POTASSIUM ION STIMULATION OF OXIDATIVE PHOSPHORYLATION BY BRAIN MITOCHONDRIA

A. R. Krall, Margaret C. Wagner, and D. M. Gozansky

Departments of Biochemistry and Psychiatry
University of Miami School of Medicine
Miami 36, Florida

Received April 14, 1964

Potassium ion is a specific stimulant of the respiration of brain slices and of brain mitochondria (Hertz & Clausen, 1963; Sugawara & Utida, 1961). Recently, Utida and Sugawara (1963) have shown that the potassium ion stimulation of mitochondrial respiration is inhibited by ouabain. They conclude that the stimulation of respiration is the result of activation of a sodium-potassium activated ATPase similar to that first reported by Skou (1957) and later demonstrated in brain microsomes by Jarnefelt (1961) and Deul and McIlwain (1961).

This paper reports a specific stimulatory effect of the potassium ion on oxidative phosphorylation by rat brain mitochondrial preparations. The rates of both oxygen uptake and phosphate esterification are increased and the control of respiration by the presence of ADP is improved by the addition of optimal concentrations of potassium.

Experimental Procedure: Brain mitochondria were isolated by a slight modification of the procedure of Weinbach (1960). Usually the brain from 1 rat was homogenized in nine volumes of 0.25 M sucrose containing 10^{-4} M EDTA pH 7.4. The mitochondria were washed only once and resuspended in this solution. The same procedure was used to prepare liver or heart mitochondria. Oxy-

gen consumption was measured either by using a Clark electrode or by manometric techniques. Phosphate esterification was measured by a phosphomolybdate extraction method (Krall et al., 1961). Reagents were made sodium and potassium free by passage over a column of acid and water washed Dowex-50 and were adjusted to pH 7.4 with tris base. All water used was doubly distilled from glass. The cation concentration was the same in all experiments.

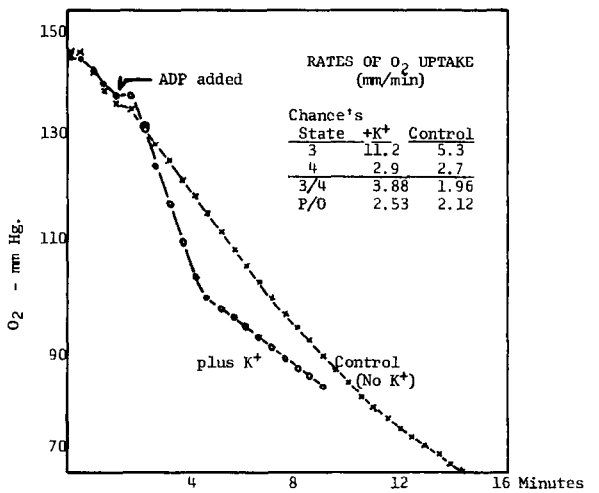

Fig. 1. Effect of potassium on oxygen uptake by rat brain mitochondria

Reaction mixtures were 0.0067M in malate, pyruvate and phosphate, about 0.07M Tris Cl pH 7.4, 0.20M sucrose, and 0.78 mg mitochondrial protein. 1 µMole ADP added, final volume 3.0 ml.

Results and Discussion: Figure 1 shows plots of the time course of oxygen uptake by brain mitochondria with and without potassium ion.

When ADP was added respiration increased about four fold (state 3, Chance & Williams, 1956) in the presence of potassium and only doubled in its absence. The rate of respiration after the ADP is all converted to ATP (state 4) is affected very

little by the presence or absence of potassium. The amount of oxygen utilized during the stimulated phase is somewhat less than without potassium, giving an apparently higher P/O ratio.

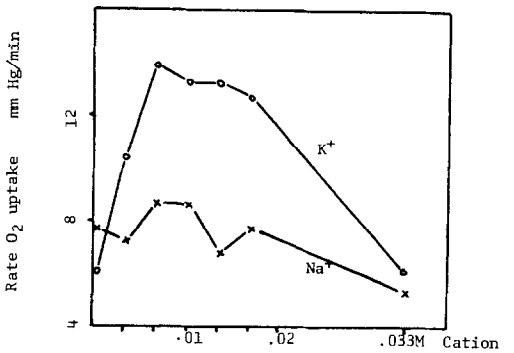

Fig. 2. Specificity of potassium for stimulation of coupled Respiration

Reaction mixture same as figure 1. Na and K added as chlorides.

Figure 2 shows that the stimulation of respiration coupled to phosphorylation i.e., stimulation of Chance's state 3 is specific for potassium. Addition of sodium gives little or no increase in rate. The stimulation is maximal at between 3 and 10 millimolar potassium. Both ions cause a drop in rate at 33 millimolar.

Table I

Effect of Addition of Potassium on Oxidative Phosphorylation.

Additions	μ atoms O	μM P_i	P/O
None	1.64	3.33	2.03
6.7mM K^+	3.23	6.96	2.16

Oxygen uptake was determined manometrically, P_i esterification as described in the text. All reaction mixtures were 0.067M in $P^{32}O_4$, malate and pyruvate and 0.033M in ADP, pH was adjusted to 7.4 with tris base. Reaction was initiated by adding phosphate and was run 20 min. at 30°C.

Table I shows the results of manometric experiments in which P/O ratios were measured directly. Addition of potassium causes an approximate doubling in rate of both oxygen and phosphate uptake. The P/O ratios are somewhat lower than those calculated from the data in Figure 1, probably because the manometric assay requires longer preincubation times and higher concentrations of reagents.

The same experiments as shown in Figure 1 and Table I have been run with mitochondria from rat heart and liver. Potassium has little or no effect on the coupled respiration in those systems.

The specific stimulatory effect of potassium on oxidative phosphorylation that is reported here could easily account for the stimulatory effect of potassium on respiratory rate of brain slices (Dickens & Greville, 1935; Hertz & Clausen, 1963). The latter authors reported from 60 to 110% stimulation of the respiration of slices from gray matter of calf brain. Their effect was specific for potassium and was not found in slices from kidney or brain white matter.

The stimulation observed here is probably the result of stimulation of the sodium-potassium activated ATPase observed first by Skou (1957). This enzyme is probably involved in transport of phosphate across the intact mitochondrial in the same way that phosphate transport accompanies magnesium transport in heart mitochondria (Brierly et al., 1962).

ACKNOWLEDGEMENT

This work was supported in part by Grants MH-5632 and RG-8510 from the National Institutes of Health and by Grant In-51 D from the American Cancer Society Florida Division.

REFERENCES

Brierley, G.P., Bachmann, E., and Green, D.E., Proc. Nat'l. Acad. Sci., U.S., 48, 1928 (1962).

Chance, B. and Williams, G.R., Advances in Enzymol., 17, 65 (1956).

Deul, D.H. and McIlwain,H.,J.Neurochem., 8, 246 (1961).

Dickens, F. and Greville, G.D., Biochem. J., 29, 1468 (1935).

Hertz, L. and Clausen, T., Biochem. J., 89, 526 (1963).

Jarnefelt, J., Biochim. et Biophys. Acta, 48, 104 (1961).

Krall, A.R., Good, N.E., and Mayne, B.C., Plant Physiol., 36, 44 (1961).

Skou, J.C., Biochim. et Biophys. Acta, 23, 394 (1957).

Sugawara, H. and Utida, S., Sci. Papers Coll. Gen. Educ., Univ. Tokyo, 11, 139 (1961).

Utida, S. and Sugawara, H., J. Biochem., 54, 553 (1963).

Weinbach, E.C., Anal. Biochem., 2, 335 (1961).