THE EFFECT OF 2-PHENYLISATOGEN ON OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

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1. Introduction

Oligomycin A, *N,N'*-dicyclohexylcarbodiimide (DCCD) and aurovertin are examples of compounds that have been shown to inhibit ATP synthesis in mitochondria [1, 2]. These compounds have proved to be useful in the elucidation of the mechanism of reactions associated with electron transport and oxidative phosphorylation such as the ATP-dependent reduction of NAD^+ by succinate and the ATP-dependent reduction of $NADP^+$ by NADH [3].

We have examined the effect of the novel heterocyclic compound 2-phenylisatogen (fig. 1) on oxidative phosphorylation in rat liver mitochondria. The reactions studied were the ADP-stimulated oxidation of succinate and the uncoupler-stimulated ATPase. ADP-stimulated succinate oxidation was inhibited to a maximum of 100% by low concentrations of the isatogen and the inhibition was reversed by uncoupling agents such as 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TTFB) and 2,4-dinitrophenol (DNP). The uncoupler-stimulated ATPase was inhibited to a maximum of 60% by the same concentrations of isatogen that were required for complete inhibition of the forward reaction.

2. Methods

Tightly coupled rat liver mitochondria were prepared by the method of Chappell and Hansford [4] and suspended in 0.25 M sucrose and 3.4 mM tris-HCl, pH 7.3, to give a protein concentration of 40 mg/ml. Oxygen consumption was measured polarographically using a Clark-type oxygen electrode; the conditions were as shown in the legends to the relevant figures. ATPase activity was determined by measuring the inorganic phosphate released from ATP in 0.5 ml aliquots of the reaction medium [5]. The concentration of protein was measured by the method of Gornall, Bardawill and David [6] after solubilisation of the mitochondria with deoxycholate (final concentration 0.16% w/v); bovine serum albumin was used as standard.

2-Phenylisatogen was prepared by the method of Bond and Hooper [7] and was added to reaction medium as its ethanolic solution. Appropriate controls carried out with equivalent amounts of ethanol had no effect on the reactions under consideration.

3. Results

Fig. 2b shows that ADP-stimulated succinate oxidation in tightly coupled rat liver mitochondria was completely inhibited by 12.5 nmole 2-phenylisatogen/mg mitochondrial protein and the inhibition was reversed in the presence of 1.3×10^{-7} M TTFB. Addition of the isatogen after ADP shows that the onset of inhibition was almost immediate (fig. 2c). Prolonged preincubation of the mitochondria with 2-phenylisatogen did not have any effect on the extent

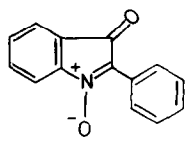

Fig. 1. Structure of 2-phenylisatogen.

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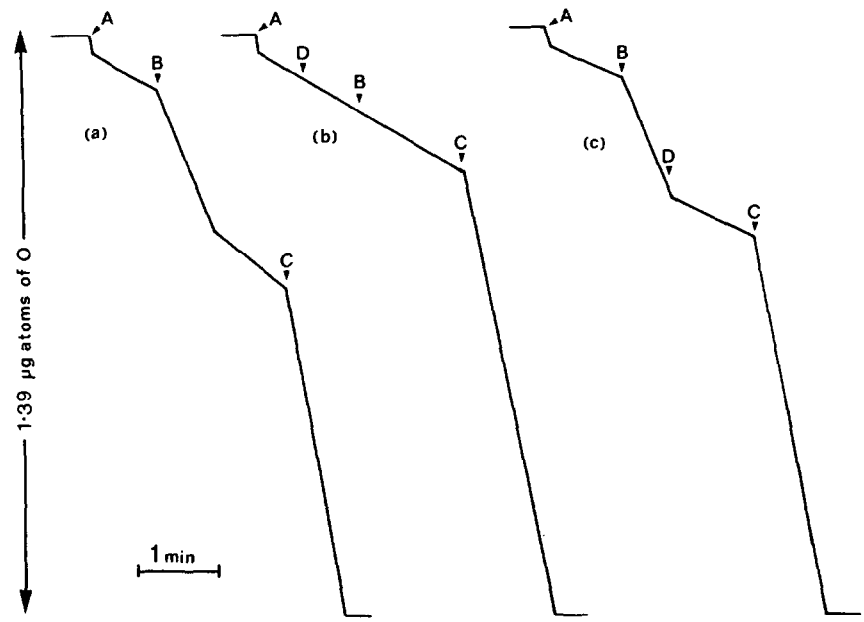

Fig. 2. The effect of 2-phenylisatogen on ADP-stimulated oxidation of succinate. In each case the medium contained 0.25 M sucrose, 3.4 mM tris-HCl pH 7.3, and 3.3 mM succinate. The final volume was 3.0 ml and the temperature was 30°. Additions: (A) 0.1 ml rat liver mitochondria, 4 mg protein; (B) ADP, 160 μM; (C) TTFB, 1.3×10^{-7} M; (D) 2-phenylisatogen, 50 nmole. (a) control; (b) 2-phenylisatogen present initially; (c) 2-phenylisatogen added after ADP (N.B. the concentration of ADP in (c) was 640 μM, sufficient to allow ADP-stimulated respiration to continue to the bottom of the trace).

of inhibition observed, for example, preincubation of mitochondria for 2 min with 5 nmole 2-phenylisatogen/mg protein gave 55% inhibition, whereas preincubation for 24 hr at 0–3° with the same concentration of isatogen gave only 50% inhibition.

Fig. 3 (curve B) shows that 2-phenylisatogen inhibited the uncoupler-stimulated ATPase to a maximum of 60% at concentrations in the region of 10–20 nmoles/mg mitochondrial protein. The effect of a range of isatogen concentrations on ADP-stimulated succinate oxidation is also shown in fig. 3 (curve A). It can be seen that both the ATPase and the ADP-stimulated reaction were inhibited over an identical concentration range, although the extent of inhibition observed was different in the two reactions.

4. Discussion

From the evidence, 2-phenylisatogen inhibits mitochondrial oxidative phosphorylation by interaction with a component of the mitochondrial energy-transfer system. This conclusion is supported by our demonstration of inhibition of both the forward and reverse reactions of oxidative phosphorylation in a manner analogous to that found with other energy-transfer inhibitors, such as oligomycin A, DCCD and aurovertin [1]. The effect of 2-phenylisatogen on ADP-stimulated respiration differs from that of DCCD which required long preincubation periods for attainment of maximum activity [8], whereas the isatogen was almost immediate in its effects. Failure to obtain 100% inhibition of the ATPase is reminiscent of the effect of aurovertin, which inhibited the DNP-stimulated ATPase of beef heart mitochondria to a maximum of 70% [9, 10]. Oligomycin A and DCCD, on the other hand, are both capable of inhibiting the ATPase 100% [1]. It has been suggested that aurovertin acts at the level of $X \sim P$ (i.e. close to the terminal ATPase) and that oligomycin A and DCCD act at the level of $X \sim I$ [eq. 1]. Further studies are in progress to investigate more precisely the site of action of 2-phenylisatogen.

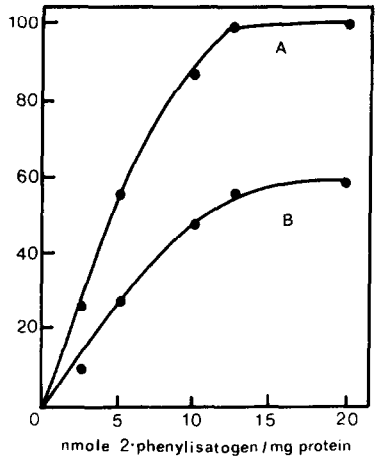

Fig. 3. The effect of 2-phenylisatogen on the uncoupler-stimulated ATPase and ADP-stimulated oxidation of succinate. Method for ATPase: mitochondria (1 mg protein) suspended in 0.1 ml 0.25 M sucrose and 3.4 mM tris-HCl pH 7.3, were added at zero time to 0.9 ml reaction medium containing 0.125 M sucrose, 60 mM tris-HCl pH 8.0, 10 μg antimycin A, 2 μg rotenone, 30 μM DNP, 3 mM ATP and 10 μl 2-phenylisatogen at the concentrations shown. After 15 min incubated at 30° the reaction was stopped by the addition of 0.1 ml 30% TCA. The orthophosphate content of the supernatant was determined by the method of Fiske and Subbarow [5]. ADP-stimulated succinate oxidation was measured as described in the legend to fig. 2. Curve A, ADP-stimulated succinate oxidation; Curve B, uncoupler-stimulated ATPase.

In conclusion, 2-phenylisatogen represents a novel heterocyclic molecule which has the advantage over many other energy-transfer inhibitors of being inexpensive and simple to prepare [7].

Acknowledgements

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