Effects of K+ on mitochondrial respiration

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The condition of isolated mitochondria is routinely assessed by measuring their respiratory control ratio (RCR) with a Clarke-type oxygen electrode (Chance & Williams, 1955). We have found that freshly prepared rat liver mitochondria cycled through respiratory States 3 and 4 (Chance & Williams, 1956) in a high-K+ medium (90 mM-KCl/10 mM-KH, PO4/75 mM-mannitol/25 mM-sucrose/10 mM-Hepes/1 mM-EDTA, pH 7.2) enter a condition which we shall term inhibited, in which the State 3 respiration rates are reduced by 20–70% (24 preparations, average 45%) (Fig. 1a). We term this condition State 3'. The RCRs and ADP/O ratios of such mitochondria are little changed, because the State 4 rates are also reduced.

We found that induction of the inhibited condition occurred during State 4 respiration on either succinate or glutamate. Inhibition developed after an initial 30 s lag phase, and reached a maximum within 5 min. The halftime for the process was 2 min. State 3' rates were found to be unaffected by the respiratory uncouplers 2,4-dinitrophenol and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP). This implied that the onset of the inhibition is not due to the ATP synthase or the adenine nucleotide translocator. Furthermore, reduction of ferricyanide (Lee et al., 1967) was unaffected, whereas oxidation of ascorbate/i-triamethyl p-phenylenediamine (Estabrook, 1961) was inhibited to the same degree as that of succinate. This indicates that the Complex IV is affected.

The inhibited condition was not attained during respiration in low-K+ (10 mM) media, where the KCl was replaced either by 90 mM-Tris/Cl or by an extra 145 mM-mannitol and 55 mM-sucrose. Respiration rates in high-K+ medium also remained uninhibited in the presence of 5 mM-Mg2+ (Fig. 1a). However, State 3' rates were unaffected either by the addition of Mg2+ or by resuspension in low-K+ medium.

State 3 respiration rates in high-K+ medium were rapidly decreased by up to 70% on addition of the K+ ionophore valinomycin (for review see Pressman, 1976) (Fig. 1b). This effect was Mg2+-independent, though competitive inhibition by Mg2+ of valinomycin action in low-K+ (10 mM) medium has been reported (Ligeti & Fonyó, 1977). The ionophore affected uncoupled respiration rates to a lesser extent, and the State 3 rates of mitochondria respiring in low-K+ medium were not reduced by valinomycin (Fig. 1b).

To explain these results, we propose a model in which State 4 respiration in high-K+ medium, in the absence of Mg2+, drives a K+ influx into the mitochondrial matrix, where K+ interacts in some way with Complex IV of the respiratory chain, inhibiting its function. The mitochondrial outer membrane is freely permeable to low molecular weight species such as K+ (O'Brien & Brierley, 1965; Pfaff et al., 1968), while the potential of the inner membrane, particularly high during State 4 respiration, should favour K+ uptake (Bernardi & Azzone, 1983). Several groups have studied K+ /H+ exchange systems of the inner mitochondrial membrane. Pertinent findings include regulation by the membrane potential (Bernardi & Azzone, 1983) and inhibition by ADP and Mg2+ (Chávez et al., 1977; Jung et al., 1977; Garlid, 1980; Nakashima et al., 1982). Brierley et al. (1984) report a 30% reduction in the State 3 respiration rate of Mg2+-depleted mitochondria, and a further 10% reduction on additional K+ -depletion. Their State 4 rates were unaffected by either treatment.

Matlib & O'Brien (1976) observed a 35% decrease in the State 3 rate of mitochondria respiring in 150 mM-K+ and 5 mM-Mg2+, compared with the rate in a sucrose medium. They considered that K+ was displacing cytochrome c from the outside of the inner membrane into the intermembrane space. Our experiments with fresh mitochondria in such a medium, however, did not confirm this reduction of the State 3 rate unless Mg2+ was omitted (data not shown). We found that 24-h-old mitochondria were much less affected by Mg2+; thus the discrepancy may arise from the mitochondria of Matlib & O'Brien (1976) being older than ours.

Abbreviations used: FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; RCR, respiratory control ratio.

Fig. 1. Typical oxygen electrode traces

Conditions were: 23°C, 3.32 ml of medium containing 6 mM-succinate, 0.3 mM-ADP, 0.75 μM-rotenone, 1.5 mg of mitochondrial protein, FCCP 0.3 μM when present. Typical traces for different conditions are shown superimposed with figures showing oxygen consumption (nmol of O2/min per mg of protein). (a) — High-K+ medium, no Mg2+; ———, high-K+ medium + 5 mM-Mg2+ or low-K+ medium; . . . . . . . . , FCCP added (either medium). (b) — High-K+ medium; ———, high-K+ medium, mitochondria uncoupled with FCCP before addition of valinomycin; . . . . . . . . low-K+ medium. Valinomycin (10 nmol/mg of protein) added to all three experiments where indicated. Respiration was initiated by adding mitochondria to the system, hence initial State 3 rates are uninhibited.
Uncoupler-induced proton permeability in mitochondria and vesicle membranes: an investigation using \(^{31}\)P and \(^{1}\)H nuclear magnetic resonance spectroscopy

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In mitochondria the synthesis of ATP via oxidative phosphorylation can be inhibited by a group of compounds known as uncouplers (Terada, 1981). These weak lipophilic acids are generally believed to act via dissipation of the proton electrochemical gradient necessary for ATP synthesis. In this communication we describe the use of n.m.r. to investigate the effect of two particular uncouplers, 2,4-dinitrophenol (DNP) and carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP) on respiring rat liver mitochondria as well as phosphatidylcholine vesicles.

The relationship between the rates of ATP synthesis and succinate utilization in partially uncoupled mitochondria can be described by the following (Wang, 1983):

\[
\frac{(H^+ /2e^-) dt}{d(Succinate)/dt} = \frac{(H^+ /ATP) dt}{d(ATP)/dt} + k(Uncoupler)
\]

\[k = \frac{d}{dt}(\text{Uncoupler})\text{ (1)}\]

'Simultaneous' n.m.r. is a convenient technique for measuring the rate of change of such metabolite levels in cellular suspensions (Oxley et al., 1984). Thus \(^{31}\)P and \(^{1}\)H n.m.r. have been used here to determine the rates of ATP synthesis and succinate utilization in dilute mitochondrial suspensions over a range of uncoupler concentrations. The mitochondria were suspended in a deuterated buffer, pH 7.2, at a total protein concentration of about 1 mg/ml. Also during experiments the sample temperature was maintained at 293 K, with oxygenation achieved by slow bubbling of air through the solution. With the rates measured and relationship given in eqn. (1) a linear plot can thus be constructed in which the gradient corresponds to \(k\), the permeability coefficient for proton transport; whilst the intercept on the y-axis provides an estimate of the \(H^+ /ATP\) stoichiometry for the \(F_o/F_1\) ATP synthase.

As a model for the mitochondrial system the effect of DNP and FCCP on the collapse of a \(\Delta pH\) of 7.2 to 6.2 induced across phosphatidylcholine vesicles was also investigated. The vesicles studied were prepared by sonicating a dispersion of phosphatidylcholine in \(1 M\)-deuterated phosphate, pH 7.2. It was thus possible to use \(^{31}\)P n.m.r. to follow the uncoupler-induced change in internal pH from the chemical shift of the internal phosphate signal (Palatini et al., 1978). In addition to the presence of uncoupler in such experiments the vesicle samples also contained excess valinomycin to prevent the generation of a membrane potential. The kinetics of \(\Delta pH\) collapse at 293 K were measured over a range of FCCP and DNP concentrations, hence allowing the determination of \(k\) for both uncouplers from plots of initial proton transport rate versus uncoupler concentration. A comparison can thus be made between vesicles and mitochondria.

In mitochondria the \(k\) values obtained for FCCP and DNP were 1.72 ± 0.17 cm/min and 0.80 ± 0.01 cm/min respectively thus giving a ratio for FCCP/DNP of 2:1.5. The comparable figures for FCCP and DNP in vesicles were 1.25 ± 0.05 cm/min and 0.54 ± 0.005 cm/min giving a ratio of 2:3.1. These values were calculated by assuming a surface area of 400 cm\(^2\)/mg of protein for mitochondria (Mitchell & Moyle, 1967) whilst for vesicles a value of 2,800 cm\(^2\)/mg of phosphatidylcholine was calculated (Nozaki & Tanford, 1981). It would appear therefore that the uncoupling activity of FCCP in relation to DNP is comparable in mitochondria and vesicles. In addition, the absolute values of \(k\) for both uncouplers are similar in the two systems. These results therefore seem to provide no evidence for a channel-forming mechanism of uncoupling by FCCP in mitochondria (Toninello & Silkprandi, 1982), but suggests that both FCCP and DNP act as simple proton shuttles (Kasianowicz et al., 1984).

The stoichiometry of the mitochondrial \(F_o/F_1\) ATP synthase with respect to the number of protons required to produce 1 ATP (\(H^+ /ATP\)) has been much studied. Values for \(H^+ /ATP\) of 2 (Mitchell & Moyle, 1973) and 3 (Sorgato et al., 1982) have been proposed depending upon experimental conditions and techniques used. In this investigation a value of 2.9 ± 0.3 was obtained with both FCCP- and DNP-induced membrane leakage in mitochondria. This was calculated by linear extrapolation of eqn. (1) to zero uncoupler concentration assuming an \(H^+ /2e^-\) of 6 for succinate (Nicholls, 1977) and also that 1 proton is required for each phosphate transported into the mitochondrion (Fonoy et al., 1975).

In the studies outlined here we have shown that a combination of \(^{31}\)P and \(^{1}\)H n.m.r. to follow changes in ATP and succinate levels in mitochondrial suspensions can provide valuable information in an unambiguous manner. Similarly the use of \(^{31}\)P n.m.r. to monitor pH changes in phosphatidylcholine vesicles gives important information from a relatively simple technique.