EFFECTS OF Na and K ON OXIDATIVE PHOSPHORYLATION IN RELATION TO RESPIRATORY CONTROL BY A CELL-MEMBRANE ATP-ASE*

BY

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The renal Na-K activated ATP-ase which is believed to be part of the mechanism for the active transport of these ions[4,7,8] acts as a pacemaker of respiration in kidney cortex.[2,9,10] Thus in homogenates, changes in the activity of the extramitochondrial ATP-ase induced by ouabain, cause parallel changes in the respiration.[2] However a difficulty arises from the fact that respiration is affected in two different ways by Na and K. Firstly, there is a stimulation due to activation of the cell-membrane ATP-ase by the combined action of the ions. This stimulation is overcome by ouabain. Secondly, when the cell-membrane ATP-ase is inhibited or absent (as in pure mitochondria) the respiration is still dependent on the Na and K concentrations, suggesting that these ions might be directly involved in mitochondrial function. As oxidative phosphorylation is a major function of mitochondria, we have investigated the mechanism of these two effects of cations by measuring the P/O ratio and the ADP concentration in homogenates incubated with either ouabain or a range of Na and K ion concentrations.

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Results and Discussion. The rate of respiration and the corresponding ADP concentration in kidney homogenates incubated with various Na and K concentrations are closely parallel at K concentrations greater than 25mM, but below 25mM K, respiration does not fall as much as the ADP concentration (Fig 1, curves a & b.) Values of the ratio of QO_2/ADP indicate that at low K concentrations, respiration is no longer a simple function of the ADP concentration (Fig.1, curve c). This is further indicated by the fact that addition of 2mM ADP to a respiring homogenate caused an increase of 150µl O_2 uptake in the presence of 100mM K, and of only 50µl O_2 in its absence.

Direct measurements of the P/0 ratio of mitochondria revealed an increase from 1.05 in the absence of K, to 1.7 - 1.8 when more than 50mM K or less than 100mM Na were present. (Fig 2, curve a).

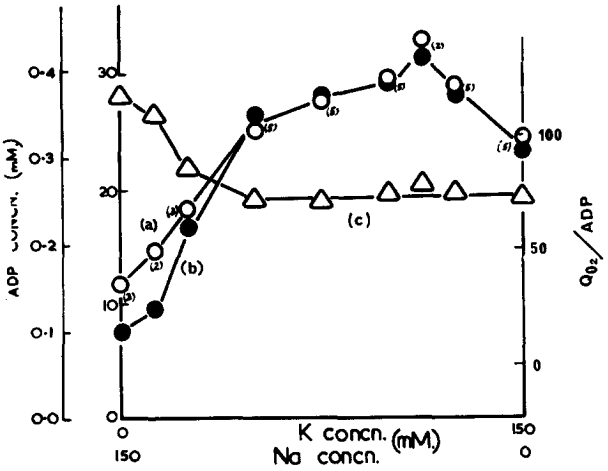

Fig.1. Respiration and ADP concentrations in kidney cortex homogenate. O_2 uptake was measured manometrically in a standard medium containing 10mM L-Malate (tris salt), 15mM iminazole-HCl at pH7.8, 2mM-$MgCl_2$, 1.25mM ATP (disodium salt), 0.125mM DPN and the amounts of NaCl and KCl as indicated. Manometer flask contents were analysed enzymically for ADP (Boehringer Test Kit). ○, QO_2(µlO_2/mg. dry wt/hr); ● , ADP concn. ; △ , ratio of QO_2/ADP concn. Points are means of values from the number of experiments indicated in parenthesis.

There is a possibility that ouabain might affect the mitochondrial ATP-ase and the efficiency of phosphorylation. The data shown in Fig.2 indicate however, that it is without effect on either of these processes,

thus strengthening the view that effects of ouabain on the respiration of homogenates and slices are indirect, and due only to inhibition of the Na-K activated ATP-ase.

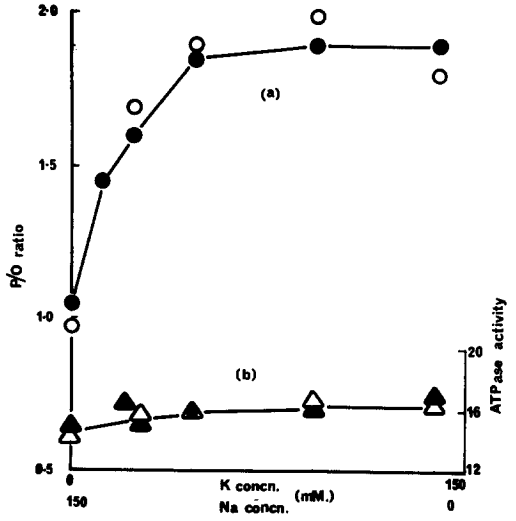

Fig.2. The effect of Na and K on the P/O ratio and ATP-ase activity of kidney mitochondria. For P/O measurements, mitochondria were incubated in the standard medium (see Fig.1.) supplemented with 20mM glucose and 20mM tris-phosphate at pH 7.8. 30 units of hexokinase (Sigma, Grade III) were added after thermal equilibration. All points are means; the number of separate determinations is indicated in parenthesis. ATP-ase activity was measured by the method employed previously,[2] and is expressed as µMoles P_i released/mg.dry wt./hr.

P/O ratio { ● control
○ with 250 µM ouabain

ATP-ase activity { ▲ control
△ with 125 µM ouabain

To distinguish whether the effect on the P/O ratio was due to stimulation by K^+ ions or to release of inhibition by Na^+ ions the latter were replaced by an osmolar equivalent of sucrose. Addition of 25mM K stimulated respiration and raised the P/O ratio by similar amounts either in the presence of sucrose or NaCl. This observation shows that the increased P/O ratio is the result of a stimulatory effect of K^+ ions.

Pressman and Lardy[6], using liver mitochondria, have previously reported a dependence of QO_2 and P/O ratio on K^+ ions, but only when

respiration was stimulated by DNP or a microsomal extract. In the present work it has not been necessary to stimulate the respiration in order to evoke the K effect. Krall et al.[5] have reported that oxidative phosphorylation in brain mitochondria is enhanced by low K concentrations.

These results suggest that a linear response of mitochondrial respiration to ADP is only to be expected with at least 50mM K when the mitochondria approach Chance's[3] state 3. Changes in the ADP concentration could arise physiologically from changes in the activity of the Na-K ATP-ase. Experimentally, the latter can be varied in two ways : by changes in the Na and K concentrations or by partial inhibition with ouabain.

The respiration and ADP concentration were compared when the homogenate was incubated with 50 - 150 mM K^+ and 0 - 100mM Na. The K concentration was not lowered below 50mM in order to maintain a constant P/O ratio. A linear relationship between ADP content and O_2 uptake was

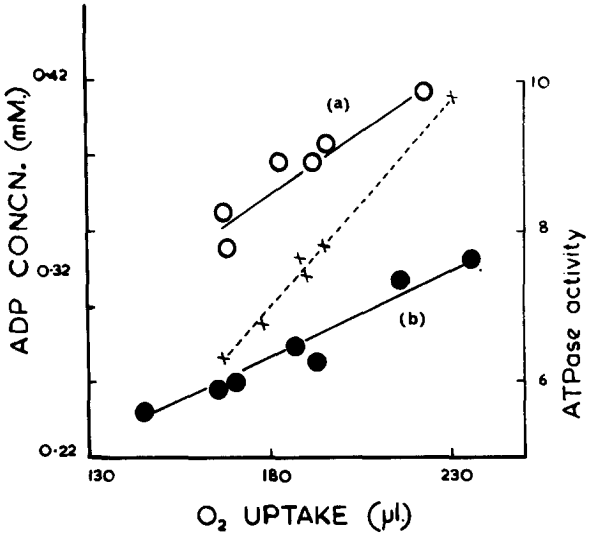

Fig.3. The dependence of O_2 uptake on ADP concentration controlled by Na-K activated ATP-ase. O, with variations in the K^+ concentration from 50-150mM and Na^+ from 0-100mM. ●, graded inhibition by submaximal concentration of ouabain, at 100mM K^+, 50mM Na^+. The dotted line shows the dependences of respiration on the Na-K activated ATP-ase activity, when this also is partially inhibited by ouabain.[2] Units as in Fig.2.

found. (Fig.3 curve a). Partial inhibition of optimum Na-K ATP-ase activity with ouabain also resulted in a similar linear relationship. (Fig. 3, curve b).

These observations are in keeping with those of Aubert, Chance and Keynes[1], who showed that spectral changes in DPNH were correlated with an increase in Na efflux from the electric eel.

These results throw light on two aspects of respiratory control by K^+ ions. Mitochondria appear to require K to promote tight coupling of phosphorylation to respiration. Secondly, in optimum conditions for oxidative phosphorylation, respiration is dependent on the concentration of ADP generated specifically by the Na-K activated ATP-ase. Thus the ADP concentration provides the link between Na-K ATP-ase activity and respiration in homogenates. Functional changes in Na-K ATP-ase activity occur in the intact tissue in which respiration would be controlled by active cation transport acting as a membrane ATP-ase regulating the ADP concentration.

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