EFFECT OF ADP ON THE INHIBITION OF OXIDATIVE PHOSPHORYLATION BY POTASSIUM ATRACTYLATE

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Potassium atractylate has been shown to be a potent inhibitor of oxidative phosphorylation in whole mitochondria and submitochondrial particles (Bruni, Contessa, Luciani 1962; Vignais, Vignais and Stanislas 1962). Inhibition of oxidative phosphorylation by atractylate in whole mitochondria may be partially removed by high concentration of ADP (Bruni, Contessa, Luciani 1962) a conclusion which is in agreement with the fact that ATP synthesis coupled to respiration in submitochondrial particles and in the presence of large amounts of ADP (7.10^{-3}M) is less inhibited by atractylate than partial reactions bringing ATP into play such as ATP-P_i exchange, or ATP hydrolysis (Vignais, Vignais and Stanislas 1962).

Data reported in this paper concern the effect of various concentrations of ADP on ATP synthesis coupled to β-hydroxybutyrate oxidation and on reoxidation of respiratory carriers in rat liver mitochondria pretreated by atractylate.

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EXPERIMENTAL METHODS

Rat liver mitochondria were prepared in 0.25 M sucrose according to Hogeboom (1955). Experiments on oxidative phosphorylation with β-hydroxybutyrate as substrate were carried out as described previously (Vignais and Viganis 1960). Acetoacetate formed during the oxidation of β-hydroxybutyrate was estimated by the method of Walker (1954). Cycles of reduction and oxidation of respiratory carriers upon addition of succinate and ADP respectively were measured with a double beam spectrophotometer (Chance 1954) with an attached recording fluorometer (Chance, Conrad and Legallais 1958). Reduction of NAD was estimated fluorometrically at 450 mμ with 365 mμ excitation light. Cytochrome b was measured in terms of the difference in absorbancies at 430 mμ and 410 mμ. Oxygen uptake was recorded polarographically by means of the vibrating platinum electrode. Potassium atractylate was isolated from the rhizome of Atractylis Gummifera and crystallized according to Angelico (1910).

RESULTS

Table I shows the results of a typical experiment of oxidative phosphorylation where glucose and hexokinase were added to trap the ATP formed and to keep constant the concentration of ADP. With 5.10^{-4} M ADP, atractylate at 4 μg/ml inhibited 70 per cent of the phosphate uptake coupled to β-hydroxybutyrate oxidation. In agreement with preceding results (Viganis, Vignais and Stanislas 1962) it is seen that the inhibition of ATP synthesis by atractylate is accompanied by a similar inhibition of acetoacetate formation without any uncoupling effect. Increasing the concentration of ADP resulted in a striking decrease of the inhibition of both respiration and phosphorylation caused by atractylate. A complete release of inhibition was obtained when the concentration of ADP reached a value of 2.10^{-3} M. The effect of ADP

could not be duplicated by orthophosphate. Furthermore, the action of oligomycin on the coupling mechanism, although partially similar to that of atractylate, was not altered by ADP (unpublished results).

TABLE I

EFFECTS OF VARIOUS AMOUNTS OF ADP ON THE OXIDATION OF β-HYDROXYBUTYRATE AND THE COUPLED PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA TREATED OR NOT WITH ATRACTYLATE.

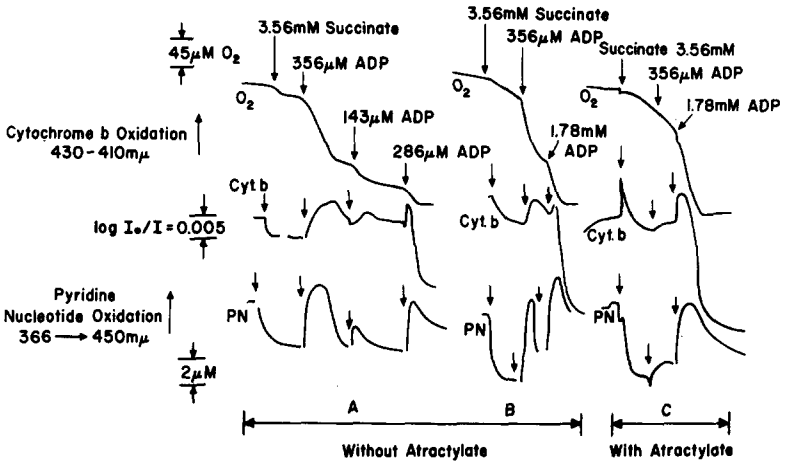
ADP final conc.	Atractylate µg/ml	Phosphate uptake mµmoles	Acetoacetate mµmoles	P/O
5.10^{-4} M	0	1180	504	2.34
5.10^{-4} M	4	370	153	2.42
1.10^{-3} M	0	1250	455	2.75
1.10^{-3} M	4	560	210	2.67
2.10^{-3} M	0	1130	430	2.63
2.10^{-3} M	4	1140	433	2.63

The incubation medium contained 0.013 M β-hydroxybutyrate, 0.070 M KCl, 0.013 M Tris pH 7.3, 0.010 M K phosphate pH 7.3 labeled with ^{32}P (500,000 cpm), 0.002 M $MgCl_2$, 0.02 M glucose, 150 K. M. units hexokinase, ADP and atractylate in 10 µl of 60 per cent ethanol as indicated in the Table. The reaction was started by the addition of mitochondria (0.9 mg of protein) in 0.1 ml of 0.25 M sucrose. Final volume 1.5 ml. Incubation for 6 min at 30°.

Another experiment (Fig. 1) was carried out to check if, as it could be predicted, respiratory carriers reduced by a substrate would be reoxidized by large amounts of ADP in the

FIGURE 1

COMBINED POLAROGRAPHIC, SPECTROSCOPIC AND FLUOROMETRIC RECORDINGS ILLUSTRATING THE EFFECT OF CALIBRATED AMOUNTS OF ADP ON RESPIRATION, CYTOCHROME b AND PYRIDINE NUCLEOTIDES OXIDATION IN RAT LIVER MITOCHONDRIA TREATED OR NOT WITH ATRACTYLATE.



Mitochondria (corresponding to 1/20 of a rat liver) in 0.2 ml of 0.25 M sucrose were added to 1.2 ml of a mixture of 0.08 M KCl, 0.02 M Tris and 0.05 M phosphate; pH 7.8 - temperature 25°. When indicated, 5 μl of atractylate in 60 per cent ethanol was added prior to the addition of succinate. O_2 consumption was measured by the vibrating platinum electrode, cytochrome b by the double beam spectrophotometer, and pyridine nucleotide fluorometrically, (optical path: 5 mm). The data recorded on separate charts, were superimposed. The pyridine nucleotide oxidation was calculated from independent fluorometric calibrations using free NADH.

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presence of atractylate. For this purpose, succinate was chosen as a substrate because it is capable of reducing DPN to a greater extent than any other DPN-linked substrate (Chance 1956) (Klingenberg and Slenczka 1959). As shown in Fig. 1 (A and B, control

experiment) addition of various amounts of ADP to mitochondria preincubated with succinate resulted in an immediate increase of the rate of respiration and in cyclic oxidation-reduction responses of pyridine nucleotides (PN) and cytochrome b, the amplitude of which was roughly maximal at a concentration of ADP of 356 μM. In contrast, in the presence of 3.5 μg/ml of atractylate (Fig. 1C), 356 μM ADP, there is only a slight reoxidation of PN and cytochrome b (5 times less than in the control experiment). However, with 1.78 mM ADP, the inhibition due to atractylate was totally overcome and the extent of reoxidation of both PN and cytochrome b reached the maximum value observed in the control experiment (Fig. 1B); this effect of ADP was accompanied by a nearly complete restoration of the respiratory control (respiratory control ratio $\simeq$ 4).

On the middle traces on which are recorded the responses of cytochrome b, it is seen that the upward deflection of the traces are larger for the higher concentrations of ADP. If, however, ADP is added when the oxygen concentration is rather low, as occurs in the last addition of each of the first two experiments, the full value for the oxidation of cytochrome b is not obtained. For example, 1.8 mM ADP added in the absence of atractylate does not give as large an effect as it does in the presence of atractylate. This is because of the low oxygen concentration at the time of the addition of ADP.

DISCUSSION

The conclusions drawn from experiments on ATP synthesis are extended and supported by the spectrophotometric data which indicate that the inhibition of energy transfer by atractylate is reversed by ADP not only at the cytochrome b site but also at the DPNH site involved in the energy-linked reduction reaction. It is obvious that, even in the presence of a large concentration of atractylate capable of inhibiting nearly completely both

respiration and phosphorylation, the coupling potentiality of mitochondria is kept intact. This property, as well as the releasing action of ADP on the inhibition of ATP synthesis caused by atractylate, strongly suggests that atractylate combines reversibly with a phosphorylated intermediate involved in oxidative phosphorylation.

We find a parallel between the activation of respiration by high concentrations of ADP in the atractylate-inhibited systems and the delayed activation of respiration in the guanidine-inhibited systems (Chance and Hollunger 1963). In the latter experiments, it was observed that maximal rates of phosphorylation and respiration were delayed for about 20 seconds following the addition of ADP. Also, the respiratory carriers clearly showed this delayed response, particularly cytochrome a and cytochrome b. Apparently the guanidine intermediate dissociates slowly whereas the inhibitory compound formed with atractylate dissociates rapidly. However, it is likely that guanidine and atractylate combine reversibly with a phosphorylated intermediate in both cases.

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