MITOCHONDRIAL K^+ TRANSPORT: EFFECT OF N-ETHYL MALEIMIDE ON ^{42}K FLUX

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SUMMARY

The energy-linked flux of K^+ into rat liver mitochondria is found to be stimulated by the sulfhydryl reagent, N-ethyl maleimide. The stimulation of K^+ influx by N-ethyl maleimide is observed only at alkaline external pH. N-ethyl maleimide also stimulates efflux of K^+ from the mitochondria. The stimulation by N-ethyl maleimide of K^+ influx, but not K^+ efflux, is dependent on the availability of metabolic energy. It is suggested that the effect of N-ethyl maleimide on K^+ influx may be secondarily the result of an inhibition of phosphate-hydroxyl exchange. The dependence of energy-linked K^+ influx on the external pH may be interpreted as evidence for a role of OH^- as a counterion accompanying K^+ through the mitochondrial pump mechanism.

INTRODUCTION

The unidirectional flux of K^+ into isolated rat liver mitochondria is dependent on the availability of metabolic energy (1-3). The K^+ influx appears to involve a mechanism at least partially distinct from the mechanism mediating energy-dependent Ca^{++} uptake (4). Nevertheless, the mitochondrial K^+ transfer resembles other mitochondrial cation transport processes in its energetic requirements and in the direction of energized transport, which is inward (1-3,5,6). The only possible exception to the inward direction of energized cation flux is the metabolism-dependent change in pH which has been interpreted by Mitchell and others as evidence for an outward directed proton pump (7).

The energy-linked K^+ influx is observable under approximately steady state conditions (2,3), and hence is a suitable model system for examining the possible role of counterions in the energized cation fluxes. Under steady state conditions, cation transfer should not require an associated anion flux unless transfers of the cation and anion are mechanistically linked. On the basis of observations of net cation fluxes, a direct role of anion movements

has previously been suggested (8,9). For example, P_i was postulated to be primarily involved in the energized accumulation of Ca^{++} by mitochondria (8). More recently, an apparent sensitivity of the respiratory carriers to fluxes of P_i across the mitochondrial membrane has been reported (10,11).

The energy-linked influx of K^+ into mitochondria has been shown to be little affected by the presence or absence of P_i in the external medium (2). On the other hand, a stimulation of K^+ influx by elevated external pH was noted (2). Tannic acid, which inhibits (12) a postulated (13) P_i-dicarboxylate exchange mechanism, has been found to stimulate the energy-linked K^+ influx (12). The sulfhydryl reagent, NEM[1], is thought to block an alternate pathway of P_i transfer, a P_i-OH^- exchange mechanism (13). The present investigations have examined the effect of NEM on K^+ flux.

METHODS

Rat liver mitochondria were isolated by standard procedures (14). Following incubation in media containing isotopic labels, the mitochondria were separated by centrifugation through silicone (15). Radioactivity was assayed by liquid scintillation counting and total K^+ was determined by atomic absorption, as in previous investigations (3,12). ^{42}K was utilized to detect K^+ influx. The distribution space of ^3H_2O provided a measure of the total water volume sedimented, while the contaminating extramitochondrial volume was estimated from the distribution space of (^{14}C)sucrose (3,15). All radioisotopes were obtained from New England Nuclear.

RESULTS

Measurements of ^{42}K influx from media of varied pH, containing succinate as energy source, are shown in Figure 1. The values of labeled K^+, represented by the triangular symbols, are not corrected for potassium in the

[1]Abbreviation used: NEM, N-ethyl maleimide.

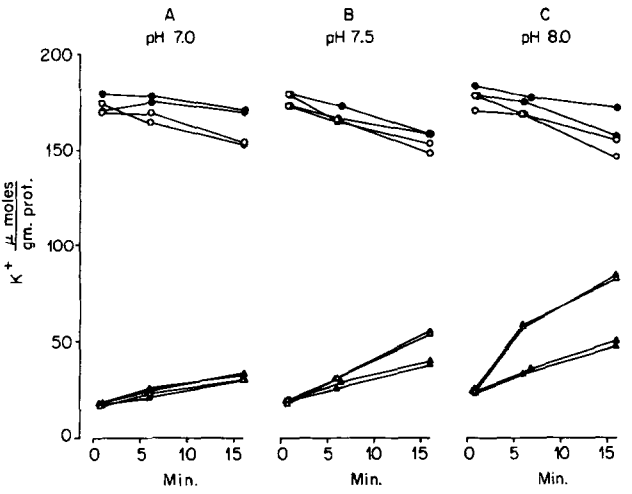

Figure 1. Effect of NEM and varied pH on uptake of labeled K^+. Mitochondrial K^+, in units of μmoles per gram of protein is plotted as a function of incubation time. The circular symbols represent values of total K^+ in the sedimented mitochondria: ●, control; ○, NEM added. The triangular symbols represent values of labeled K^+ taken up, calculated on the basis of the ^{42}K counts sedimented and the initial specific activity of the supernate: ▲, control; △, NEM added. The mitochondria (5.2 mg protein/ml) were incubated in medium containing 200 mM sucrose, 1.7 mM succinic acid, 30 mM Tris, 4.8 mM KCl and the isotopic labels ^{42}K (approx. 8 μCi/ml), ^{14}C-sucrose (approx. 0.3 μCi/ml) and $^{3}H_2O$ (approx. 2.5 μCi/ml). The medium was adjusted to pH 7.0, 7.5 or 8.0 with HCl as indicated. NEM, when present, was at a concentration of 0.5 mM.

sucrose-penetrable space. The contaminating extra-mitochondrial K^+ is estimated to be 11 μmoles per gram of protein in the experiment shown. The control values, represented by the solid triangles, are consistent with previous observations indicating that K^+ influx increases with increasing pH (2). The uptake of K^+, in the presence of a concentration of NEM that would block P_i-OH^- exchange (13), is indicated by the open triangles. A stimulatory effect of NEM is seen, which increases with increasing pH. At pH 7.0 NEM does not stimulate K^+ influx at all. At pH 8.0 there is an increase of approximatly 140 % in the amount of labeled K^+ taken up between the first samples after 45 seconds and the final samples after 16 minutes of incubation.

A slight stimulation of net K^+ efflux by NEM is seen at all pH values tested, as indicated by the circular symbols in Figure 1. Since the stimulated ^{42}K influx is not accompanied by an increase in total K^+, it is clear that the measured influxes reflect an enhanced exchange of endogenous K^+.

Table I. Effect of NEM and Antimycin A on K^+ Flux

Medium pH	Additions	^{42}K Influx	Net Flux	Unidirectional Efflux
7.0	None	+ 16	- 13	- 29
		+ 18	- 3	- 21
	NEM	+ 14	- 26	- 40
		+ 15	- 21	- 36
	Antimycin A	+ 1	- 18	- 19
		+ 3	- 18	- 21
	NEM & Antimycin A	+ 7	- 86	- 93
		+ 7	- 66	- 73
8.0	None	+ 30	- 21	- 51
		+ 26	- 22	- 48
	NEM	+ 48	- 42	- 90
		+ 41	- 53	- 94
	Antimycin A	+ 8	- 23	- 31
		+ 6	- 37	- 43
	NEM & Antimycin A	+ 22	- 66	- 88
		+ 19	- 99	-118

The indicated flux rates represent the changes in K^+ between samples taken after 45 seconds incubation and after 16 minutes incubation at 20 °C. The data are expressed in units of μmoles K^+ per gram of protein. The values of ^{42}K Influx represent the change in labeled K^+, calculated from the ^{42}K counts sedimented and the initial specific activity of the medium. The values of Net Flux represent the change in total K^+ sedimented, determined by atomic absorption. The values of Unidirectional Efflux are calculated as the difference between the values of Net Flux and ^{42}K Influx. The mitochondria (4.7 mg protein/ml) were incubated in the same medium as in the experiment of Figure 1. NEM when present was at a concentration of 0.5 mM. Antimycin A when present was at a concentration of 0.3 μgm per ml. All samples were pre-incubated for 2 minutes before addition of the ^{42}K at zero time.

The energy-dependence of the effect of NEM on K^+ flux is examined in the experiments depicted in Tables I and II. The data as represented in Tables I and II more clearly distinguish effects on influx and efflux rates. The

Table II. Effect of NEM and Dinitrophenol on K^+ Flux

Medium pH	Additions	^{42}K Influx	Net Flux	Unidirectional Efflux
7.0	None	+ 15	- 8	- 23
		+ 13	+ 2	- 11
	NEM	+ 11	- 30	- 41
		+ 13	- 14	- 27
	DNP	+ 3	- 57	- 60
		+ 4	- 50	- 54
	NEM & DNP	+ 4	- 57	- 61
		+ 4	- 76	- 80
8.0	None	+ 22	- 6	- 28
		+ 22	- 33	- 55
	NEM	+ 56	- 23	- 79
		+ 56	- 23	- 79
	DNP	+ 4	- 53	- 57
		+ 5	- 47	- 52
	NEM & DNP	+ 6	- 69	- 75
		+ 7	- 71	- 78

The indicated flux rates, representing the changes in K^+ between samples taken after 45 seconds incubation and 16 minutes incubation at 20 °C were calculated as described in the legend to Table I, and expressed in units of μmoles K^+ per gram of protein. The mitochondria (4.9 mg protein/ml) were incubated in the same medium as in the experiment of Figure 1 except that the concentration of K^+ was 3.6 mM. NEM when present was at a concentration of 0.5 mM. Dinitrophenol (DNP) when present was at a concentration of 0.1 mM.

unidirectional influx and efflux rates depicted in the Tables should be considered approximate, as they are calculated relative to the initial specific activity of the medium and do not take into account the recycling of K^+ in those samples where relatively large fluxes have occured. As indicated in Tables I and II, the K^+ influx at both neutral and alkaline pH is inhibited by treatment with either the respiratory inhibitor, antimycin A, or the

uncoupler, dinitrophenol, in agreement with previous studies (1-3,12). The stimulation of K^+ influx by NEM is largely blocked in the presence of either antimycin A or dinitrophenol. These findings support the conclusion that NEM alters K^+ influx via the energy-linked mechanism.

It is also apparent from Tables I and II that NEM stimulates the unidirectional efflux of K^+ at both neutral and alkaline pH. With the stimulated influx blocked, there is a large net K^+ efflux when antimycin A and NEM are simultaneously present. Qualitatively similar results are obtained with dinitrophenol. The uncoupler itself stimulates K^+ efflux, as has been reported (1-3,12,16). The effects of dinitrophenol and NEM on the unidirectional K^+ efflux do not appear to be additive.

DISCUSSION

It is not clear whether the effect of NEM on K^+ flux is related to its effect on P_i-OH exchange. No P_i is included in the medium in the experiments described here. Driven by the existing gradients, an exchange of endogenous P_i with external OH^- might alter the transmembrane pH gradient. Considering the dependence of the energy-linked K^+ influx on pH, it is possible that an inhibition of P_i-OH^- exchange might result in an enhancement of the effectiveness of a high external pH. The stimulatory effect on K^+ influx of tannic acid (12) is less readily explained in this manner. Although possibly a coincidence, the finding that inhibitors of two different mechanisms of phosphate transfer stimulate K^+ influx is perhaps significant. Alternatively, NEM, which is known to interact with many other catalytic mechanisms (17), may directly affect the mechanism mediating K^+ flux in the mitochondrial membrane. Since the K^+ influx stimulated by NEM remains sensitive to pH and to metabolic inhibitors, it is at least clear that NEM, directly or indirectly, affects the specific energy-linked transport mechanism.

Much evidence indicates that large membrane potentials do not exist across the mitochondrial membrane, even under energized conditions (18-20). It is thus reasonable to speculate that the mitochondrial ion pump may be non-elec-

trogenic. On the basis of the dependence of K^+ influx on an alkaline external pH, and the energy-linked pH changes that have been described (7), it is suggested that a non-electrogenic pump mechanism might effect an inward coupled transfer of K^+ and OH^-. Although mechanistically different, an outward movement of protons linked to an influx of K^+ would constitute the same overall reaction. It is unlikely that K^+ influx is driven by a separate electrogenic proton pump (7), since metabolic energy is required to support K^+ influx even under conditions of a favorable external pH (see above). It seems more likely that the cation fluxes and pH changes are directly coupled. The OH^- moved inward by a postulated K^+-OH^- pump could exchange for other anions via the known exchange reactions (11,13,21,22), in accordance with the known effects of a pH gradient on anion accumulation (23). Thus the increase in the anion distribution ratios which accompanies cation uptake (24) could be explained. The suggested role of the postulated cation-OH^- pump in promoting accumulation of the substrate anions could finally provide a raison d'etre for the so-called proton pump, whose postulated (7) role in oxidative phosphorylation has remained uncertain (25). It is hoped that future studies of the relationship between OH^- (or H^+) flux and the energy-linked flux of K^+ across the mitochondrial membrane will aid in elucidating the as yet hypothetical ion pump mechanism.

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