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Biochemical Pharmacology, Vol. 31, No. 22, pp. 3703–3705, 1982.
Printed in Great Britain.

0006-2952/82/223703-03 $03.00/0

Effects of niridazole and 5-nitroimidazoles on heart mitochondrial respiration

(*Received* 12 *May* 1982; *accepted* 29 *June* 1982)

Nitroimidazole derivatives are used extensively to treat infections caused by anaerobic protozoa and bacteria [1]. These drugs were found to be selectively adsorbed and have a cytotoxic action on anaerobes [2]. Recently a number of nitroimidazoles have been used as radiosensitizers of hypoxic cells [3], since these are believed to limit the effectiveness of radiation therapy in certain tumors [4]. However, in antineoplastic chemotherapy, large amounts of drugs are required [5], increasing the risk of toxic effects. In fact some nitroimidazoles have shown a weak carcinogenic effect [6, 7], whilst for most of them a mutagenic action has been demonstrated [8, 9]. Moreover ECG abnormalities were observed in patients under either niridazole or metronidazole therapy [10, 11]. Although the mechanism of antibacterial and antiprotozoal action is still under investigation, it is generally assumed that the nitrogroup of these compounds must be reduced and the highly reactive products possibly bind to DNA or other biopolymers [12, 13]. The reduction of the nitrogroup is a necessary step in the induction of mutagenicity in *Salmonella typhymurium* [14]. Adams *et al.* [15] pointed out a relationship between electroreduction potential and efficiency as radiosensitizers.

The purpose of the present paper was to study the effect of one nitrothiazole and five nitroimidazole derivatives on oxidative processes in rabbit heart mitochondria and the relationship with their electroreduction potentials.

Materials and methods. Female rabbits weighing 3–4 kg were used. While niridazole, ipronidazole, 1-methyl-2-formyl-5-nitroimidazole (MFNI), ornidazole and metronidazole were obtained from commercial sources, DA 3851 was a gift of De Angeli (Milano, Italy). Stock solutions were made in methanol or dimethylsulphoxide (niridazole). ADP, dinitrophenol (DNP) and respiration substrates were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. Mitochondria were prepared from rabbit heart, according to Tyler and Gonze [16], and rapidly utilized. Oxygen consumption was recorded at 30° using a Clark-type oxygen electrode obtained as an integral part of a thermostated vessel. The reaction mixture consisted of 0.25 M sucrose, 5 mM K_2HPO_4, 5 mM $MgCl_2$, 10 mM KCl, 0.5 mM EDTA and 10 mM Tris–HCl at pH 7.4, added to 1 mg mitochondria, in a total volume of 2.8 ml. Substrates were either 8 mM glutamate–malate or 20 mM succinate in the presence of 2 μM rotenone. Mitochondrial swelling was tested by measuring extinction changes at 546 nm [17] by means of a Zeiss PMQ 3 spectrophotometer. Mitochondrial protein was determined by the biuret method [18], using deoxycholate for solubilization.

The electroreduction potentials had been determined at pH 7.4 by means of cyclic voltametry using a mercury dropping electrode vs a saturated calomel electrode [8]. Only peaks between −200 and −900 mV had been considered.

Results and discussion. The structures, electroreduction potentials and per cent inhibitions of oxygen uptake are reported in Table 1. MFNI considerably affected mitochondrial respiration in the presence of glutamate–malate as substrate; effectiveness of inhibition of ADP-stimulated respiration was concentration-dependent (Fig. 1). Niridazole, ipronidazole, DA 3851 and ornidazole at concentrations up to 0.8 mM led to falls of only 10–20% in oxygen uptake; metronidazole did not seem to affect the process at all. The *in vitro* concentrations were fairly close to the serum levels which occur *in vivo* after administration of

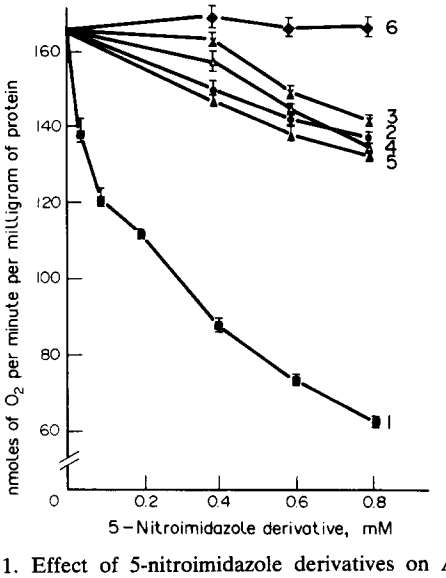
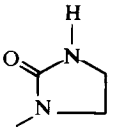
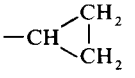


Fig. 1. Effect of 5-nitroimidazole derivatives on ADP-stimulated respiration. One milligram mitochondria was incubated at 30° in 2.8 ml of reaction medium (see Materials and Methods) containing 5-nitroimidazoles as indicated and 8 mM glutamate–malate as substrate. Respiration was stimulated by addition of 5 μl 0.1 M ADP. The points represent mean values ± S.E.M. for four separate experiments.

Table 1. Niridazole and 5-nitroimidazoles: structural formulae, electroreduction potentials and per cent inhibitions of ADP-stimulated mitochondrial respiration

Compound No.	Name	X	R_1	R_2	E^l_{cp}* (mV)	% inhibition†
1	1-Methyl-2-formyl-5-nitroimidazole	N	$-CH_3$	$-CHO$	−465	61.8
2	1-(5-Nitro-2-thiazolyl)-2-imidazolidinone (niridazole)	S	—		−490	16.7
3	1-Methyl-2-(1-methylethyl)-5-nitroimidazole (ipronidazole)	N	$-CH_3$	$-CH(CH_3)_2$	−585	13.9
4	1-Methyl-2-cyclopropyl-5-nitroimidazole (DA 3851)	N	$-CH_3$		−597	18.2
5	1-[α-(Chloromethyl)idroxyethyl]-2-methyl-5-nitroimidazole (ornidazole)	N	$-CH(CH_2Cl)-CH_2OH$	$-CH_3$	−630	19.4
6	1-(2-Hydroxyethyl)-2-methyl-5-nitroimidazole (metronidazole)	N	$-CH_2-CH_2OH$	$-CH_3$	−695	0.0

* E^l_{cp} is the electroreduction potential determined at pH 7.4 by means of cyclic voltametry using a mercury dropping electrode vs a saturated calomel electrode.

† Per cent inhibition of ADP-stimulated mitochondrial respiration with 0.8 mM test compounds. Reaction mixtures were as described in Materials and Methods; 8 mM glutamate–malate was the substrate.

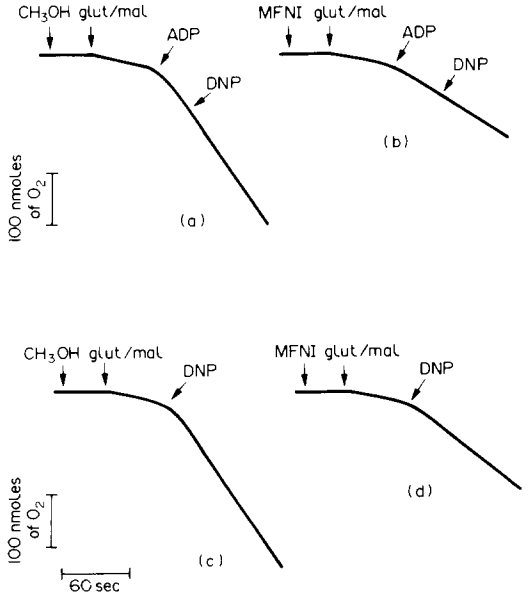


Fig. 2. Effect of 1-methyl-2-formyl-5-nitroimidazole on mitochondrial uncoupled respiration. Experimental conditions were as described in Materials and Methods. Addition of 1 μl 0.1 M DNP to the reaction mixture for ADP-stimulated respiration, in the presence of 0.4 mM 1-methyl-2-formyl-5-nitroimidazole (b): (a) control. Respiration rate of uncoupled mitochondria (1 μl 0.1 M DNP), in the presence of 0.4 mM 1-methyl-2-formyl-5-nitroimidazole (d): (c) control.

therapeutic doses [19]. Since interference with mitochondrial oxygen uptake often leads to extensive structural modification of mitochondria, we investigated the effect of the test compounds on the conformation of the mitochondrial membrane, by measuring changes in volume of the particles suspended in isotonic medium. Lehninger [17] reported that mitochondrial swelling is associated with large-amplitude extinction changes at 546 nm. The test compounds (2 mM) did not change the extinction at 546 nm of 0.2 mg/ml mitochondria suspended in 0.1 M NH_4Cl solution, thus suggesting a lack of effects on the membrane permeability. In Fig. 2 it is shown that mitochondrial respiration with glutamate–malate as substrate is inhibited by MFNI even when the uncoupler 2,4-dinitrophenol was present (state 3 u respiration). Both state 3 and state 3 u respiration were inhibited to the same extent, indicating that the active compounds exerted their influence directly on the respiratory chain rather than on the coupling mechanism. In order to better localize which region of the chain was affected by the action of MFNI, we carried out a number of experiments evaluating mitochondrial oxygen uptake with 20 mM succinate as substrate in the presence of 2 μM rotenone. As a result we could not observe any significant inhibitory effect under any of the test conditions. Therefore we could draw the conclusion that inhibition by nitroimidazoles and niridazole possibly was exerted at the NADH oxidation level (site I respiration).

All these findings might be related to the redox properties of the nitroderivatives. In fact when comparing the E^l_{cp} values with the inhibitory efficiency of nitroderivatives on electron transport, we may observe that MFNI, the most powerful inhibitor, has the least negative E^l_{cp}, due to the presence of an aldehyde group in position 2. Metronidazole, which did not affect the respiratory chain at all, has the most negative E^l_{cp}. However a significant linear relationship

could not be shown between E^{1}_{cp} values and respiratory inhibition. Other chemical and/or physicochemical parameters must be involved in the inhibitory process. In particular the lipophilic character of the molecules might be important in determining the penetration through the mitochondrial membrane. At the present time we are carrying on further experiments with submitochondrial particles in order to study the possible relationship between lipophilicity and inhibitory effects of the compounds.

In summary, 2.3 micromoles/mg protein of MFNI induced a 60% decrease in the heart mitochondrial ADP-stimulated oxygen uptake using glutamate–malate as substrate. The same amount of niridazole, ipronidazole, DA 3851 and ornidazole led to falls of less than 20% in the oxygen uptake, whilst metronidazole was ineffective. State 3 and state 3 u (uncoupled) respiration were affected to the same extent. Oxygen-uptake using succinate as substrate was not inhibited indicating that the action was exerted at the NADH oxidation level. The relationship between electroreduction potentials of the test compounds and inhibition of respiration has been studied.

Acknowledgements—Appreciation is expressed to Professors G. L. Biagi and G. Lenaz for their valuable discussions.

This work was supported by a grant from the Italian CNR.

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Biochemical Pharmacology, Vol. 31, No. 22, pp. 3705–3707, 1982.
Printed in Great Britain.

0006–2952/82/223705–03 $03.00/0

The effect of various calmodulin inhibitors on the response of adrenal glomerulosa cells to angiotensin II and cyclic AMP

(*Received* 7 *May* 1982; *accepted* 2 *July* 1982)

Involvement of calcium ions in the stimulatory action of angiotensin II, corticotropin (ACTH) and potassium ions on adrenal glomerulosa cells has been amply verified [1]. Nevertheless, the precise role of calcium ions in the stimulation of adrenal steroidogenesis is still a matter of controversy. In several tissues many calcium-mediated cell activities have been shown to be controlled by the calcium-dependent regulator protein calmodulin [2]. In a previous study [3] we demonstrated that the tranquillizer drug trifluoperazine, an inhibitor of calmodulin, completely prevented the aldosterone-stimulating action of angiotensin II and potassium ions, both agents supposedly acting via calcium ions rather than cyclic AMP (cAMP). At the same concentration trifluoperazine inhibited only partially the response to ACTH or its second messenger cAMP. These results raise the possibility that calmodulin may participate in the stimulation of aldosterone production. Moreover, it was also assumed that calmodulin plays a different role in the mode of action of angiotensin II and potassium ions on the one hand and of ACTH or cAMP on the other hand. To test whether the effect of trifluoperazine was due to the inhibition of calmodulin in the present experiments we examined the effect of three calmodulin antagonists of partly heterologous structure (penfluridol, pimozide and trifluoperazine) chosen on the basis of the studies of Weiss *et al.* [4]. In order to compare the mode of action of stimuli