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The inhibition of succinate oxidation in isolated mitochondria by uncouplers

It has often been reported that uncouplers of oxidative phosphorylation cause an inhibition of the oxidation of succinate by mitochondria[1–5], which can be abolished by preventing oxaloacetate formation with Amytal[1,2] or rotenone[4,5], or by removing oxaloacetate with glutamate[4–6]. Furthermore, it has been shown directly that oxaloacetate accumulates in the inhibited phase of succinate oxidation[4,5]. It is, therefore, conceivable that in the presence of uncouplers inhibition of succinate dehydrogenase by oxaloacetate occurs[7]. The inhibition of succinate oxidation by uncouplers can also be abolished by ATP; however the mechanism of this effect of ATP is still a matter of debate[2,3–5,7,8].

Recently it has been observed that at low concentrations of succinate, uncouplers cause inhibition of respiration also in the presence of rotenone[9]. The inhibition has been demonstrated to be competitive with respect to succinate[10–12]. It has also been shown that mitochondria accumulate succinate from the suspending medium and that this process is inhibited by uncouplers[13,14]. On the basis of these and related findings it has been proposed that the inhibition of succinate oxidation by uncouplers in the presence of rotenone is due to inhibition of an energy-dependent uptake of succinate[8,13,14]. Furthermore, it has been suggested that depression of succinate uptake, rather than oxaloacetate inhibition, also explains the inhibition in the absence of rotenone[8].

In this paper the inhibition of succinate oxidation by dicoumarol, either in the presence or in the absence of rotenone, has been studied. It is shown that the actual mechanism of inhibition is different in the two cases.

Fig. 1 illustrates the effect of dicoumarol on the oxidation of 10 mM succinate by rabbit-kidney mitochondria. Respiration was recorded with a Clark O_2 electrode. Preincubation of mitochondria with dicoumarol caused a marked inhibition of succinate oxidation (traces a and b). The inhibition could be largely prevented by oligomycin (trace c). Since oligomycin inhibits the ATPase induced by uncouplers, its effect suggests that the inhibition of respiration was dependent upon hydrolysis of endogenous ATP. In fact the addition of ATP in the presence (trace d) or absence of oligomycin (not shown) completely abolished the inhibition of respiration. Direct analysis[4] showed that 5–10 nmoles oxaloacetate accumulated in the mitochondrial

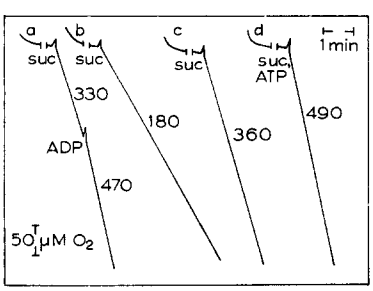

Fig. 1. Effect of dicoumarol on succinate oxidation by rabbit-kidney mitochondria in the absence of rotenone. Mitochondria (3.9 mg protein) were suspended in 1.5 ml of a medium containing 130 mM KCl, 20 mM Tris–HCl, 10 mM potassium phosphate buffer (pH 7.4) and 1 mM arsenite (arsenite was added to abolish substrate-level phosphorylation). Before adding succinate, mitochondria were preincubated in the medium with the following additions: Expt. a, no additions; Expt. b, 20 μM dicoumarol; Expts. c and d, dicoumarol and 15 μg oligomycin. Final concentration of additions: 10 mM succinate (suc), 2 mM ADP, 2 mM ATP. Final pH, 7.2. Temperature 25°. The values on the traces are respiratory rates expressed as mμgatoms oxygen per min. The break in each curve (in this and the following figure) represents a time interval of 3 min.

suspension after preincubation with dicoumarol. Some more oxaloacetate was formed during the inhibited phase of succinate oxidation.

In the experiments of Fig. 2A the effect of dicoumarol on the oxidation of succinate in the presence of rotenone was studied. The preincubation of mitochondria with dicoumarol caused a slight stimulation of the oxidation of 10 mM succinate (traces a and c). On the other hand, at 1 mM succinate, dicoumarol caused a marked inhibition of respiration (traces b and d). In contrast to what was observed in the absence of rotenone, the addition of ATP had no effect on the inhibited respiration. The inhibition could, however, be released by the addition of serum albumin (trace e), which binds dicoumarol and restores the coupled state[11,15]. Traces f and g show that the addition of dicoumarol in the presence of oligomycin caused a slight inhibition of respiration, which again was abolished by albumin (trace h).

Traces a, b and c of Fig. 2B show that the inhibition of oxidation of 10 mM succinate caused by dicoumarol in the absence of rotenone was almost completely released by albumin. In the experiments illustrated by traces e and f of Fig. 2B, mitochondria were preincubated 4 min with dicoumarol, then oligomycin and rotenone were added followed by succinate or succinate *plus* albumin. Under these conditions

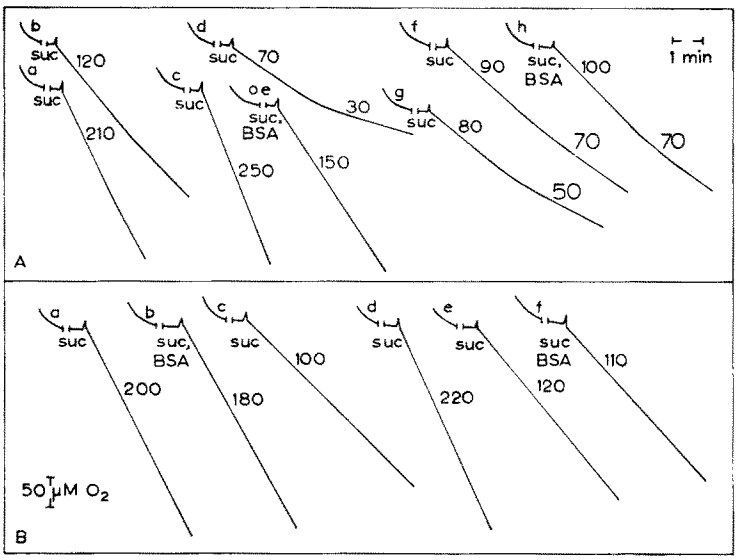

Fig. 2. A. Effect of dicoumarol on succinate oxidation by rabbit-kidney mitochondria in the presence of rotenone. Mitochondria (2 mg protein) were suspended in 1.5 ml of a medium containing 250 mM sucrose, 20 mM Tris–HCl, 5 mM KCl, 10 mM potassium phosphate buffer and 1 μg rotenone. Before adding succinate, mitochondria were preincubated in the medium with the following additions: Expts. a and b, 2 mM ADP; Expts. c–e, 2 mM ADP and 20 μM dicoumarol; Expt. f, 15 μg oligomycin and 1 mM arsenite; Expts. g and h, dicoumarol, arsenite and oligomycin. In Expts. a and c, 10 mM succinate was added and in the other experiments, 1 mM succinate. The amount of bovine serum albumin (BSA) added was 8 mg. Final pH, 7.2. B. Effect of albumin on the inhibition by the dicoumarol of succinate oxidation in rabbit-kidney mitochondria. Mitochondria (2.4 mg protein Expts. a–c and 1.7 mg Expts. d–f) were preincubated in the medium of Fig. 1 with the following additions: Expts. a and d, no additions; Expts. b, c, e and f, 20 μM dicoumarol. In the Expts. d–f, after 4 min preincubation 15 μg oligomycin and 1 μg rotenone were added, followed 30 sec later by succinate. The concentration of succinate was 10 mM, the amount of albumin 8 mg. Control experiments showed that albumin had no significant effect when added in the absence of dicoumarol.

rotenone and oligomycin did not abolish the inhibition of succinate oxidation by dicoumarol (compare traces d and e), but they did prevent completely the restoration of O_2 uptake by albumin (trace f). This indicates that the removal of the inhibition by albumin observed in trace b was not due to removal of dicoumarol *per se* but depended upon resynthesis of ATP and/or energy-dependent succinate-linked NAD reduction[16].

These results give consistent evidence that the inhibition of succinate oxidation by dicoumarol in the absence of rotenone and at a succinate concentration of 10 mM is due to inhibition of succinate dehydrogenase by oxaloacetate. The release of the inhibition by ATP, which is insensitive to dicoumarol *plus* oligomycin, can hardly be ascribed to restoration of succinate uptake[8]. The uptake of succinate by mitochondria has been correlated with energy-linked uptake of cations[8,13,14]. ATP-driven cation uptake is inhibited both by uncouplers and oligomycin. It has been found that oligomycin inhibits the ATP-dependent uptake of succinate[13]. The inhibition of succinate oxidation by dicoumarol in the presence of rotenone can be due to inhibition of the energy-dependent uptake of succinate. In this case the inhibition, which occurs only at low concentration of succinate, was not reversed by ATP, but it was released by removing dicoumarol with albumin either in the presence or absence of oligomycin. However, the possibility that dicoumarol competes with succinate for its binding site on succinate dehydrogenase[10] cannot be dismissed on the basis of the data available. Our results support the proposal that ATP, as such or after transphosphorylation with other nucleoside diphosphates, activates succinate oxidation by preventing inhibition of succinate dehydrogenase by oxaloacetate[7,3]. We have presented evidence that ATP can act by supporting reactions which remove oxaloacetate[4]. On the other hand, it has been proposed by AKERBLOM *et al.*[17] that ATP promotes reactions which regulate the sensitivity of succinate dehydrogenase towards oxaloacetate.

S. PAPA
N. E. LOFRUMENTO
G. PARADIES
E. QUAGLIARIELLO

Department of Biochemistry,
University of Bari,
Bari (Italy)

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