K^+ FLUXES AND THE MITOCHONDRIAL MEMBRANE POTENTIAL

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Received 21 October 1975

1. Introduction

It has been proposed that the transport of K^+ in isolated mitochondria is a passive process driven by the electrical potential difference across the mitochondrial membrane [1]. This conclusion is based on questionable premises [2]. Most significantly, the concept is based on the idea that the distribution of K^+ in the presence of valinomycin during energized conditions (e.g., see [1,3]) is the result of a potential across the mitochondrial membrane. In fact, the potassium distribution can be accounted for quantitatively by the internal negative charges generated by an H^+ pump operating in an electroneutral exchange with K^+ [4]. The distribution is not consistent with the presence of an electrogenic pump [4].

The theory governing the relationship between passive influx and efflux of ions in the presence of a membrane potential is relatively well understood (e.g., see [5–7]). An application of this theory to the mitochondrial K^+ fluxes measured in the absence of added ionophores, with and without the availability of metabolic energy, yields results inconsistent with a model which assumes that these ion fluxes are the passive result of a potential across the mitochondrial membrane. A similar approach has been used to demonstrate that the membrane potential across the frog skin drives the Cl^- [8] but not the Na^+ [9] fluxes. Analogous flux equations have been previously used to calculate the flux of Na^+ in giant squid axons [6].

2. Methods

Rat liver mitochondria isolated by standard procedures [10] were incubated in media containing ^{42}K, ^3H_2O and [^{14}C]sucrose. The media included 200 mM sucrose, 31 mM Tris, 8 mM succinic acid and approx. 2–4 mM K^+ (see values of $(K)_0$ in table 1), and were adjusted to pH 8.0 with HCl. The concentrations of antimycin A, NaCN (or NaCl) and 2,4-dinitrophenol (DNP) when present were 0.25 μg/ml, 1 mM and 0.1 mM respectively. The mitochondria were combined with the medium containing isotopes at zero time and samples taken after 0.75 and 7 min of incubation, except in the determinations with antimycin A and NaCN where the ^{42}K was not added until after a 2 min preincubation with the inhibitor to allow dissipation of endogenous ATP. Samples were then taken at 0.75 and 7 min after the addition of the ^{42}K. The mitochondrial samples were separated from incubation media by rapid centrifugation through silicone [11,12]. Total K^+ levels were determined by atomic absorption spectroscopy. Radioisotopes were assayed by liquid scintillation counting. All isotopes were obtained from New England Nuclear.

The amount of labeled K^+ taken up was calculated from the ^{42}K counts sedimented and the initial (0.75 min) specific activity of the supernate, as in previous experiments [12,13]. The K^+ influx (J_i) was calculated as the difference in labeled K^+ taken up between the 0.75 min and 7 min samples. The net change in mitochondrial K^+ (J_{net}) was calculated as the difference in total mitochondrial K^+, determined

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by atomic absorption, between the 0.75 min and 7 min samples. The K^+ efflex (J_o) was calculated as equal to $J_i - J_{net}$. In calculating the internal K^+ concentration, $(K)_i$, it was necessary to correct the measured mitochondrial K^+ for external K^+ and rapidly exchangeable adsorbed K^+ [14]. An estimate of the zero time labeled K^+ taken up was obtained by extrapolation and subtracted from the measured (0.75 min) K^+ content of each sample. This corrected value of K^+ content was divided by the difference between the ^3H_2O and [^{14}C]sucrose distribution spaces, assumed to be equal to the intramitochondrial fluid volume [11]. All data are expressed as means of six determinations ± standard deviations.

3. Results and discussion

Following the Goldman constant field assumption (see [5,6]) the net flux of K^+ is expressed by equation 1:

$$J_i - J_o = PQ\,[(K)_o - (K)_i e^{\beta}] \quad (1)$$

P is the permeability coefficient, $(K)_o$ and $(K)_i$ are the external and the internal K^+ concentrations respectively. Q is $\beta/(e^{\beta}-1)$ and $\beta = F\Delta E/RT$, where F is the Faraday, R the gas constant, T the temperature (°K) and ΔE is the potential across the mitochondrial semipermeable membrane. Accordingly, the unidirectional influx, J_i, and the unidirectional efflux, J_o, are:

$$J_i = PQ(K)_o \quad (2)$$

$$J_o = PQ(K)_i e^{\beta} \quad (3)$$

The experimental data can be most easily summarized in terms of the flux constants k_i and k_o where $k_i = J_i/(K)_o$ and $k_o = J_o/(K)_i$. From Eqns (2) and (3), $k_i = PQ$ and $k_o = PQe^{\beta}$. The ratio of k_i/k_o expressed in Eqn (4) (see below) can be used to calculate ΔE.

If there exists an electrogenic potential across the mitochondrial membrane that is dependent on oxidative metabolism, some useful relationships between the flux constants in the presence of metabolism, k_M, or in its absence, k_O, may be calculated. The ratios of these constants are solely a function of membrane potential, and further, they can be determined experimentally. It can be shown that $k_{i_M}/k_{i_O} = Q$ and $k_{o_M}/k_{o_O} = Qe^{\beta}$. These ratios confirm the intuitive deduction that in the presence of a ΔE, negative inside, the k_i can only be decreased and the k_o can only be increased by blocking metabolism. The calculated values of k_{i_M}/k_{i_O} equal approximately 1, 2 and 12 respectively for membrane potentials of 0, 50 and 300 mV, negative inside. For k_{o_M}/k_{o_O} corresponding values are 1, 0.3 and 0.0001. The experimentally determined J_i's and J_o's are listed in table 1.

Table 1
Effects on K^+ fluxes of antimycin A, NaCN and 2,4-dinitrophenol

Expt.	mg mito. protein/ml	mM $(K)_o$	mM $(K)_i$	Conditions	μmoles (g protein)$^{-1}$ min^{-1} J_i	J_o
I	5.7	2.43	108 ± 11	Control	1.03 ± 0.03	2.2 ± 0.8
				Antimycin A	0.32 ± 0.03	1.3 ± 0.4
				DNP	0.25 ± 0.02	2.5 ± 0.3
II	4.9	2.36	106 ± 8	Control	1.03 ± 0.03	1.8 ± 0.4
				Antimycin A	0.38 ± 0.03	1.6 ± 0.7
				DNP	0.30 ± 0.03	2.9 ± 0.4
III	7.3	4.39	151 ± 38	Control (NaCl)	1.36 ± 0.08	1.9 ± 0.3
				NaCN	0.20 ± 0.04	1.3 ± 0.3
IV	5.3	4.29	104 ± 11	Control (NaCl)	1.73 ± 0.14	2.5 ± 1.0
				NaCN	0.23 ± 0.08	1.7 ± 0.5
I–IV				Control	1.29 ± 0.33	2.1 ± 0.3
				NaCN or Antimycin A	0.28 ± 0.08	1.5 ± 0.2

Table 2

Part A: Flux constants and apparent potentials calculated from data in table 1

Expt.	Conditions	μmoles (g prot.)$^{-1}$ min^{-1} mM^{-1} k_i	k_o	mV $-(RT/F) \ln (k_i/k_o)$
I	Control	0.43 ± 0.01	0.021 ± 0.008	−77 ± 9
	Antimycin A	0.13 ± 0.01	0.013 ± 0.004	−60 ± 8
	DNP	0.10 ± 0.01	0.023 ± 0.003	−39 ± 3
II	Control	0.44 ± 0.01	0.017 ± 0.004	−82 ± 6
	Antimycin A	0.16 ± 0.01	0.015 ± 0.006	−62 ± 13
	DNP	0.13 ± 0.01	0.027 ± 0.005	−40 ± 5
III	Control (NaCl)	0.31 ± 0.02	0.014 ± 0.005	−79 ± 7
	NaCN	0.04 ± 0.01	0.008 ± 0.002	−43 ± 6
IV	Control (NaCl)	0.40 ± 0.03	0.024 ± 0.009	−73 ± 9
	NaCN	0.05 ± 0.02	0.016 ± 0.003	−29 ± 11

Part B: Change in flux constants with inhibitors and DNP

Reagent	k_{i_M}/k_{i_O}	k_{o_M}/k_{o_O}
Antimycin A	3.2	1.6
	2.7	1.2
NaCN	7.0	1.8
	7.6	1.5
DNP	4.1	0.9
	3.4	0.7

The values of k_i and k_o calculated from these data are shown in table 2, along with values of k_{i_M}/k_{i_O} and k_{o_M}/k_{o_O}. The results do not conform to the expectations when antimycin A or cyanide is used as inhibitor. Although k_i decreases when metabolism is blocked, k_o does not increase as predicted by the presence of a metabolism-dependent membrane potential. When all experiments are pooled, it is clear that J_o actually decreases significantly when metabolism is blocked with antimycin A or NaCN, leading to values of k_{o_M}/k_{o_O} greater than 1. In other experiments similar to those depicted in tables 1 and 2 except that the medium was at pH 7, antimycin A similarly failed to cause an increase in K^+ efflux rate. The results can be explained most easily by postulating that the K^+ influx is the result of a metabolism-dependent active transport and is not a passive process.

In the presence of DNP, the k_o increases. The effect is small, although the increase is statistically significant. It may correspond to a special effect of DNP itself on K^+ transport. Alternatively, it seems reasonable to hypothesize that if the K^+ efflux involves reversal of a metabolism-linked pump mechanism, then dissipation of a high energy intermediate of this mechanism might conceivably accelerate the reverse reaction, i.e. K^+ efflux.

The data are clearly inconsistent with the presence of a metabolically dependent membrane potential. Nevertheless it might be of interest to calculate the highest possible potential assuming that the influx is driven by the electrical potential across the mitochondrial membrane. This can be done most simply from the ratio k_i/k_o, since from Eqns (2) and (3) (e.g. see [7]):

$$\Delta E = -\frac{RT}{F} \ln \frac{J_i}{J_o} \frac{(K)_i}{(K)_o} = -\frac{RT}{F} \ln \frac{k_i}{k_o} \quad (4)$$

This equation yields membrane potentials in the absence of inhibitors in the range of −70 to −80 mV. These are likely to be overestimates since there is an apparent membrane potential in the presence of concentrations of antimycin A or NaCN that inhibit respiration completely, or in the presence of uncoupling concentrations of DNP. Correcting for these, the maximally allowable membrane potential ranges

from about −20 to −40 mV. It is evident also from the values given in table 2 that estimates of ΔE that would be consistent with the relationships $k_{i_M}/k_{i_O} = Q$ and $k_{o_M}/k_{o_O} = Qe^{\beta}$ would be substantially different from values of ΔE calculated from Eqn (4), which is further evidence against the validity of the major assumption upon which all of these equations are based, namely that the only driving force for the K^+ flux is the electrochemical potential gradient.

It should also be noted that for a more complex model for the passive diffusion of ions (e.g. a model in which the diffusion is restricted to small channels, the so called single file model (see [15]) the magnitude of the estimated potentials would be even smaller than those listed in table 2.

These results are in agreement with estimates from microelectrode and fluorescent probe studies that indicate that an electrogenically generated membrane potential is not significant in isolated mitochondria [16–18]. A kinetic analysis of the dependence of the K^+ influx on several biochemical parameters (e.g. K^+ concentration, pH, phosphate concentration) suggests a saturable, carrier mediated process [19], as will be detailed in a separate communication [14].

Acknowledgements

This work was supported by grants from the U.S. Public Health Service, #GM-20726, and from the American Cancer Society, Inc., #BC-161. The authors are grateful to Robert I. Macey and Charles Edwards for their most helpful discussions of this problem.

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