TRANSIENT PERMEABILITY CHANGES OF MITOCHONDRIA INDUCED BY UNCOUPLING AGENTS

A. H. Caswell and B. C. Pressman

Johnson Research Foundation, University of Pennsylvania

Philadelphia, Pennsylvania

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The permeability of ions across the mitochondrial membrane has been widely associated with mechanisms of energy conservation and mitochondrial control (Mitchell 1961, Harris et al. 1967). In most cases examined the ion permeability has either been an inherent characteristic of the mitochondrial membrane as in Ca^{++} transport or has been induced by addition of agents that specifically transport ions (Moore and Pressman 1964). It has been recognized that, unless specifically treated to alter the membrane characteristics, mitochondria have only slight K^+ permeability (Harris et al. 1966). Addition of uncoupling agents to mitochondria has been shown to give rise to K^+ extrusion (Judah et al. 1965, Kimmich and Rasmussen 1967). Evidence will be presented that uncoupling agents induce a new type of permeability change that is not a direct transport of ions by the reagent, but a response of the mitochondrial membrane to changes in the metabolic state induced by uncoupler. The specific permeability changes in the membrane will be related to the inhibitory effects on respiration of high concentrations of the uncoupling agent trifluoromethoxycarbonylcyanide phenylhydrazone (FCCP).

METHODS

Rat liver mitochondria are prepared by the method of Schneider (1948). Mitochondria are incubated in a cuvette containing a K^+ electrode, a pH electrode and a vibrating platinum potentiometric electrode, all used in conjunction with a common calomel reference electrode. The reference electrode is connected by a short agar-salt bridge to the reaction medium since this configuration

minimizes the streaming potential artifacts caused by stirring. The platinum electrode is used to measure the redox potential of cytochrome c by the method described by Caswell and Pressman (1968) employing tetramethyl-p-phenylenediamine (TMPD) as a mediator of electrons from cytochrome c to the electrode. Oxygen consumption is measured with a teflon covered Clark electrode. Each electrode is connected to an appropriate amplifier which drives an oscillographic recorder (cf. Pressman 1967).

RESULTS

Mitochondria incubated in a medium devoid of added K^+ and containing P_i with glutamate and malate as substrates lose K^+ extensively when treated with a high concentration of FCCP. Figure 1A shows this effect and the extensive oxidation of cytochrome c that accompanies the loss of K^+. The oxygen electrode trace shows an initial stimulation of respiration followed by a progressive inhibition paralleling the oxidation of cytochrome c; a slight alkalinization of

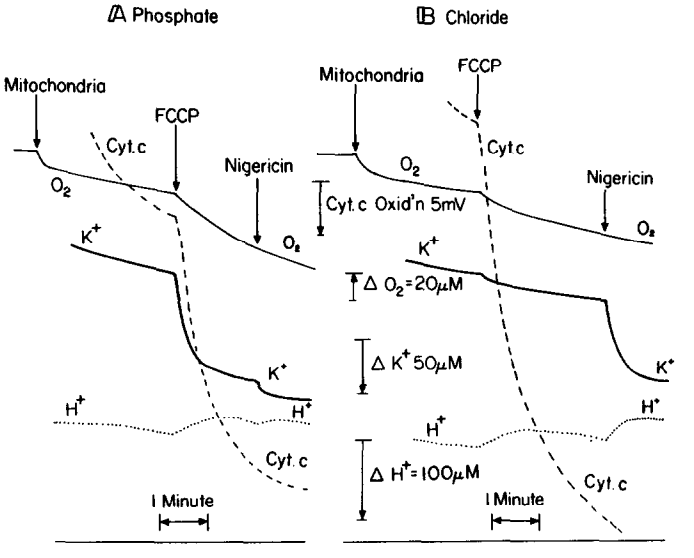

Figure 1. Multichannel traces showing the response of ions to FCCP addition. The incubation medium contains: 300 mM sucrose; 2.5 mM Tris glutamate; 2.5 mM Tris malate; 14 μM TMPD and either 2 mM Tris P_i or 4 mM Tris Cl. The pH is 7.4 and temp. 22°. Additions to the medium are: mitochondria 1.5 mg protein/ml; FCCP 1.7 μM; nigericin 0.033 μg/ml. The direction of the arrows giving the scales in the figure indicates increase in the external medium.

the medium also occurs. Nigericin is then added to show the maximum K^+ movement and H^+ uptake which is obtainable, since this reagent equilibrates the pH and K^+ gradients across the mitochondria (Pressman et al. 1967). It is seen that, despite the substantial K^+ movement after FCCP addition, complete equilibration has not been achieved. In a medium devoid of added P_i (Figure 1B) the same stimulation followed by inhibition of respiration and the same extensive oxidation of cytochrome c occurs as in Figure 1A. However, in contradistinction to the movements in the P_i medium, minimal K^+ movement occurs. The uptake of H^+ into the mitochondria is similar to that observed in Figure 1A. The subsequent addition of nigericin gives rise to the extensive release of K^+ and uptake of H^+. It follows from the K^+ traces that inhibition of respiration by high concentrations of FCCP does not correlate with K^+ loss from the mitochondria.

Concentrations of acetate up to 40 mM fail to mimic the effects of P_i on K^+ movement. Mg^{++} ion inhibits the K^+ loss from the mitochondria upon FCCP addition when P_i is present in the medium and yet has no effect on the pH, oxygen electrode or cytochrome c traces. A further striking feature of the K^+ movements is illustrated in Table I. Not only can the rate of K^+ movement be altered by varying the P_i or Mg^{++} concentrations, but so also can the extent of K^+ movement. The cutoff in K^+ movement is sharp and occurs after the same time interval following FCCP addition regardless of the amount or rate of K^+ extrusion.

The transitory nature of the K^+ permeability induced by FCCP is illustrated in Figure 2. If P_i is added prior to FCCP, then substantial K^+ release occurs (cf. Fig. 1A). If however P_i is added after FCCP, then no movement of K^+ is seen whether the FCCP concentration is sufficient to cause inhibition (Fig. 2A), or exerts a substantial stimulation of respiration (Fig. 2B). On the other hand the P_i addition produces a significant increase in the rate of respiration and the cytochrome c redox level becomes more reduced. Further addition of FCCP after P_i causes K^+ loss, but only if the previous aliquot was insufficient to cause respiratory inhibition.

TABLE I

Potassium Movements Following FCCP Addition

Phosphate Concentration	Rate of K^+ Movement	Net K^+ Movement
μM	μmoles / gm protein / min	μmoles / gm protein
0	12.3	3.1
20	12.9	3.7
60	14.3	6.1
200	30	8.5
600	40	12.2
2,000	55	25
(nigericin)		56

Incubation conditions are the same as in Figure 1B.

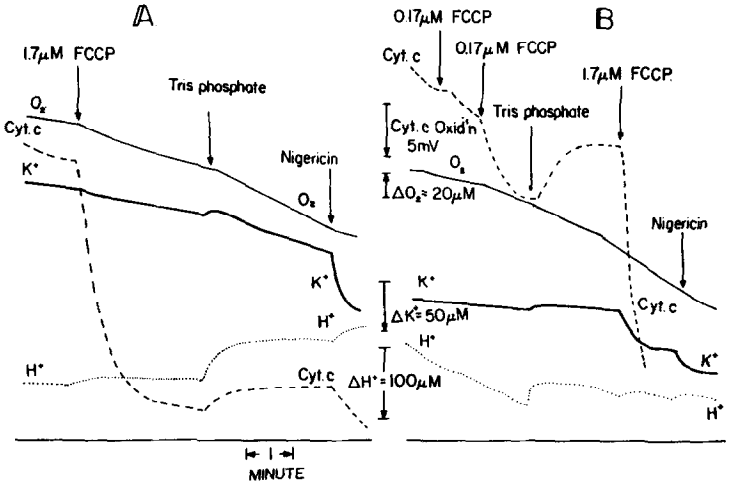

Figure 2. Multichannel traces showing the effect of P_i addition. The medium is the same as for Figure 1B. Additions to the medium are: mitochondria, 1.5 mg protein/ml; Tris P_i pH 7.4, 2 mM; nigericin 0.033 μg/ml.

DISCUSSION

The data described above show that there is no direct correlation between uncoupling of respiration in mitochondria and K^+ extrusion and there is no correlation between K^+ loss from the mitochondria and inhibition of respiration (cf. Kimmich and Rasmussen 1967).

The pH change on adding uncoupler appears to be largely independent of the K^+ movement and hence the K^+ must be moving in association with an anion. Since mitochondria contain a high concentration of phosphate, this is likely to be transported with K^+. Mitchell (1966) and Bielawski et al. (1966) have proposed that uncouplers exert their effect on respiration by increasing H^+ permeability. However the failure of correlation of K^+ movement with H^+ movement or respiration rate suggests that the K^+ permeability changes induced by uncoupling agents should not be considered to be coupled K^+/H^+ movements, but are a secondary phenomenon of the metabolic changes induced by uncoupler. We propose that the extent of K^+ permeability is controlled by the potential of high energy intermediate of the energy conservation process.

The ion permeability is dependent on other factors including the external phosphate and magnesium concentrations. The influence of external phosphate upon K^+ permeability is distinct from any effect of phosphate as a permeant anion since the anion movement accompanying K^+ extrusion is an outflow. Furthermore we have shown that the effect is not observed with acetate which is also a permeant anion and phosphate, when added after uncoupler, can affect the cytochrome c potential and respiration rate without concomitant ion movement.

Whereas the induction of K^+ movement is dependent on a number of factors which do not correlate with the effects of uncoupling agents on respiration and energy conservation, the inhibition of K^+ movement coincides with metabolic changes induced by uncoupler. In the presence or in the absence of phosphate or Mg^{++} ions the cessation of K^+ movement is accompanied by cessation of respiration and oxidation of cytochrome c. We propose that a possible mechanism of inhibition of K^+ movement is through inhibition of anion permeability which

prevents coupled K^+/anion extrusion and that since substrates of respiration are themselves anions they are no longer transported across the mitochondrial membrane to their site of oxidation and consequently inhibition of respiration occurs.

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