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EFFECT OF ANTIDEPRESSANT AND NEUROLEPTIC DRUGS ON RESPIRATORY FUNCTION OF RAT HEART MITOCHONDRIA

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Abstract—The effects of five antidepressants, two phenothiazines and one butyrophenone neuroleptic drug on respiratory functions of rat heart mitochondria were studied *in vitro* using four different substrates. All compounds caused uncoupling of oxidative phosphorylation with comparable no-effect levels ranging from 1×10^{-6} to 5×10^{-5} moles per mg mitochondrial protein. The uncoupling effect was accompanied by increased oxygen consumption. The same drugs were given orally twice daily to rats. Uncoupling of oxidative phosphorylation was observed in mitochondria isolated from hearts of the treated animals. Oxygen consumption was not altered. Serum and myocardial tissue levels were determined with one of the test compounds (protriptyline). Serum concentrations in rats were higher than those observed in patients treated therapeutically, but well below those observed in human overdose situations. The effects on heart mitochondria are considered to be an indicator of altered membrane functions resulting from an accumulation of the drugs in lipid membranes.

Antidepressants of the imipramine-type and phenothiazine neuroleptics have a marked influence on many functions of the cardiovascular system. Both classes of drug affect central and peripheral autonomic receptors, and inhibit norepinephrine reuptake into adrenergic neurons [1, 2]. They also have a direct depressant effect on the heart, which is often described as "quinidine-like". It is responsible for the reduced cardiac contractility and the electrocardiographic changes, e.g. prolongation of PQ and QT intervals and lengthening of the QRS complex [1, 3–5]. The mechanism of the cardiodepressant effect is not yet fully explained. It was suggested that the drugs may alter the function of the cell membrane, and impair the transmembranal flux of sodium and potassium ions [6]. Antidepressants and phenothiazines have a high affinity for myocardial tissue where they tend to accumulate [7–9]. It was, therefore, of interest to investigate their effects on an intracellular membrane structure, the mitochondria. Four tricyclic and one tetracyclic antidepressant and two phenothiazine neuroleptics, all drugs that are widely used in clinical practice, were studied for their effect on oxygen consumption coupled to ATP production in rat heart mitochondria *in vitro* and *in vivo*. Haloperidol, a neuroleptic of the butyrophenone class, was included for comparison. The structural formulae of the test compounds are shown in Fig. 1.

MATERIALS AND METHODS

Female rats of the ZUR–SIV–Z strain with a starting weight of 100–110 g were housed in pairs in Macrolone® cages with wood shavings as bedding. They had free access to Nafag 890 rat pellets and water. For the *in vitro* experiments mitochondria were isolated from hearts of untreated rats by differential centrifugation. They were suspended in an incubation medium as described previously [10]. Oxygen consumption coupled to oxidative phosphorylation was measured in a Gilson oxygraph (Gilson Medical Electronics, Middleton, WI) equipped with a Clark electrode. Succinate, β-hydroxybutyrate, 2-oxo-glutarate, and DL-glutamate were used as substrates [10]. Drugs were dissolved in distilled water (amitriptyline, dibenzepine, imipramine, maprotiline, protriptyline), ethanol (chlorpromazine, thioridazin), or dimethyl-sulfoxide (DMSO) (haloperidol). Serial dilutions were made with the respective solvents. After a control run with substrate and ADP, 20 μl of the test compounds were added to the incubation mixture to estimate the immediate effects on electron transfer reactions and oxidative phosphorylation. Control experiments with equal volumes of ethanol and DMSO indicated that these solvents had no adverse effect on mitochondrial functions. Figure 2 shows a typical record demonstrating the sequence of the experimental procedure. The solutions of the test compound were diluted until a "no-effect level" was reached. This was defined as the highest drug concentration, calculated in moles per mg mitochondrial protein that had no measurable effect on electron transfer reactions and oxidative phosphorylation.

For the *in vivo* experiments the drugs were dissolved in water, or, if poorly water soluble, suspended in 0.5% methylcellulose. Groups of 32 rats were dosed twice daily (8 a.m., 3 p.m.) by gavage on 5 days per week for 4 weeks. The dose selection was based on a previous experiment [11], with the intention to reach maximally tolerated levels. In two cases the initially selected dose had to be reduced because of oversedation and loss of appetite. With the other compounds it was necessary to increase the dose in the course of the experiment. All drugs

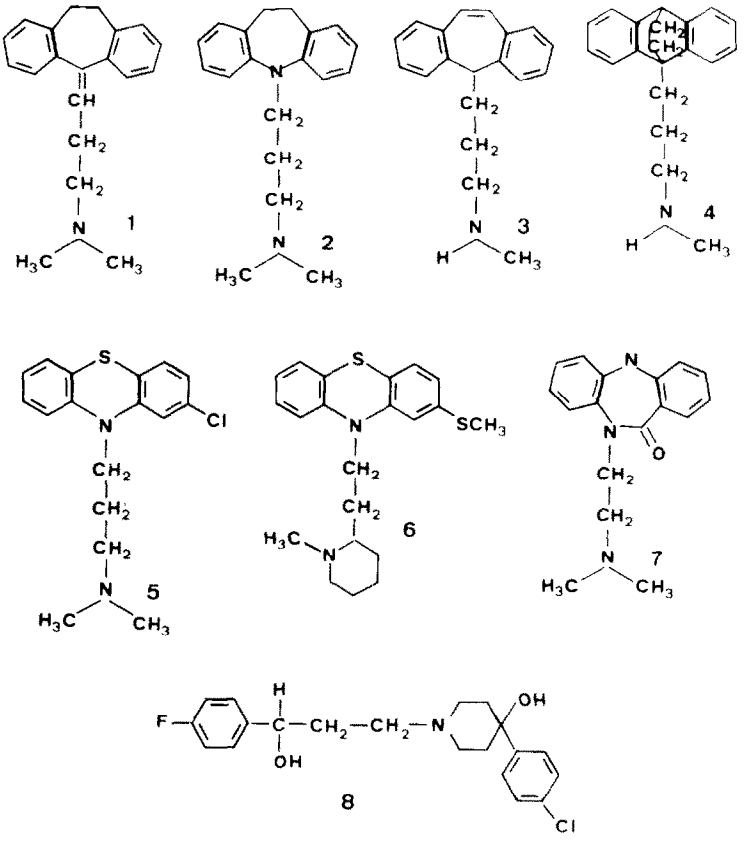

Fig. 1. The chemical structures of the antidepressant and neuroleptic drugs used in this study:
1 = Amitriptylin, 2 = Imipramin, 3 = Protriptylin, 4 = Maprotilin, 5 = Chlorpromazin, 6 = Thioridazin, 7 = Dibenzepin, 8 = Haloperidol.

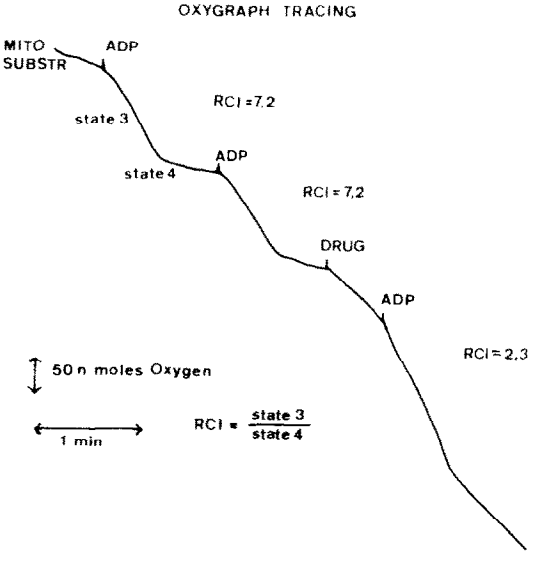

Fig. 2. Oxygraph tracing, showing oxygen consumption by rat heart mitochondria coupled to oxidative phosphorylation (upon the addition of ADP), under control conditions and upon the addition of a drug leading to uncoupling of oxidative phosphorylation (ADP/O ratio and respiratory control index (RCI) are decreasing).

were administered in a volume of 2 ml/kg. Control groups received equal volumes of water or 0.5% methylcellulose. On the second and fifth day of each week four rats per group were killed by cervical dislocation 17 to 18 hr after the second treatment of the previous day. Hearts were excised immediately. Mitochondria were isolated from the pooled hearts. Respiratory function was measured as described for the *in vitro* experiments.

An additional group of rats was treated twice daily with 32 mg/kg protriptyline by gavage. Eighteen hours after the 2nd, 4th, 6th and 8th dose 4 animals were anesthetized with ether. Blood was collected by open heart puncture and was permitted to clot. Hearts were excised, cut open, blotted dry and homogenized in cold 0.9% NaCl. Protriptyline concentrations were measured in serum and myocardial tissue after methylene chloride extraction by fluorescence spectrophotometry (excitation at 300 nm, emission at 360 nm) [12]. Protein determinations were done by the Biuret method [13] using small amounts of deoxycholate to solubilize the samples. All chemicals were of reagent grade. ADP was purchased from Sigma Chemical Co., St. Louis, MO.

RESULTS

In vitro *experiments*

After incubation of the heart mitochondria isolated from untreated animals, with all test drugs,

Table 1. *In vitro* effect of test drugs on rat heart mitochondria*

Drug	Substrates used to test electron-transfer activities and oxidative phosphorylation			
	Succinate (moles/mg) (μg/mg)	β-Hydroxy-butyrate (moles/mg) (μg/mg)	2-Oxo-glutarate (moles/mg) (μg/mg)	DL-Glutamate (moles/mg) (μg/mg)
Amitriptylin	$1 \times 10^{-5} = 2.77$	$1 \times 10^{-5} = 2.77$	$1 \times 10^{-5} = 2.77$	$1 \times 10^{-5} = 2.77$
Imipramin	$5 \times 10^{-5} = 14.0$	$2.5 \times 10^{-5} = 7.0$	$2.5 \times 10^{-5} = 7.0$	$2.5 \times 10^{-5} = 7.0$
Chlorpromazin	$1 \times 10^{-6} = 0.32$	$5 \times 10^{-6} = 1.59$	$5 \times 10^{-6} = 1.59$	$1 \times 10^{-6} = 0.32$
Thioridazin	$5 \times 10^{-6} = 1.85$	$2.5 \times 10^{-6} = 0.93$	$5 \times 10^{-6} = 1.85$	$1 \times 10^{-6} = 0.37$
Protriptylin	$1 \times 10^{-6} = 0.26$	$2.5 \times 10^{-6} = 0.65$	$1 \times 10^{-6} = 0.26$	$1 \times 10^{-6} = 0.26$
Haloperidol	$1 \times 10^{-5} = 1.48$	$2.5 \times 10^{-5} = 9.4$	$2.5 \times 10^{-5} = 9.4$	$1 \times 10^{-5} = 3.75$
Dibenzepin	$5 \times 10^{-6} = 1.48$	$2.5 \times 10^{-6} = 0.74$	$2.5 \times 10^{-6} = 0.74$	$5 \times 10^{-6} = 1.48$
Maprotilin	$1 \times 10^{-5} = 2.77$	$5 \times 10^{-6} = 1.38$	$5 \times 10^{-6} = 1.38$	$5 \times 10^{-6} = 1.38$

* No-effect levels in moles/mg protein and μg/mg protein. The primary effect in all cases was uncoupling of oxidative phosphorylation. N = 6.

uncoupling of oxidative phosphorylation was observed. The no-effect levels ranged from 5×10^{-5} to 1×10^{-6} moles per mg mitochondrial protein and was similar with all four substrates (Table 1). Oxygen consumption was markedly increased with all drug concentrations that caused uncoupling of oxidative phosphorylation.

In vivo *experiments*

Uncoupling of oxidative phosphorylation was also observed in heart mitochondria isolated from drug treated rats. The effect was again comparable with all four substrates. In Table 2 the results obtained with 2-oxoglutarate as substrate are shown. Uncoupling of oxidative phosphorylation ranging from 60 to 80 per cent of the controls was reached already after the second dose and remained essentially unchanged throughout the 4-week experiment. The only exception was maprotiline: with this drug ATP production decreased slowly reaching the lowest levels towards the end of the experiment. Oxygen consumption during state III respiration [14] was unchanged in comparison with the controls. This indicates that the electron-transfer chain is also affected by these compounds.

Protriptyline concentrations in serum and heart

Eighteen hours after the second dose of 32 mg/kg the serum contained 0.532 ± 0.041 μg/ml protriptyline. There was only a slight increase after continued treatment (Table 3). Marked accumulation of the drug occurred in the heart during the first 2 days. A further, though slight, increase was measured on days 3 and 4. After the first day protriptyline concentrations in the heart were five times higher than in the serum. On the fourth day a twelve fold increase in drug concentration in the heart over the serum levels was measured. Heart mitochondria isolated from animals having received eight doses, contained 0.06 and 0.105 μg protriptylin per mg protein (two separate experiments).

DISCUSSION

The uncoupling effect of chlorpromazine on oxidative phosphorylation *in vitro* has been known for a long time. Abood [15] and Andrejew [16] demonstrated it in brain and liver mitochondria at concentrations that were similar to those of the classical uncoupler 2,4-dinitrophenol (DNP). The authors also observed inhibition of mitochondrial ATPase and cytochrome oxidase. The present study showed that chlorpromazine also uncoupled oxidative phosphorylation in heart mitochondria. This effect was also present with another phenothiazine (thioridazine), four tricyclic and one tetracyclic antidepressant and the butyrophenone derivative haloperidol. A marked increase in oxygen consumption accompanied the uncoupling effect of these psychotropic drugs. This indicates an uncontrolled dissipation of energy in the form of heat and is well known from *in vitro* studies with DNP [17].

In the *in vivo* experiments mitochondria were isolated from hearts of rats that were treated with maximally tolerated doses of the test compounds. Uncoupling of oxidative phosphorylation was observed with all drugs. With the exception of maprotiline the effect was established already after the second dose, and did not increase markedly thereafter. With maprotiline uncoupling of oxidative phosphorylation developed slowly, but this may in part be due to the low initial dose selected for this compound. Determinations of protriptylin in mitochondria showed that the uncoupling occurred at concentrations comparable to the no-effect levels measured *in vitro*.

In contrast to the *in vitro* experiments no increase in oxygen consumption during state III respiration [14] accompanied the uncoupling effect in heart mitochondria isolated from *in vivo* treated animals. In some cases respiration rates were even slightly decreased with respect to the corresponding controls.

The psychotropic drugs used in this study are known to be highly membrane-soluble. They accumulate markedly in membranes and interfere with many membrane-associated biological events [18, 19]. Depending on the concentration they either stabilize or labilize erythrocyte membranes in hypoosmotic solutions [18], they alter trans-membrane fluxes and inhibit nervous excitations [19]. This nonspecific membrane effect is probably also the cause of the electrocardiographic changes, i.e. prolongation of conduction time of the electric impulses and disturbances of depolarization and repolarization [1, 3, 4, 6, 11, 20]. The uncoupling of oxidative phosphorylation without concomitant increase in oxygen consumption which was observed in the present *in vivo* experiments may also be regarded as a conse-

Table 2. Effect of test drugs on oxygen consumption (O_2) and ADP/O ratios in heart mitochondria isolated from treated rats*

Drug	Daily treatment (mg/kg) week 1	week 2	week 3	week 4	Heart mitochondrial function measured with 2-oxoglutarate as substrate week 1 (2) O_2	ADP/O	week 1 (8) O_2	ADP/O	week 2 (12) O_2	ADP/O	week 2 (18) O_2	ADP/O	week 3 (22) O_2	ADP/O	week 3 (28) O_2	ADP/O	week 4 (32) O_2	ADP/O	week 4 (38) O_2	ADP/O
Control					124	2.96	111	2.98	111	2.99	118	2.95	114	2.96	115	2.98	114	2.96	119	2.96
Amitrip.	2 × 16	2 × 16	2 × 32	2 × 32	99	2.30	101	2.29	102	2.13	115	2.12	98	2.13	89	2.22	102	2.25	121	2.19
Chlorpr.	2 × 16/2 × 8	2 × 8	2 × 16	2 × 16	130	2.42	110	2.23	104	2.13	110	2.10	96	2.13	99	2.10	96	2.05	116	2.13
Haloper.	2 × 4/2 × 2 2 × 1	2 × 1	2 × 1	2 × 1	102	2.43	102	2.25	107	1.99	101	1.85	102	1.78	95	1.94	105	2.02	109	2.15
Control					115	2.96	114	2.98	110	2.97	115	2.96	109	2.95	122	2.98	113	2.94	111	2.98
Maprot.	2 × 8	2 × 16	2 × 32	2 × 32	117	2.82	100	2.54	94	2.32	102	2.18	109	1.93	108	1.69	118	1.73	117	1.81
Control					110	2.96	115	2.98	127	2.98	111	2.96	118	2.98	118	2.98	120	2.99	111	2.96
Imipram.	2 × 16	2 × 32	2 × 32	2 × 32	125	2.31	118	2.25	126	2.32	99	2.28	107	2.25	112	2.19	104	2.18	102	2.21
Dibenz.	2 × 8	2 × 16	2 × 16	2 × 16	114	2.32	109	2.32	121	2.18	132	2.08	134	2.04	111	2.02	102	2.10	103	2.25
Protrip.	2 × 16	2 × 32	2 × 32	2 × 32	106	2.25	120	2.28	133	2.02	118	2.12	129	2.22	118	2.25	124	2.39	125	2.22
Thiorid.	2 × 8	2 × 16	2 × 16	2 × 16	116	2.08	118	2.08	121	2.10	109	2.15	106	2.13	105	2.13	107	2.22	116	2.52

* The data obtained with 2-oxoglutarate as substrate are shown. They are representative for all others obtained with succinate, β-hydroxybutyrate, and DL-glutamate.

Oxygen consumption in state III: nmoles oxygen mg protein min.

In parentheses: number of doses administered.

Table 3. Serum and myocardial tissue levels in rats receiving 2 × 32 mg/kg protriptylin per day

Number of doses	Time after last dose (hr)	Serum level (μg/ml)	Heart level (μg/g fresh tissue)
1 × 2	18	0.532 ± 0.041	2.695 ± 0.051
2 × 2	18	0.632 ± 0.022	6.701 ± 0.212
3 × 2	18	0.732 ± 0.060	7.925 ± 0.110
4 × 2	18	0.695 ± 0.035	8.330 ± 0.591

quence of membrane changes induced by the test compounds. It is well established that mitochondria undergo conformational changes during electron transfer and oxidative phosphorylation [21–28]. This implies that the enzyme proteins need a certain freedom of movement in the lipid bilayer in order to transfer charges within the electron transfer chain or in the enzyme sequence leading to fixation of energy in an ADP–P bond [29–33]. The accumulation of the highly lipid soluble drugs in the lipid bilayer renders the membrane less flexible and thus disturbs the function of the membrane-associated enzymes. It is also possible that the transfer of ATP, formed on the inside of the inner mitochondrial membrane, to the outside of the inner membrane (ATP-transferase reaction) is inhibited by the psychotropic drugs through their interaction with the lipids of the membrane. Such a disturbance is likely to occur, since these transfer reactions require a certain mobility of the proteins involved within the membrane [32, 34].

In the present experiment psychotropic drugs of three distinctly different classes (phenothiazines, tri- and tetracyclic antidepressants, butyrophenones) were used. In order to compare their effects at equitoxic levels, an attempt was made to treat the animals with maximally tolerated doses that had visible but not incapacitating effects on general behavior, e.g. sedation, nervousness, aggressiveness [11]. In an effort to judge the toxicological relevance of the biochemical findings obtained with such doses, drug concentrations were determined repeatedly in the serum and the heart muscle in rats treated with protriptyline. It was found that the concentrations in the serum increased rapidly during the first 2 days, but rose only slightly on continued treatment. In humans, steady state plasma levels are only reached after 3 weeks [35]. The maximal concentration of approximately 0.73 μg/ml observed in the rat experiment is about twice as high as the steady state plasma levels of protriptyline measured in patients treated with 0.90 mg/kg (60 mg per day) [35, 36]. In human overdose situations associated with serious cardiovascular side-effects considerably higher plasma levels (approximately 1.200 μg/ml) were found [37]. From these comparisons it is apparent that the doses used in the rat experiments induced serum levels that were higher than those reached in humans receiving therapeutic doses, but were well below those occurring after intentional or accidental overdosage.

The experiment with protriptyline also showed that high concentrations of the drug in the myocardial tissue were reached after the second day of treatment, and that there was only a slight increase on continued treatment. This is in good agreement with the observation that uncoupling of oxidative phosphorylation in heart mitochondria was established after the second day and did not change much on continued treatment.

The question must also be asked whether the effect of the psychotropic drugs on mitochondrial function is related to their cardiotoxic properties. Unfortunately, there are few studies in which the cardiovascular toxicity of these substances has been compared. In acute experiments in which drugs were injected intravenously every 15 sec into mice, amitriptyline proved to be considerably more cardiotoxic than protriptyline [7, 8]. In a similar experiment in rats imipramine was more cardiotoxic than chlorpromazine [38]. It is obvious from our data, however, that such marked differences between these drugs did not exist with regard to their effect on mitochondrial function. On the contrary, all compounds tested had a very similar uncoupling effect on oxidative phosphorylation in heart mitochondria. This is also true for the butyrophenone neuroleptic haloperidol which is rarely if ever associated with serious cardiovascular side-effects in man [2, 39]. The uncoupling of oxidative phosphorylation in heart mitochondria demonstrated in the rat experiments must, therefore, not be regarded as an indicator of potential cardiotoxicity, but as an expression of an altered membrane function resulting from the marked accumulation of these drugs in the lipid membranes.

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