EFFECT OF CHELERYTHRINE ON MITOCHONDRIAL ENERGY COUPLING

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Received 8 January 1972

1. Introduction

Chelerythrine is a 1:2-benzo-phenanthridine alkaloid found in Papaveraceae and Rutaceae [1, 2]. It is a powerful fungicide [1] and might be responsible, together with related alkaloids, for endemic glaucoma [3].

In a study of the effect of several alkaloids on yeast metabolism, chelerythrine was found to be one of the stronger inhibitors of yeast respiration [4]. This inhibition seemed to take place in yeast mitochondria.

We report here a study of the effect of chelerythrine on mitochondrial energy coupling mechanisms. Low concentrations of chelerythrine inhibited energy transfer and coupled respiration in rat liver mitochondria. Inhibition of respiration was not reversed by uncouplers of oxidative phosphorylation. Chelerythrine did not inhibit electron transport in submitochondrial particles. It behaved as an uncoupler at high concentrations but caused only a transient stimulation of state "4" [5] respiration.

2. Experimental procedure

Rat liver mitochondria were prepared as described by Vallejos and Stoppani [6].

Oxygen consumption was determined manometrically in a Warburg apparatus at 30°. The reaction medium (2.5 ml) contained 0.25 M sucrose, 30 mM KCl, 6 mM $MgCl_2$, 1 mM EDTA, 10 mM potassium phosphate pH 7.4, 25 mM Tris-HCl pH 7.4, 5 units of hexokinase (E C 2.7.1.1), 28 mM glucose, 1 mM ATP and 4–8 mg protein of rat liver mitochondria. Substrate was either succinate (10 mM) or malate–glutamate (5 mM each plus 5 mM malonate). 0.1 ml 10% NaOH was put in the central well. The reaction was started after 5 min of preincubation by tipping glucose, ATP and hexokinase from the side arm and was stopped after 40 min with 5% trichloroacetic acid (w/v, final concentration). Phosphorylation was measured by the disappearance of inorganic phosphate according to Slater [7].

Measurement of ATPase activity was carried out at 30° in a reaction mixture (1 ml) containing 100 mM KCl, 50 mM Tris-HCl pH 7.4 and 5 mM ATP. The reaction was started, after 5 min preincubation, by adding mitochondria (about 1 mg) and stopped after 5 min with 5% trichloroacetic acid (w/v). After cooling and centrifugation, inorganic phosphate was determined in the supernatant and in zero-time controls according to Sumner [8]. Protein was determined by a modification of the biuret method [9].

3. Results

Fig. 1 shows the titration curves of the inhibitory effect of chelerythrine on active respiration, phosphorylation and P/O ratios of rat liver mitochondria with malate–glutamate as substrates. 10 nmoles of chelerythrine per mg of protein completely abolished phosphorylation and P/O ratio and inhibited oxygen uptake by 75%. Similar results were obtained with succinate or N, N, N′, N′-tetramethyl-*p*-phenylenediamine-ascorbate as substrates except that state "3" respiration was less sensitive.

The inhibition of active respiration by chelerythrine (with malate–glutamate or succinate as substrates) was time-dependent (fig. 2A) and was preceded by a short burst of respiration. Once the inhibition had set in it was not reversed by uncouplers like 2-4-dinitrophenol (DNP), carbonylcyanide *p*-tri-

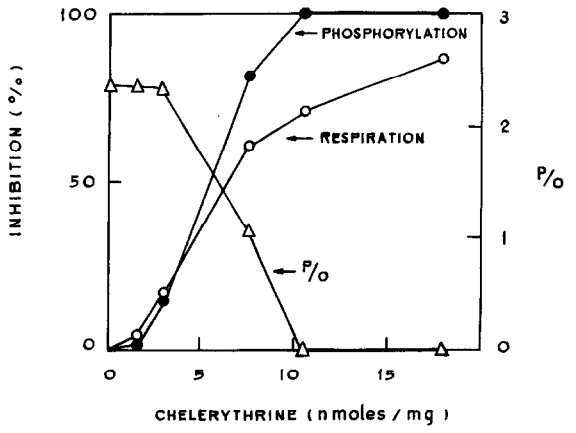

Fig. 1. Effect of chelerythrine on phosphorylation and coupled respiration of rat liver mitochondria. Experimental conditions were as described in Experimental procedure. The substrate was malate–glutamate. Inhibition (%) of phosphorylation (●–●–●) and respiration (○–○–○) are represented on the left ordinate and P/O ratios (△–△–△) on the right one.

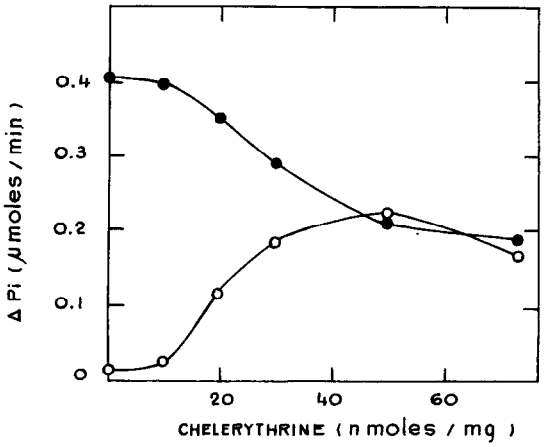

Fig. 3. Effect of chelerythrine on mitochondrial ATPase. ATPase activity was determined as described in the text with the amounts of chelerythrine stated. (○–○–○): Latent ATPase; (●–●–●): ATPase activity in the presence of 150 μM DNP.

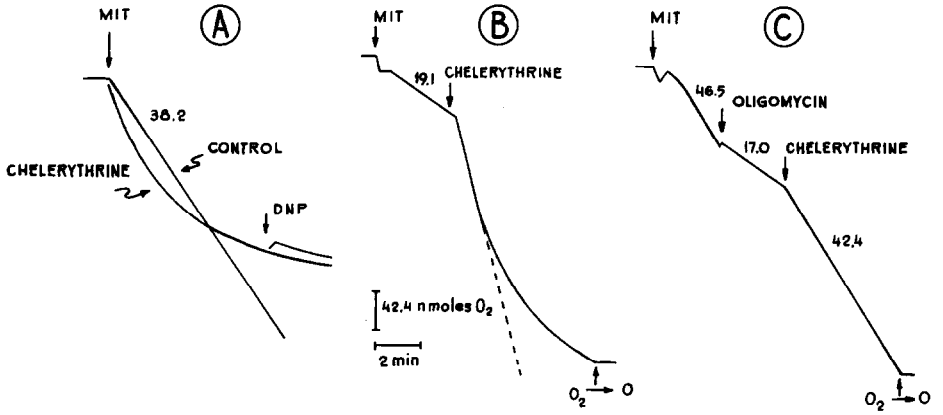

Fig. 2. Effect of chelerythrine on mitochondrial respiration. Oxygen uptake was determined at 30° with a Clark electrode in a Gilson Oxygraph. The reaction mixture was as used for manometric experiments except that hexokinase, glucose and ATP were not added. The final volume was 1.65 ml. Numerals on the slopes are nmoles O_2/min. Exp. A) substrate, succinate and 0.65 mM AMP; chelerythrine (24 nmoles/mg protein) was added before starting the experiment (not added in the control run); DNP was 90 μM. Exp. B) substrate, malate-glutamate (state "4"); chelerythrine was 36.8 nmoles/mg protein. Exp. C) substrate, malate–glutamate and 0.65 mM AMP; oligomycin was 2.5 μg; chelerythrine was 33 nmoles/mg protein.

fluoromethoxyphenylhydrazone or 5-chloro-3-t-butyl-2′-chloro-4′-nitro salicylanilide.

The alkaloid behaved like an uncoupler at higher concentrations, i.e., it stimulated controlled respiration and reversed oligomycin inhibition of active respiration as shown in fig. 2B and C. However these effects are transitory and, in a few minutes, the respiration was inhibited below the state "4" level.

It should be pointed out that chelerythrine did not inhibit NADH or succinate oxidation by rat liver mitochondria treated by ultrasonic vibration for 2 min at 0° (MSE, 100 W ultrasonic disintegrator).

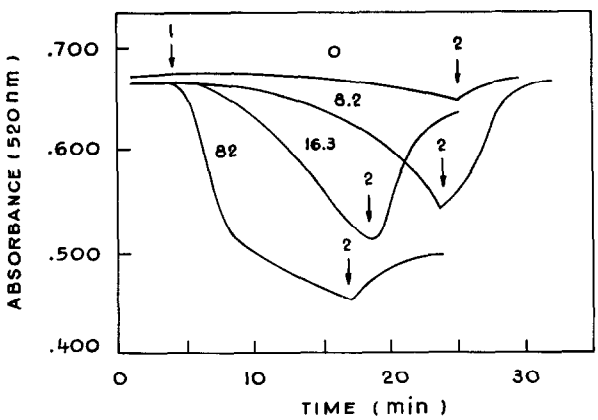

Fig. 4. Effect of chelerythrine on mitochondrial swelling. Absorbance at 520 nm of a mitochondrial suspension was followed in a Beckman DU spectrophotometer at 30°. The reaction mixture (3 ml) contained 140 mM KCl, 10 mM Tris-HCl pH 7.4 and 1.6 mg protein of rat liver mitochondria. The amounts of chelerythrine stated (nmoles per mg of protein) were added at arrow 1; 2.5 mM ATP and 4 mM $MgCl_2$ were added at arrow 2.

Chelerythrine at concentrations that completely abolished phosphorylation had no effect on ATPase activity but at higher concentrations stimulated the latent ATPase of rat liver mitochondria (fig. 3). The maximum stimulation of latent ATPase by chelerythrine was obtained with 49 nmoles/mg of protein and was about half of that observed with DNP. On the other hand the DNP-stimulated ATPase was inhibited by the same concentrations of chelerythrine that stimulated the latent ATPase (fig. 3).

Chelerythrine also induced swelling of rat liver mitochondria in KCl-Tris-HCl medium (fig. 4). Concentrations that completely inhibited phosphorylation induced a minor swelling which was reversed by ATP-Mg. Higher concentrations induced a larger and more rapid swelling which was only partially reversed by ATP-Mg.

Discussion

Low concentrations of chelerythrine inhibited phosphorylation and coupled respiration like oligomycin [10]. However, uncouplers were not able to reverse the chelerythrine induced inhibition of respiration. Inhibition of DNP-stimulated ATPase is caused by both inhibitors but oligomycin does not stimulate latent ATPase as chelerythrine does.

The properties of chelerythrine resemble that of Dio-9 [11]. Inhibition of respiration by Dio-9 is also not reversed by DNP and is preceded by a short burst of oxygen uptake. However Dio-9 action depends on the presence or absence of P_i which was not the case for chelerythrine.

Guanidines, which like chelerythrine have a positive charge, have a time-lag for inhibiting coupled respiration and the inhibition is slowly reversed by DNP [12–13]. However, inhibition of ATP synthesis by chelerythrine does not seem to be site-specific as it has been claimed for guanidines [13], since it took place with malate–glutamate, succinate or ascorbate–TMPD as substrates.

We can conclude that chelerythrine is a powerful inhibitor of mitochondrial energy transfer that shares some properties of inhibitors like oligomycin, Dio-9 and guanidines but has at the same time some peculiar features which make it worth studying.

Acknowledgements

We thank Dr. A.M. Kuck (Universidad de Buenos Aires) for the generous gift of chelerythrine.

R.H. Vallejos is a Career Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina). This work was supported by grants from CONICET and Consejo de Investigaciones Científicas y Técnicas de la Provincia de Santa Fe (Argentina).

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