UNCOUPLING ACTIVITY OF THE ANTHELMINTIC OXYCLOZANIDE IN RODENTS

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The uncoupling activity of oxyclozanide in warm blooded animals has been studied in whole animals, isolated tissue *in vitro* and on mitochondrial preparations. The onset of post mortem rigidity in mice and rats is accelerated and a contracture of striated muscle is produced. Oxyclozanide (1 μM) stimulated rat liver mitochondrial respiration and stimulated an ATP-ase activity.

Introduction

Following the work of Van Miert & Groeneveld (1969) on the activity of anthelmintics as uncouplers of oxidative phosphorylation in warm blooded animals, the anthelmintic oxyclozanide (Zanil: 3,3',5,5',6-pentachloro-2,2'-dihydroxybenzanilid) has been studied, including its effects on the respiration of liver mitochondria. Oxyclozanide has structural resemblance to taeniacide Yomesan: N-(2'-chloro-4'-nitrophenyl)-5-chlorsalicylamide which is an uncoupler of the oxidative phosphorylation process in rat liver mitochondria (Gönnert & Schraufstätter, 1960). In addition there are indications from experiments with whole liver flukes under aerobic conditions that the fasciolicidal effect of oxyclozanide might be due to uncoupling of phosphorylation from electron flow (Corbett & Goose, 1971). However, it is questionable whether measurements made aerobically, reflect the circumstances under natural (anaerobic) conditions. Furthermore, Vanden Bossche & Tollenaere (1972) stated that the malate-induced phosphorylation in *Fasciola* mitochondria resembles electron transport linked phosphorylation in rat liver mitochondria in its sensitivity to uncouplers, although the 2-4-dinitrophenol (DNP) stimulated ATP hydrolysing activity of *Fasciola* mitochondria was at least 50% lower than the activity of rat liver mitochondria.

Oxyclozanide is used against *Fasciola hepatica* infections in sheep (Boray & Happich, 1968; Boray, Happich & Jones, 1969) and it would seem to be of particular value in cattle, in which species this drug has a wide safety margin, combined with a high efficacy (Sinclair, 1969).

Methods

Acute toxicity

Test substances dissolved in propyleneglycol were injected intraperitoneally into Swiss mice (average body weight 25 g) and rats (average body weight 200 g) of either sex.

Diaphragm preparation

The phrenic nerve-diaphragm preparation as described by Bülbring (1946) was used. Twitches of striated muscle produced by electrical stimulation of the phrenic nerve or by direct stimulation of the muscle were recorded as described previously by Van Miert & Groeneveld (1969). The nerve was stimulated supramaximally at 0.25 Hz.

Respiration of rat liver mitochondria

The liver homogenate was prepared according to Myers & Slater (1957). After centrifugation of the homogenate at 900 x *g* for 5 min in a cooled (−3°C) Spinco Preparative Ultracentrifuge the pellett was discarded and three-quarters of the suspension was centrifuged again at 4,500 x *g* for 10 minutes. The pellett was resuspended in sucrose (250 mM) and centrifuged for 10 min at 12,500 x *g*. Mitochondrial respiration was measured by means of a Gilson Medical Polarograph in a total volume of 1.8 ml containing (mM) KCl 15, disodium edetate (EDTA) 2, $MgCl_2$ 5, Tris buffer 50, glucose 30, phosphate buffer 25, sodium-glutamate 20 and (−)-malate 20 at 25°C and pH 7.4. Mitochondrial protein was measured according to Cleland & Slater (1953). Results are given per mg of protein. In all experiments 2-4 dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, was used for comparison.

ATP-ase induction in rat liver mitochondria

Mitochondria were incubated for 15 min at 20°C and pH 7.4 in a medium containing KCl 0.15 M, EDTA 1 mM, $MgCl_2$ 3 mM, Tris-HCl buffer 0.2 M,

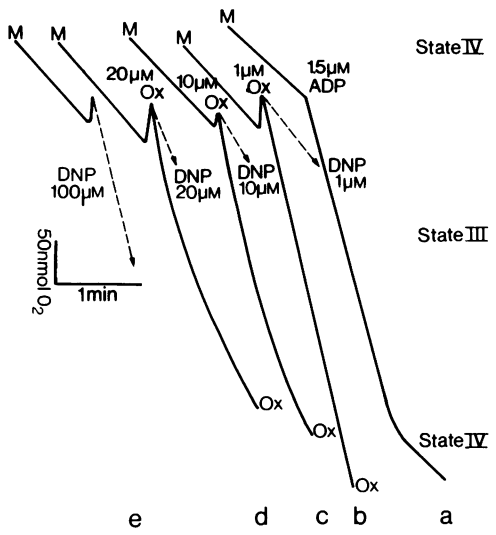

Fig. 1 Polarographic measurement of oxygen consumption rate of rat liver mitochondria.

Line (a), basic mitochondrial respiration (state IV)—respiration while phosphorylating (state III)—basic respiration (state IV).

(b) Basic respiration (state IV)—respiration after 1 μM oxyclozanide (full line) or after 1 μM 2-4 dinitrophenol (DNP) (dotted line).

(c) Basic respiration (state IV)—respiration after 10 μM oxyclozanide (full line) or after 10 μM DNP (dotted line).

(d) Basic respiration (state IV)—respiration after 20 μM oxyclozanide (full line) or after 20 μM DNP (dotted line).

(e) Basic respiration (state IV)—respiration after 100 μM DNP (dotted line).

Compounds added at arrows; Ox, oxyclozanide; M, mitochondria (0.36 ml); DNP, dinitrophenol.

sucrose 0.25 M and ATP 30 mM. Inorganic phosphate (Pi) was measured according to Sumner (1944). DNP was used for comparison.

Results

A lethal dose of DNP (50 mg/kg) or oxyclozanide (75 mg/kg) given intraperitoneally to mice and rats caused a post mortem rigidity developing within 1-3 minutes. In animals dying after 250 mg/kg of sodium pentobarbitone (intravenously) or decapitation this took 50-87 minutes.

The phrenic nerve-diaphragm preparation of the rat was used for studying effects of uncoupling agents *in vitro* on contractions of the striated muscle of the diaphragm evoked by either stimulation of the phrenic nerve or direct stimulation of the muscle. As shown by Barnes, Duff & Threlfall (1955) DNP (0.046 μmol/ml organ bath) caused a decrease in contractions: those produced by indirect stimulation via the phrenic nerve disappeared first, followed by a gradual onset of a characteristic contracture. Oxyclozanide (0.067 μmol/ml organ bath) produced a similar effect.

Polarographic measurements (six experiments) showed that oxyclozanide (10 μM) stimulates rat liver mitochondrial respiration (27.07 ± 1.31 μl O_2 mg protein^{-1} h^{-1}) (average of 6 ± standard error of the mean). The control value was 8.40 ± 0.94 and 10 μM of DNP gave a value of 19.60 ± 1.18 μl O_2 mg protein^{-1} hour^{-1}. Figure 1 shows that oxyclozanide 1 μM (line b) caused maximal stimulation of rat liver mitochondrial respiration, while higher concentrations of 10 μM (line c) and of 20 μM (line d) produced a gradual onset of inhibition of mitochondrial respiration. It seems that in this respect oxyclozanide is a more potent compound than DNP.

Experiments to test the ability of oxyclozanide to induce adenosine triphosphatase (ATP-ase) activity in liver mitochondria showed that 1 μM of oxyclozanide induced a formation of 32.2 ± 4.5 nmol inorganic phosphate per mg protein and per minute. The control value was 7.7 ± 1.0 (average of 6 ± standard error of the mean), while a maximal uncoupling dose of DNP (100 μM) gave a value of 36.1 ± 2.9. Oxyclozanide in concentrations of 10 μM and 100 μM gave values higher than control values, but lower than the effect of 1 μM, because of an inhibitory effect.

Discussion

From the accelerated onset of post mortem rigidity in mice and rats; the contracture of the rat striated muscle preparation *in vitro*; the stimulation of the rat liver mitochondrial respiration, followed by inhibition at higher doses and from the increase of rat liver mitochondrial adenosine triphosphatase activity, all of which phenomena can be regarded as characteristic of uncouplers of oxidative phosphorylation, the conclusion seems reasonable that oxyclozanide (Zanil) is an active uncoupler of oxidative phosphorylation in rodents.

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