitochondria to retain K+ and low rates of turnover of matrix K+ (5-10) also suggest that the unmodified mitochondrial membrane has a low permeability to K+. In addition, mitochondria have the capacity to carry out a net extrusion of cations by an energy-dependent reaction (1, 11-13) and the evidence suggests that this reaction depends on a cation+/H+ exchanger which responds to a transmembrane pH

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Table I - Efflux of $^{42}K^+$ from Heart Mitochondria

		Loss of Mitochondrial $^{42}K^+$ (Per Cent)			
		K^+ Medium (100 mM)		Na^+ Medium (100 mM)	
Medium and Additions	pH	No Resp.	Succinate	No Resp.	Succinate
Chloride	7.2	14	34	23	39
Cl^- + val. + CCP(10^{-7}ea.)	7.2	92	-	95	-
Cl^-	8.4	25	81	51	67
Cl^- (100 mM) + P_i(2mM)	7.2	8	72	28	34
Cl^- + P_i + CCP(10^{-7}M)	"	-	8	-	-
Cl^- + P_i + ADP(4 mM)	"	-	27	-	-
Cl^- + P_i + EDTA (1 mM)	"	-	70	-	84
Cl^- + mersalyl (20 nmoles mg^{-1})	7.2	33	93	27	27
Cl^- + mersalyl + acetate (5 mM)	"	-	18*	-	-
Acetate (100 mM)	7.2	4	42*	9*	48*
Acetate + mersalyl	"	9	18*	11*	18*

*Net salt uptake as shown by ΔA_{546} of greater than 0.1 in 3 min at 25°.

Nagarse beef heart mitochondria (14) were incubated in ^{42}KCl (40 mM), sucrose (120 mM), and Tris succinate (4 mM, pH 7.2) for 8 min at 25°, isolated by centrifugation, and washed once in cold 0.25M sucrose. These mitochondria have the same or slightly elevated K^+ content (120 nmoles/mg) relative to untreated controls, identical ADP:O and respiratory control ratios, and identical patterns of K^+ loss to a K^+-free medium as recorded with a K^+ electrode. The ^{42}K-labeled mitochondria were incubated at 1 mg/ml in either KCl, NaCl, or the acetate salts (100 mM in each case) with the pH and additions as indicated. Rotenone (3 ug/ml) was present in all incubations and when present, the Tris succinate concentration was 4 mM. After 5 min at 25° the mitochondria were sedimented at 20,000 rpm in a Sorvall SE-12 rotor (1.5 min total centrifugation time) and the radioactivity of the supernatants determined. Per cent loss was calculated from the increase in radioactivity of the supernatant (less a zero time control) as compared with the total count of the suspension (again, less the zero time control). Subtracting the zero time control eliminates extramitochondrial and intermembrane $^{42}K^+$ as well as passively bound label and assures that the data presented represent matrix $^{42}K^+$. Swelling was measured in an Eppendorf photometer under identical conditions. The abbreviations are: val, valinomycin; CCP, m-Cl-carbonylcyanidephenylhydrazone.

difference. It appears that many of the apparent experimental contradictions in regard to mitochondrial K+ movements could be resolved by postulating that both an inward K+ uniport (responsive to $\Delta\Psi$) and a K+/H+ exchanger (responsive to ΔpH) are present in the membrane and that the two activities are regulated

in such a way as to maintain a nearly constant K+ concentration in the matrix. The present preliminary communication summarizes $^{42}K^+$ efflux data which strongly support this suggestion.

RESULTS

Heart mitochondria in which matrix K+ has been labeled with $^{42}K^+$ retain 86% of the label when incubated for 5 min at 25° in 100 mM KCl (Table I) and comparable amounts in cold sucrose or NaCl. Respiration increases $^{42}K^+$ loss in both K+ and Na+ media and this respiration-dependent $^{42}K^+$ efflux is markedly activated by P_i and by mersalyl in the K+ but not the Na+ medium. The bulk of the label is mobilized by addition of valinomycin plus CCP regardless of the suspending medium (Table I). At pH 8.4 the respiration-dependent loss of $^{42}K^+$ is accelerated in both K+ and Na+, but there is also an increase in passive loss of label at this elevated pH which is consistent with increased permeability to monovalent cation. Respiration-dependent efflux of $^{42}K^+$ is sensitive to uncouplers and the P_i-dependent efflux is strongly inhibited when ADP is added to initiate oxidative phosphorylation (Table I). It is of interest that respiration-dependent efflux of $^{42}K^+$ occurs in NaCl when EDTA (15) is added to produce increased permeability to Na+ (Table I). Respiration-dependent efflux of $^{42}K^+$ in the presence of P_i is inhibited by Mg^{+2}, but that induced by mersalyl is not (data not shown).

Simultaneous light-scattering studies have established that no swelling or contraction is associated with respiration in the chloride medium used for the studies in Table I and that the respiration-dependent efflux is occuring under conditions approximating steady-state K+. Swelling equivalent to the net uptake of 60-70 nmoles/mg of K+ occurs when P_i (2 mM) is added and filtration studies of efflux kinetics (data not shown) indicate that little efflux occurs during this net K+ uptake phase (about one min. at 25°). No volume change is associated with the extensive, respiration-dependent efflux of $^{42}K^+$ which occurs when mersalyl is added to the KCl medium. Addition of acetate (2 to 5 mM) in the presence of mersalyl in the KCl medium markedly inhibits the efflux of

$^{42}K^+$ (Table I) and brings about a large net K+ uptake (2-300 nmoles/mg in 5 min, as estimated from the extent of swelling). In the absence of mersalyl (or any other inducing agent) a spontaneous, respiration-dependent accumulation of K+ occurs when mitochondria are suspended in 100 mM K+ acetate (1, 16). Mitochondria labeled with $^{42}K^+$ retain the label remarkably well during this large amplitude swelling and K+ accumulation (Table I, 42% loss of label as compared to 72% for the minimal swelling in KCl - P_i). In addition, there is essentially no loss of $^{42}K^+$ during extensive passive swelling in Na+ acetate (Table I). Addition of mersalyl during the respiration-dependent accumulation of either K+ or Na+ acetate does not inhibit ion accumulation (and actually increases swelling in K+ acetate), but markedly inhibits the efflux of $^{42}K^+$ (Table I).

DISCUSSION

These studies confirm that isolated heart mitochondria retain matrix K+ rather well in the absence of metabolic energy. The efflux of $^{42}K^+$ under near steady-state K+ conditions has the properties of an energy-linked reaction, and in fact closely resembles the steady-state influx reported by Diwan and coworkers (4,7,9,10) for liver mitochondria. Both influx and efflux require respiration and both are sensitive to uncouplers and stimulated by P_i, mersalyl, and elevated pH. Steady-state influx of $^{42}K^+$ into beef heart mitochondria shows saturation kinetics (Km of 12 mM K+) and, like the respiration-dependent efflux, the influx is inhibited by exogenous Mg^{+2} (K_i of 3 mM) and by ADP (17). Efflux of $^{42}K^+$ stimulated by P_i or by mersalyl requires external K+ (Table I), but the concentrations required are considerably higher (Km of about 30 mM) than those for the influx reaction (17). The retention of a relatively constant pool of K+ in the matrix and the close correspondence between the properties of $^{42}K^+$ influx and efflux suggest that, in contrast to the pump-leak system found in erythrocytes and other cells, mitochondrial K+ is maintained by an energy-dependent K+/K+ exchange system. Since heart mitochondria <u>in situ</u> are faced with 140 mM K+, it would seem that the possibilities for regulation of membrane potential and pH gradients in the mitochondria by displacement of the K+ influx

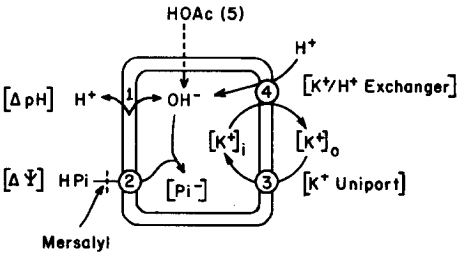

Fig. 1 - Propsed model for K+ influx and efflux in mitochondria. See the text for details.

and efflux reactions under various metabolic conditions should be explored.

The presence of both a K+ uniport and a K+/H+ exchanger is in accord with chemiosmotic coupling principles (3), provided that some regulatory factors are present which prevent futile cycling of K+. The model shown in Fig. 1 can be used to rationalize the $^{42}K+$ turnover data just described, as well as net uptake and extrusion of K+.

It is proposed that [1] respiration produces a ΔpH, [2] the P_i and substrate transporters convert a portion of the ΔpH to ΔΨ, [3] a voltage-gated K+ uniport (18) permits electrophoretic K+ entry when ΔΨ exceeds a certain limiting value, and [4] a K+/H+ exchanger comes into play when ΔpH attains sufficient magnitude. In addition, when acetate [5] is present, the equilibrium concentration of free acetic acid will convert ΔpH into an acetate gradient ($\Delta\Psi_A$)(see Fig. 1).

In this model, spontaneous net accumulation of cations (1,16) will occur when ΔΨ is large compared to ΔpH (isotonic K+ acetate or phosphate, for example) and net extrusion (1,12,13) when most of the protonmotice force is reflected in a ΔpH (mitochondria swollen in isotonic K+ nitrate, for example). Whereas these net uptake and extrusion reactions occur at near physiological concentrations of K+ (100-150 mM) they are dependent on the ability of free acetic acid to discharge ΔpH and the permeable nitrate ion to equilibrate ΔΨ. In a chloride medium anion movements are minimal and the presence of both ΔpH and ΔΨ components (3) would produce a balance between K+ influx and efflux. Oxidative phosphorylation

would lower the protonmotive force (3) and decrease exchange (Table I). In the absence of ADP, P_i (2 mM) first increases $\Delta\Psi$ and produces net K+ uptake. As the accumulation of P_i produces a high ratio of interior to exterior P_i, the activity of the transporter diminishes and a new steady state is established which has a significant ΔpH component and which promotes K+ turnover (Table I).

The high steady-state turnover produced by mersalyl (Table I) would be explained by the well-established ability of the mercurial to block conversion of ΔpH to $\Delta\Psi$ on the phosphate and dicarboxylate transporters. The resulting increase in ΔpH (visible as a pH transient, Ref. 19) activates K+/H+ exchange [4]. Since anion compensation does not occur in the chloride medium the net efflux of K+ produces a $\Delta\Psi$ which would promote K+ influx through the uniport [3] and increase steady-state $^{42}K+$ turnover. It should be noted that mersalyl strongly stimulates net extrusion of K+ in a nitrate medium in which anion compensation eliminates $\Delta\Psi$ (17). In the presence of acetate, the mersalyl-dependent increase in ΔpH would be rapidly dissipated by HOAc penetration (to the exclusion of K+/H+ exchange). The increased $\Delta\Psi_A$ would bring about increased influx through the uniport [3] and produce net ion accumulation with decreased $^{42}K+$ turnover. Mersalyl at these concentrations is clearly not activating ion uptake by increasing cation permeability as we have suggested for other mercurials under different conditions (19).

A more complete account of these experiments and a more extended development of the model is obviously necessary and will be presented elsewhere.

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