INDUCTION OF RESPIRATORY CONTROL BY K^+ IN MITOCHONDRIA

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Received June 9, 1969

Summary

The effect of K^+ on the respiratory control of rat liver mitochondria partially depleted of K^+ has been studied. Although the P/O ratios are not ostensibly affected by K^+, the State 3/State 4 respiratory ratio is considerably enhanced by the addition of the cation to the incubation mixture with NAD dependent substrates. The results are suggestive that K^+ is not required for the formation of ATP during coupled electron transport, but that the respiratory control of mitochondria depends on the K^+ content of the mitochondria.

The role of K^+ in the oxidative phosphorylation process of mitochondria and of submitochondrial particles has been a controversial subject. Although Pressman and Lardy (1955) reported higher rates of oxidative phosphorylation in mitochondria incubated with K^+ than with other cations, Opit and Charnock (1965) did not confirm their results. In submitochondrial particles, Smith and Beyer (1967) reported that K^+ up to a concentration of 30 mM did not affect oxidative phosphorylation, but Papa et al (1969) found inhibition of the process by higher concentrations of K^+. In brain and kidney mitochondria, a favorable effect of K^+ on oxidative phosphorylation has been described (Krall et al, 1964; Blond and Whittam, 1965). In this paper, the effect of K^+ on the respiratory control of mitochondria partially depleted of K^+ will be described.

Methods

Rat liver mitochondria were isolated according to Schneider and Hogeboom (1950) in 0.25 M sucrose and 0.001 M ethylene-diamine-tetra acetate (EDTA) adjusted to pH 7.3 with Tris. Settlemire et al (1968) found that the addition of EDTA to mitochondria incubated with Na^+ produced extrusion of K^+ from the

mitochondria. Accordingly, the mitochondrial pellet from approximately 7 g of rat liver was suspended in 5 ml of sucrose-EDTA and added to 50 ml of a mixture which contained 0.1 M NaCl, 0.01 M glutamate, 0.01 M H_3PO_4 (adjusted to pH 7.3 with Tris), 0.02 M Tris-HCl (pH 7.3), and 0.1 M sucrose. The mixture was vigorously agitated for three minutes at room temperature and then diluted to approximately 300 ml with sucrose-EDTA and centrifuged for 10 minutes at 12,000 x g. The pellet was washed with 150 ml of sucrose-EDTA and centrifuged again. The pellet was suspended for subsequent assays with 1.0 ml of sucrose-EDTA. Respiration was measured polarographically (Yellow Springs Instrument Co.). K^+ was measured in mitochondrial perchloric acid extracts with a Zeiss PF 5 Flame Photometer.

Results

In confirmation of the data of Settlemire et al (1968), it was found that mitochondria incubated in the conditions described above had a low K^+ content, approximately 20 μeq per gram of protein which contrasts with the K^+ content of mitochondria in which the preincubation was not made (70 μeq per gram of protein).

The State 3/State 4 respiratory ratio of mitochondria treated with Na^+ and EDTA was significantly enhanced by added KCl up to a point at which further additions of KCl resulted in inhibition of respiration (Figure 1). Similar results were obtained with α-ketoglutarate, malate-pyruvate, and citrate-malate as substrates. On the other hand, K^+ failed to increase the respiratory control of mitochondria oxidizing succinate.

The inhibition of respiration produced by high concentrations of KCl is probably due to an increase in the tonicity of the media, since increases in sucrose concentration produced also inhibition of respiration. Figure 2 illustrates the Lineweaver-Burke plot for K^+ in the region where activation of State

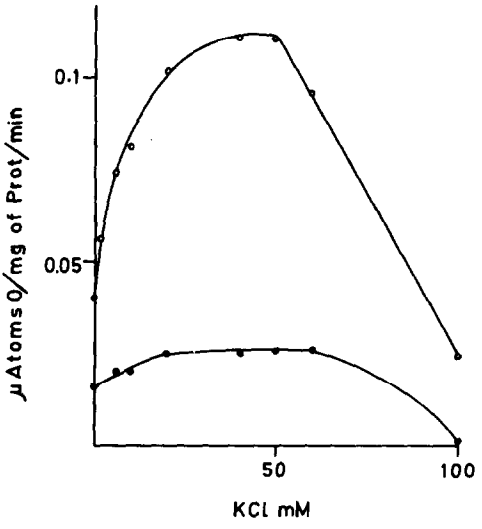

Figure 1. Effect of KCl on the Oxygen Uptake of Mitochondria. The respiration of mitochondria treated as described under Methods was measured with 0.005 M H_3PO_4 (adjusted to pH 7.3 with Tris), 0.005 M glutamate, 0.001 M EDTA, 0.04 M sucrose, and the indicated concentration of KCl in a final volume of 5.0 ml. Temperature 25°. The respiratory rate before (closed circles) and after (open circles) the addition of 1 X 10^{-4} M 2,4-dinitrophenol is plotted.

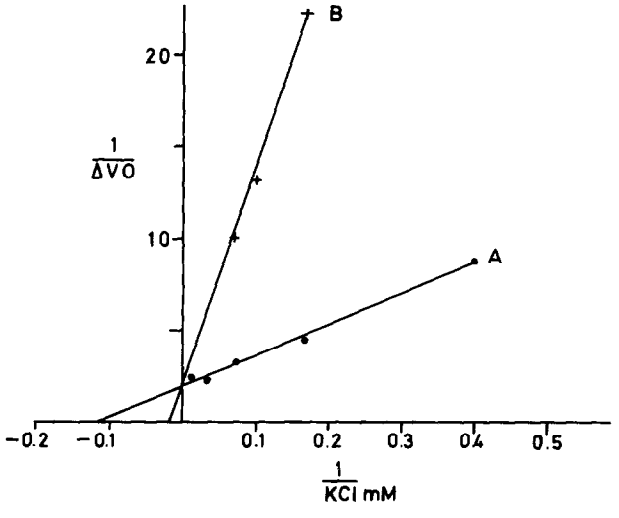

Figure 2. Effect of Tonicity on the Lineweaver-Burke Plot for K^+ on Mitochondrial Respiration. In A, the experimental conditions were as in Figure 1. In B, the concentration of sucrose was 0.14M. The reciprocal of the increase of the 2,4-dinitrophenol stimulated respiration per minute by 6.7 mg of mitochondrial protein produced by K^+ is plotted.

3 respiration by K^+ is attained. It may also be observed that the inhibition of respiration produced by the increase in the tonicity of the media is of the competitive type.

As will be described elsewhere, mitochondria incubated with Na^+ and EDTA have a high rate of oxygen uptake and loss of respiratory control. In the experiment shown in Table I, mitochondria previously treated with Na^+ and EDTA were incubated with various concentrations of NaCl;it may be observed that as the concentration of NaCl is increased, the higher the rate of State 4 respiration and the lower the response to ADP become. The addition of KCl to actively respiring mitochondria induced a lower rate of respiration and at this stage, the addition of ADP (or dinitrophenol) induced the well studied release of oxygen uptake. In these particular experiments, the State 3/State 4 ratio

Table I

Induction of Respiratory Control by K^+ in Mitochondria

		Respiratory rate μatoms O/mg/min		
NaCl	KCl	Before ADP	After ADP	State 3/State 4
6	-	0.026	0.051	2.0
6	40	0.026	0.087	3.3
14	-	0.038	0.066	1.7
14	40	0.026	0.079	3.0
40	-	0.055	0.075	1.4
40	40	0.034	0.060	1.8
60	-	0.070	0.070	1.0
60	40	0.030	0.043	1.4

The oxygen uptake of mitochondria treated as described under Methods was measured as in Figure 1, except that the mixture contained the indicated concentration of mM NaCl. To obtain the State 3 respiratory rate 2.0 micromoles of ADP were added. To mitochondria respiring with the indicated concentration of NaCl, 40 mM KCl was added at approximately 70% oxygen saturation in the medium which resulted in the indicated respiratory rate; the subsequent addition of 2.0 micromoles of ADP resulted in the indicated State 3 respiratory rate.

Table II

Dependency of Respiratory Control on the K^+ Content of Mitochondria

Exp.	Preincubation Conditions	μeq of K^+/gm of protein	μatoms O/mg of Protein/minute		State 3/State 4
			Before ADP	After ADP	
1.	Complete	21.1	0.019	0.040	2.1
	" -EDTA	81.4	0.020	0.061	3.0
	" + $MgCl_2$	84.6	0.019	0.069	3.6
	" -Glutamate	32.2	0.020	0.042	2.1
2.	Complete	15.8	0.018	0.029	1.6
	" - Na^+	69.3	0.020	0.052	2.6
	" -Glutamate	67.5	0.019	0.044	2.3

Mitochondria were treated as described in Methods except that the volume of the preincubation mixture and the subsequent washings were made with half the volume described. From the final suspension, aliquots were taken to measure oxygen uptake without added K^+ as indicated in Figure 1. State 3 respiration was released by the addition of 2.0 micromoles of ADP. Another aliquot was mixed with an equal volume of 20% perchloric acid and K^+ was determined in the supernatant after suitable dilutions. From the complete preincubation media described under Methods, the indicated substances were omitted; where indicated 0.008 M $MgCl_2$ was added.

was enhanced by K^+ by lowering the State 4 rate of respiration.

It has been possible to control roughly the content of K^+ in the mitochondria by varying the composition of the preincubation mixture (Table II). The omission of glutamate from the preincubation media resulted in mitochondria with a variable concentration of K^+. This is probably due to the different content of endogenous substrates in various mitochondrial preparations. Nevertheless, it may be observed that the State 3/State 4 respiratory ratio of mitochondria depends on the intramitochondrial concentration of K^+.

Discussion

The results of this paper indicate that K^+ enhances the respiratory control of rat liver mitochondria provided that the K^+ content in the mitochondria is

kept at a low level. This effect of K^+ may be due to some extent to an increase in the entrance or oxidation of substrates (Lynn and Brown, 1966; Graven et al, 1966; Harris et al, 1967). However, in mitochondria that have a high rate of respiration in the absence of agents that stimulate oxygen uptake , K^+ induces a significant increase in the State 3/State 4 ratio by lowering the rate at State 4.

Papa et al (1969) reported that K^+ inhibited oxidative phosphorylation in submitochondrial particles, however, this inhibiting effect was also observed with other cations. In our experimental conditions, the P/O ratio measured polarographically (Estabrook, 1967) was not ostensibly affected by K^+. Furthermore, oxidative phosphorylation has been observed in submitochondrial particles which are virtually devoid of K^+ (Lee and Ernster, 1968). These latter observations seem to indicate that K^+ is not required for the formation of ATP during coupled electron transport, but, the presently described results suggest that K^+ is required for appropiate respiratory control in mitochondria.

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