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The action of cations and anions on the respiration and ATPase activity of submitochondrial particles

Recently the effect that salts have on the oxidative phosphorylation process of submitochondrial particles has been studied in several laboratories. Although SMITH AND BEYER[1] reported that K^+ did not affect the process, the data of PAPA *et al.*[2] indicate that salts at higher concentrations inhibit the phosphorylation reaction. On the other hand, CHRISTIANSEN *et al.*[3], using low concentrations of salts in their incubation media, found that different salts diminish the P:O ratios of submitochondrial particles either by increasing the respiratory rate or by decreasing the phosphorylation rate or both. As these results are in apparent contradiction with those obtained in our laboratory in which K^+ was found to increase the State 3/State 4 ratio of K^+-depleted mitochondria[4], the effect that various salts have on the respiration and ATPase activity of submitochondrial particles was examined.

Bovine heart mitochondria were prepared according to the method of LÖW AND VALLIN[5] and were stored for at least 24 h in 0.25 M sucrose and 1.0 mM EDTA (pH 7.3) at —4°. EDTA sonic particles were prepared according to the method of LEE *et al.*[6]. Respiration was measured polarographically and ATPase was measured as indicated in Fig. 1. Inorganic phosphate was determined in the 6% trichloroacetic acid supernatant by the method of SUMNER[7].

In submitochondrial particles oxidizing NADH, the tested salts, KCl, KNO_3, potassium formate and potassium acetate, stimulated O_2 uptake to a similar extent (Fig. 1). On the other hand, the salts tested inhibited ATPase to a different degree;

the nitrate salts inhibited more effectively than the chloride salts and formate salts, while the acetate salts had a very small effect on ATPase (Fig. 1).

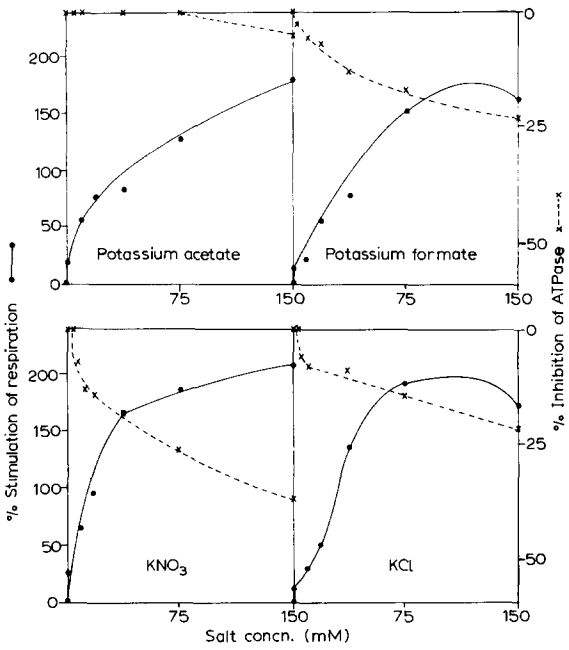

Fig. 1. Effect of various potassium salts on the ATPase activity (×---×) and O_2 uptake (●—●) of submitochondrial particles. In a final volume of 1.5 ml the following components were added for the assay of ATPase activity: 0.33 μmole $MgSO_4$; 0.27 μmole Tris–HCl (pH 7.4); 5.4 μmoles ATP and the indicated concentrations of the respective salts. Incubation time, 15 min; temperature, 25°. Respiration, in a final volume of 3.0 ml the following components were added: 0.13 μmole Tris–HCl (pH 7.4), 1.91 μmoles NADH and the indicated concentrations of the respective salts. The results are expressed either as percentual stimulation of respiration or percentual inhibition of ATPase activity.

The same type of results were obtained with the nitrate, chloride, formate and acetate salts of sodium and Tris (results not shown). Apparently, the effect of salts on respiration is unspecific while the effect of salts on ATPase is highly dependent on the anion. It is logical to conclude, if one takes into consideration that K^+ enhances the State 3/State 4 respiratory ratios of mitochondria[4], that cations are responsible for the stimulation of respiration and that anions are involved in the inhibition of ATPase activity, since the latter depends on the anion included. In this respect, Fig. 1 shows that considerable stimulation of O_2 uptake takes place at concentrations of salts in which an effect on ATPase is hardly noticeable. Furthermore, the order by which anions inhibit ATPase activity corresponds very closely to the order reported by CHRISTIANSEN *et al.*[3] by which anions inhibit the phosphorylation of ADP also in submitochondrial particles.

Fig. 2A shows a Lineweaver–Burk plot for the effect of KNO_3 on the O_2 uptake of submitochondrial particles and Fig. 2B illustrates that the inhibiting effect of KNO_3 on ATPase activity is of the uncompetitive type with ATP. These results confirm the aforementioned suggestion that salts have two independent effects on submito-

chondrial particles: a stimulatory effect on respiration which would be due to the cation and an inhibiting effect on ATPase due to the anion.

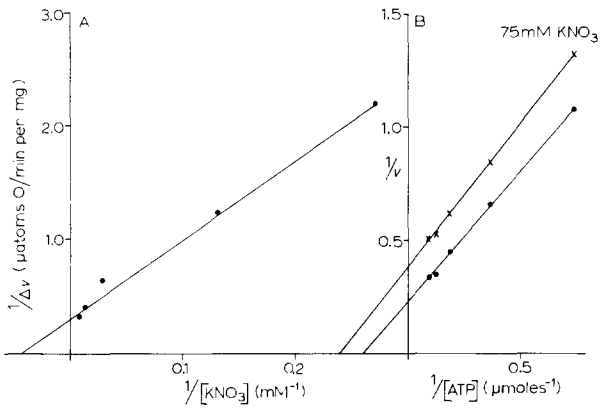

Fig. 2. Lineweaver–Burk plots for KNO_3 on the respiration of submitochondrial particles and for the effect of KNO_3 on the ATPase activity. The experimental conditions were as in Fig. 1, except that the indicated concentrations of KNO_3 and ATP were included. The time of incubation in the ATPase experiment was 3 min.

The data of CHRISTIANSEN *et al.*[3] also show that salts inhibit the succinate-associated phosphorylation; thus anions, NO_3^- being the most effective, may be considered as inhibitors of the three phosphorylating sites in submitochondrial particles.

On the other hand, K^+ (ref. 4), as well as other cations[8], independent of their action on substrate transport[9], increase the State 3/State 4 ratio of K^+-depleted mitochondria at the expense of an increase in the State 3 rate only with NAD-dependent substrates. In submitochondrial particles, the stimulatory effect of cations is also limited to NADH; no such effect has been observed with succinate as substrate. Apparently, cations exert their action at Site I of oxidative phosphorylation, most probably by increasing the rate of the energized state.

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