EFFECT OF POTASSIUM AND OUABAIN ON SWELLING OF RAT LIVER MITOCHONDRIA

Eva M. Suranyi and Y. Avi-Dor

Department of Chemistry, Israel Institute of Technology, Haifa, Israel

Received February 12, 1965

Pressman and Lardy (1955) have demonstrated that maximum rates of respiration and phosphorylation in liver mitochondria could only be obtained in the presence of potassium. The potassium effect was found to be enhanced by the addition of sensitizing agents, such as microsomal particles. The above mentioned authors also showed a correlation between the effect of the sensitizers and loss of intramitochondrial potassium. These findings, and a previous observation that microsomal particles induce swelling (Avi-Dor, 1960), prompted us to investigate the effect of K^+ ions on swelling of liver mitochondria.

This report presents results which show that the time-course of swelling is greatly affected by the nature of the alkali metal ion present in the suspending medium, potassium ions causing the highest degree of stabilization. The effect of potassium depends on the presence of ATP, it is abolished by ouabain, oligomycin and 2,4-DNP, and it is most prominent under hypotonic conditions.

Materials and Methods

Rat-liver mitochondria were isolated from homogenate prepared in 0.25 M sucrose by differential centrifugation according to the method of Ernster and Löw (1955). The mitochondria were suspended in 0.25 M sucrose. 3.0 ml of the final suspension corresponded to 1.0 g of liver (wet wt.).

The composition of the incubation medium was as described in the legends to the figures. Swelling and contraction was followed by measuring changes in the extinction at the wave-length of 520 mμ (for the reliability

of the optical method see: Bartley and Enser, 1964).

ATP, ADP, AMP and ouabain were products of Sigma Chemical Company. Oligomycin was a generous gift of Dr. H. A. Lardy (Inst. for Enzyme Research, University of Wisconsin).

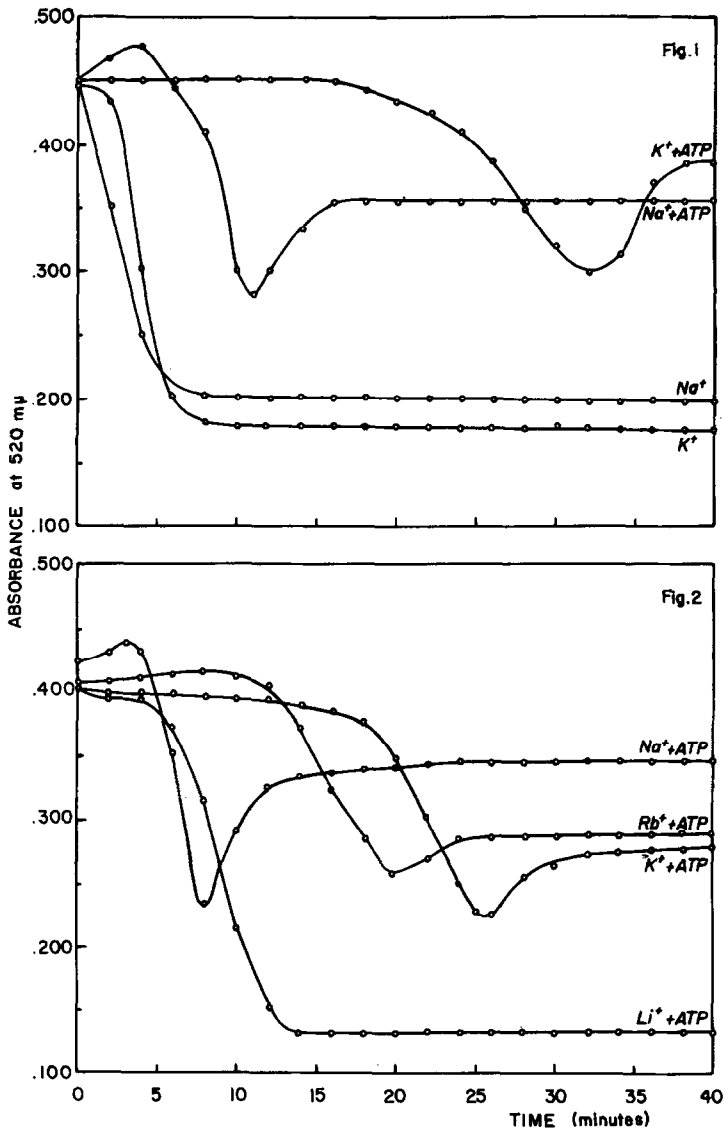
Results

In preliminary experiments, in which isotonic (0.15 M) KCl or NaCl solution was used as the assay medium, some ion specificity could be observed if the reaction mixture also contained ATP. Under these conditions the lag which preceded the onset of swelling was slightly longer in KCl compared to that in NaCl.

In the attempt to find conditions for a more clear-cut demonstration of the potassium effect, we varied systematically both the total salt concentration and the ratio between the concentration of the alkali metal salt and the tris buffer. From such experiments it was found that when the osmolarity of the suspending medium was lowered, the stability of the mitochondria became strikingly dependent both on ATP and on the nature of the alkali metal ion. For example, in the presence of ATP and at a total salt concentration of 0.09 M onset of swelling occurred after 22 min in 0.03 M KCl; in an equal concentration of NaCl swelling started after only 5 min. In the absence of ATP immediate swelling occurred independently of the nature of the alkali metal ion (Fig. 1). Rb^+ ions were found to increase the stability to nearly the same extent as K^+ ions, whereas Li^+ behaved more like Na^+ (Fig. 2).

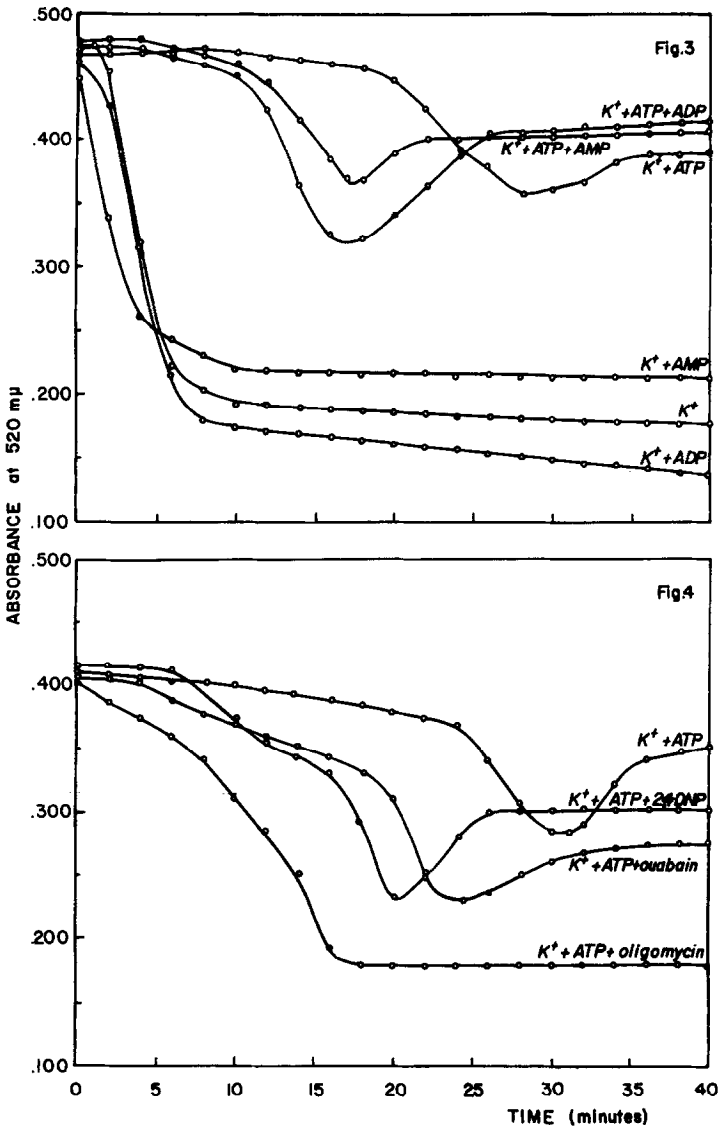
ADP or AMP did not increase the lag phase, and when added simultaneously with ATP the stability was less than with ATP alone (Fig. 3).

The apparent specificity for both K^+ ions and for the stabilization of mitochondria led us to try agents which are known to interfere with phosphorylation processes in which potassium or ATP are involved. It can be



Figures 1, 2, 3 and 4. The assay medium contained 6×10^{-2} M tris buffer, pH 7.4. When it is indicated on the respective curve the following substances were added: NaCl, KCl, RbCl, LiCl (3×10^{-2} M); ATP, ADP, AMP (10^{-3} M); 2,4-DNP (10^{-5} M); ouabain (10^{-4} M) and oligomycin (15 ug). Total volume, 3,0 ml. Temperature, 20°. The reaction was started by adding mitochondria in a quantity to give an optical density between 0.400-0.500 at the wave-length of 520 mμ in a cell of 1 cm light-path against a blank containing all the components with exception of mitochondria.

seen from Fig. 4 that the specific inhibitor of $Na^+ + K^+$-accelerated microsomal ATP-ase - ouabain (Hokin and Hokin, 1963), the uncoupler - 2,4-DNP, and the inhibitor of oxidative phosphorylation - oligomycin, all decreased the



duration of the lag preceding swelling. The lowest concentraction at which the above agents still had a significant effect was found to be 10^{-5} M for ouabain, 5.10^{-6} M for 2,4-DNP and approximately 1 μg/ml for oligomycin.

In experiments in which the incubation medium contained ATP and either Na^+ or K^+ ions, swelling was followed by a spontaneous recontraction as reflected by a gradual increase in optical density (Figs. 1, 2, 3 and 4). Recontraction also occurred in ATP-Rb^+ but not in ATP-Li^+ (Fig. 2). A

closer inspection of the curves showing such swelling-recontraction cycles also reveals that agents which increase the rate of swelling generally also accelerate the recontraction. An exception to this rule is oligomycin, which causes an acceleration of swelling, but inhibits recontraction (Fig. 4).

An ATP-dependent swelling-recontraction cycle has also been noted to occur in isotonic KCl (Kindler and Avi-Dor, unpublished results). However, in the isotonic medium the extent of reversal was smaller.

Discussion

Ample information is available in the literature on the role of potassium and ATP in the preservation of the mitochondrial structure. Amoore (1960) found that the decline in the concentration of bound, intramitochondrial potassium coincides with the onset of swelling and with an abrupt change in the permeability of the mitochondrial membrane. Swelling was also shown to be connected with the breakdown of intramitochondrial ATP (Raaflaub, 1953) and it was inhibited by external ATP (Witter and Cottone, 1956; Price et, al., 1956; Avi-Dor, 1960; Connelly and Lardy, 1964).

The findings described in this paper provide support for the contention that both potassium ions and ATP are involved in the regulation of membrane permeability. It seems likely that 2,4-DNP and oligomycin interfere with a reaction in which ATP is involved, whereas ouabain probably acts as an antagonist to potassium, as in the case of microsomal ATPase (Hokin and Hokin, 1963). However, the possibility that ATP and K^+ act at a common site cannot be excluded by the experimental data available at present.

It has been found during the present investigation that ATP, which can delay but not prevent swelling, recontracts the mitochondria after the volume has increased to a certain extent. A comparison of this spontaneous reversal of swelling with the ATP-induced recontraction of swollen

mitochondria studied by Lehninger and his group (for rev. see Lehninger, 1962) reveals great similarities: inhibition by oligomycin (Neubert and Lehninger, 1962), lack of effect of 2,4-DNP (Lehninger, 1959). It is probable that we are dealing with the same phenomenon in both cases, and that the delay in the recontraction observed in this study is caused by a permeability barrier, present in the non-swollen particles, which prevents the exogenous ATP to reach the site where it activates the contractive mechanism.

The reason for the increased ion specificity of the swelling--recontraction cycle at low osmotic pressures remains to be established. One possible explanation is the facilitation of access of extramitochondrial potassium to the site of binding.

Reference

Amoore, J. E. (1960). Biochem. J. 76, 438.
Avi-Dor, Y. (1960). Biochim. Biophys. Acta 39, 53.
Bartley, W. and Enser, M. B. (1964). Biochem. J., 93, 322.
Connelly, J. L. and Lardy, H. A. (1964). J. Biol. Chem., 239, 3065.
Ernster, L. and Löw, H. (1955). Exptl. Cell Research, Suppl. 3, 133.
Hokin, L. E. and Hokin, M. R. (1963). Ann. Rev. of Biochemistry 32, 553.
Kindler, S. and Avi-Dor, Y. (1962). Unpublished data.
Lehninger, A. L. (1959). J. Biol. Chem. 234, 2187.
Lehninger, A. L. (1962). Physiol. Rev. 42, 467.
Neubert, D. and Lehninger, A. L. (1962). Biochim. Biophys. Acta 62, 556.
Pressman, B. C. and Lardy, H. A. (1955). Biochim. Biophys. Acta 18, 482.
Price, C. A., Fonnesu, A. and Davies, R. E. (1956). Biochem. J. 64, 754.
Raaflaub, J. (1953). Helv. Physiol. Pharmacol. Acta 11, 142.
Witter, R. F. and Cottone, M. A. (1956). Biochim. Biophys. Acta 22, 364.