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EFFECTS OF TRICYCLIC ANTIDEPRESSANT DRUGS ON ENERGY-LINKED REACTIONS IN MITOCHONDRIA

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Abstract—The effects of impramine and chlorimipramine on energy-linked reactions in mitochondria were characterized. Both compounds exhibited some characteristics of classical uncouplers of oxidative phosphorylation, i.e. they released respiratory control, hindered ATP synthesis, and enhanced ATPase activity of isolated rat liver mitochondria. Unlike classical uncouplers, however, these compounds only weakly stimulated proton uptake in intact mitochondria. They also exhibited unusual effects on energy-linked reactions in beef heart submitochondrial particles (SMP). Both compounds inhibited NADH oxidation in SMP in an "oligomycin-like" manner, and inhibited ATPase activity of SMP and the soluble F_1-ATPase. In contrast, the drugs weakly inhibited ATPase activities of bovine adrenal gland chromaffin granules and resealed granule ghosts. The mechanisms responsible for the multiple effects on mitochondrial energy-linked processes are unclear. They may be related to the hydrophobicity of the drugs, as has been shown for other hydrophobic amines.

There is abundant evidence that various drugs exert their pharmacological action by interfering with mitochondrial energy-linked functions [1, 2]. Among these drugs are numerous psychoactive compounds in clinical use that have been shown to uncouple oxidative phosphorylation in isolated mitochondria. This phenomenon was first reported by Maina [3] for reserpine, and subsequently by Bachmann and Zbinden [4] for several butyrophenones, phenothiazines, and tricyclic antidepressant drugs.

Earlier studies in our laboratory investigated the mechanism of action of numerous agents that uncouple oxidative phosphorylation in rat liver mitochondria (summarized in Ref. 5). Recently, our interest has focused on the psychoactive drugs. A previous study [6] explored in detail the effects of reserpine on isolated mitochondria and submitochondrial particles. Because of the unusual multiple effects of reserpine on bioenergetic processes, it was of interest for us to examine the effects of other psychoactive drugs. The tricyclic antidepressant compounds seemed of particular interest because of their potential for toxicity when given (or taken) at high doses. The present report describes the actions of imipramine and chlorimipramine, tricyclic antidepressants, on mitochondrial energy-linked reactions. The two compounds have multiple effects on these reactions similar to those shown by reserpine, and they differ in other respects from classical uncouplers of oxidative phosphorylation.

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MATERIALS AND METHODS

Chemicals

Imipramine was purchased from the Sigma Chemical Co. (St. Louis, MO), and chlorimipramine was obtained through the courtesy of the Ciba-Geigy Corp. (Summit, NJ). All other compounds were of the highest quality commercially available.

Mitochondria

Intact mitochondria were isolated from rat liver in 0.25 M sucrose as described previously [7]. Beef heart was the source of both submitochondrial particles isolated with EDTA [8] and the soluble F_1-ATPase [9].

Chromaffin granules

The P3 pellet was isolated from bovine adrenal glands [10]. Chromaffin granule ghosts were prepared from isolated granules by resealing after hypotonic lysis. Resealed ghosts were purified on a sucrose/^2H_2O gradient [11].

Assays

Oxygen consumption was determined polarographically with the Clark oxygen electrode [12]. Respiratory ratios and states were calculated by the method of Chance and Williams [13], modified as described previously [6]. Proton gradients of mitochondria were determined with a Gilson Oxygraph, model 5/6H (Gibson Medical Electronics, Middletown, WI). Drug effects on respiratory control induced by oligomycin in beef-heart submitochondrial particles were assessed as described by Lee and

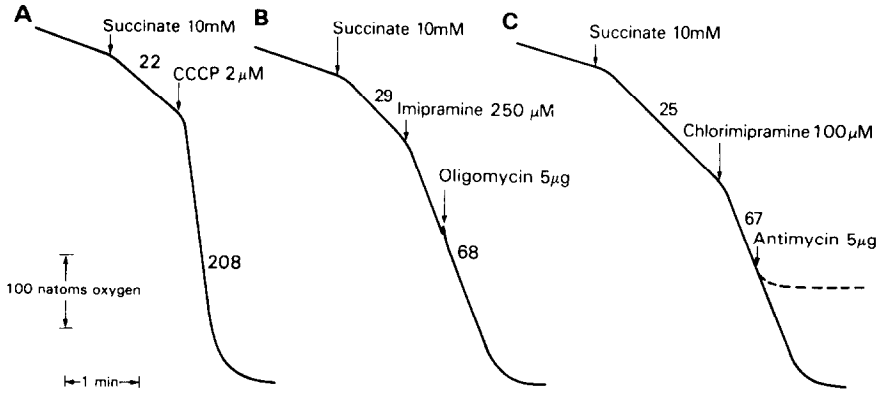

Fig. 1. Effects of imipramine and chlorimipramine on respiration of rat liver mitochondria: The release of respiratory control. The reaction mixture consisted of 20 mM HEPES (pH 7.4), 120 mM KCl, 5 mM $MgCl_2$, 15 mM potassium phosphate (pH 7.4), and 0.2 ml of mitochondria (4 mg protein; 50 mM sucrose), plus other additions as indicated, in a final volume of 1.0 ml. Succinate was added as the potassium salt. The numbers on the tracings express specific activities as natoms of oxygen consumed/min/mg protein at 24°.

Ernster [8]. NADH oxidation in these particles was determined spectrophotometrically at 340 nm. ATPase of intact rat liver mitochondria was assayed chemically by determining the amount of P_i released from ATP [14]. Soluble F_1-ATPase and ATPase activity of chromaffin granules and ghosts were assayed enzymatically by coupling the reaction to pyruvate kinase and lactate dehydrogenase systems and measuring NADH oxidation spectrophotometrically [15]. Other details are given in the legends to the tables and figures. All data were calculated as the average of at least three independent experiments.

Protein determinations

Soluble protein was determined by the procedure of Lowry *et al.* [16], and particulate protein by a modified biuret method [17]. Bovine serum albumin was the standard for both procedures.

RESULTS

Energy metabolism in intact rat liver mitochondria

Imipramine and chlorimipramine increased the rate of oxygen consumption by rat liver mitochondria in the presence of appropriate substrates. The effect was observed with succinate, as shown in panels B and C of Fig. 1, and with the NAD^+-linked substrates L-glutamate and DL-β-hydroxybutyrate. The increased respiration resembled that induced by classical uncouplers of oxidative phosphorylation, illustrated in panel A of Fig. 1 with carbonylcyanide 3-chlorophenylhydrazone (CCCP*). The increased rate of oxygen consumption was unaffected by oligomycin, and was inhibited by antimycin (Fig. 1, B and C). Both drugs, therefore, appeared to release respiratory control in intact mitochondria [18]. Chlorimipramine was the more potent of the two: a concentration of 52 μM was sufficient to produce one-half the maximal rate of uncoupled respiration, as compared with 88 μM for imipramine.

As is characteristic of classical uncouplers [19], both imipramine and chlorimipramine hindered the phosphorylation of ADP. The drugs, however, were less potent than CCCP, requiring much higher concentrations to release respiratory control (Fig. 1). They also adversely affected mitochondrial oxidative phosphorylation (Fig. 2). Both the ADP/O (P/O) ratios and the RCI values (Fig. 2A) were diminished in the presence of chlorimipramine, as shown here (Fig. 2B), and by imipramine (data not shown).

Similar to our findings with reserpine [6], but in contrast to the behavior of classical uncouplers [12], the uncoupling action of chlorimipramine was not counteracted by a 3-fold molar excess of defatted bovine serum albumin (data not shown). A large molar excess (6-fold) of the albumin partially protected intact rat liver mitochondria from uncoupling by chlorimipramine, but attempts to demonstrate specific binding of the drug to the serum protein by difference spectrophotometry [20] were not successful.

On the other hand, the antidepressant drugs appear to be tightly bound to mitochondria. As shown in Table 1, impairment of respiratory control could not be restored after five washings of mitochondria that had been reacted with these drugs.

Unlike CCCP and other classical uncouplers, imipramine and chlorimipramine were weakly effective in stimulating proton uptake by rat liver mitochondria which were actively accumulating calcium. Chlorimipramine, for example, elicited a slow reentry of protons during accumulation of calcium by intact mitochondria (Fig. 3). This effect was not the characteristic rapid action seen with CCCP and other known uncouplers.

Energy metabolism in submitochondrial particles

Uncoupling reagents released the oligomycin-induced respiratory control of beef heart sub-

* Abbreviations: CCCP, carbonylcyanide 3-chlorophenylhydrazone; FCCP, carbonylcyanide 4-trifluoromethoxyphenylhydrazone; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonate; and SMP, submitochondrial particles.

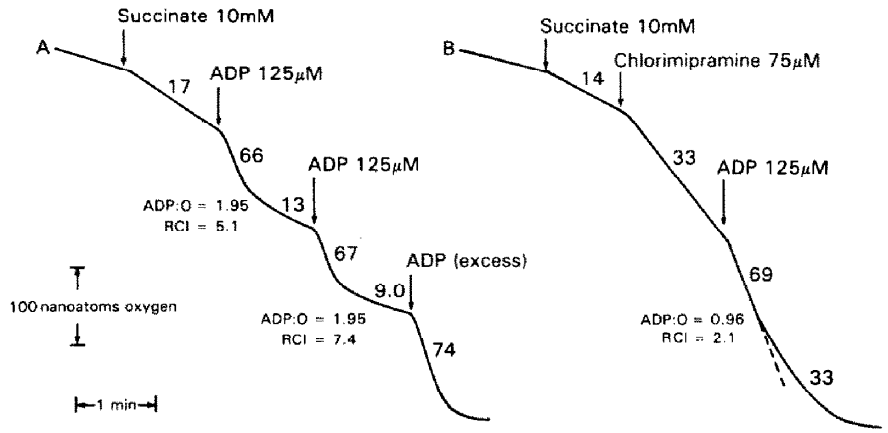

Fig. 2. Effect of chlorimipramine on oxidative phosphorylation. The composition of the reaction mixture was the same as given in the legend of Fig. 1. Tracing A is a control. Tracing B illustrates the effect of chlorimipramine on the respiratory response to ADP. RCI = respiratory control index [13].

Table 1. Binding of tricyclic drugs to mitochondria

Conditions	Respiratory control indicies after 1 to 5 washes					
	0	1	2	3	4	5
Control	4.3	4.2	4.5	4.1	3.2	3.3
Imipramine	2.2	2.2	1.9	2.0	1.7	1.4
Chlorimipramine	2.4	2.1	2.2	2.0	1.8	1.4

Suspensions of intact rat liver mitochondria (20 mg protein/ml) were divided into two equal portions. One portion was reacted with either imipramine (84 nmoles/mg protein) or chlorimipramine (42 nmoles/mg protein) which were dissolved in ethanol. An equal amount of ethanol was added to the other (control) portion of the mitochondrial suspension. Samples were removed for the initial (O-wash) assays after 3 min. Mitochondria were washed by successive centrifugations (8500 *g* for 10 min) and resuspensions in fresh media (0.25 M sucrose). Polarographic assays were done as described in the legend of Fig. 1. The experiments were repeated twice, and the numbers shown are average values.

mitochondrial particles which were oxidizing NADH [21]. In contrast, the tricyclic antidepressants *inhibited* the oxidation of NADH in these particles (Fig. 4) in an "oligomycin-like" manner. As seen here, the inhibition associated with both imipramine and chlorimipramine was reversed by the subsequent addition of FCCP. The inhibition obtained with higher concentrations of the drugs (200 μM imipramine and 100 μM chlorimipramine), however, was not reversed by FCCP.

The drugs did differ in their effects on the increased rate of NADH oxidation observed when

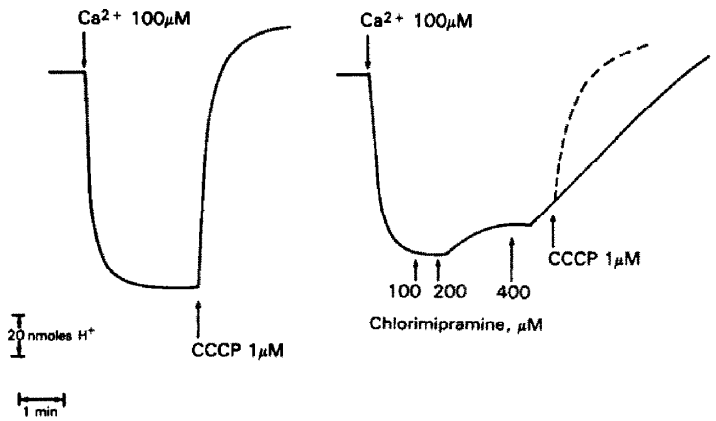

Fig. 3. Effect of chlorimipramine on proton uptake. The reaction mixture contained 3 mM HEPES (pH 7.0), 60 mM $LiCl_2$, 1.74 mM KCl and 0.2 ml rat liver mitochondria (4 mg protein) in a final volume of 2.0 ml. Other additions were made as indicated. Ca^{2+} was added as the chloride.

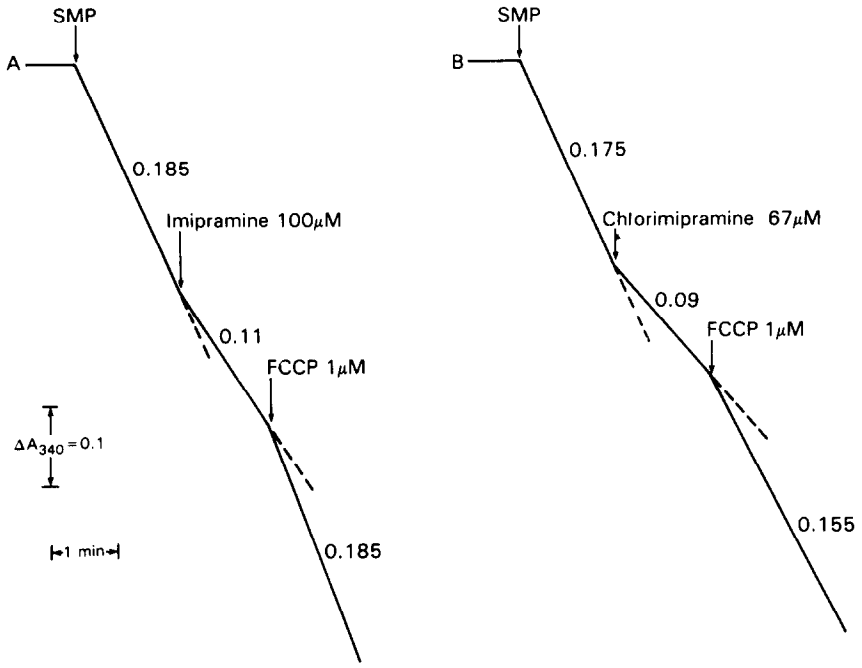

Fig. 4. Effect of imipramine on NADH oxidation in beef heart submitochondrial particles. The reaction mixture consisted of 50 mM Tris–Cl (pH 7.6), 0.2 mM NADH, and 250 mM sucrose, plus additions as indicated, in a final volume of 3.0 ml. Submitochondrial particles (SMP), 122 μg protein, were added to initiate the reaction which was determined at 30° by the decrease of absorbance at 340 nm in a recording spectrophotometer. The numbers on the tracing refer to ΔA per min per mg protein.

FCCP was present initially. Imipramine markedly inhibited the FCCP-stimulated oxidation (Fig. 5), whereas chlorimipramine had little effect on the uncoupler-stimulated oxidation (Fig. 6).

ATPase activities

Intact mitochondria. Classical uncouplers of oxidative phosphorylation enhance the hydrolysis of ATP in freshly isolated rat liver mitochondria [14, 19]. As summarized in Table 2, both imipramine and chlorimipramine increased the ATPase activity of intact mitochondria. Higher absolute rates of ATP hydrolysis were observed with imipramine when the concentrations of the two drugs were identical. At 500 μM (the highest concentration tested), the ability of chlorimipramine to enhance ATPase activity diminished, whereas that of imipramine continued to increase (Table 1). This finding suggested that chlorimipramine at the higher concentrations used in these experiments may have begun to inhibit the

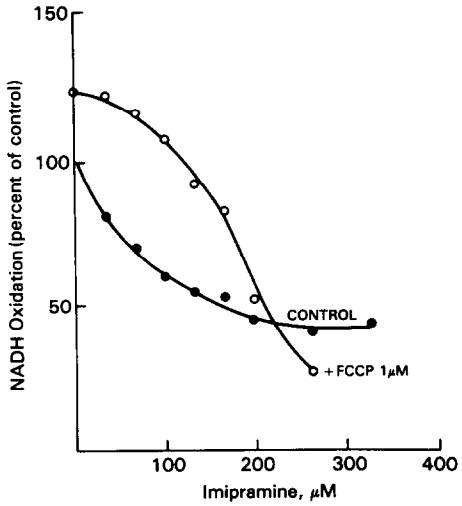

Fig. 5. Inhibition of NADH oxidation by imipramine. Experimental conditions were as described in the legend to Fig. 4.

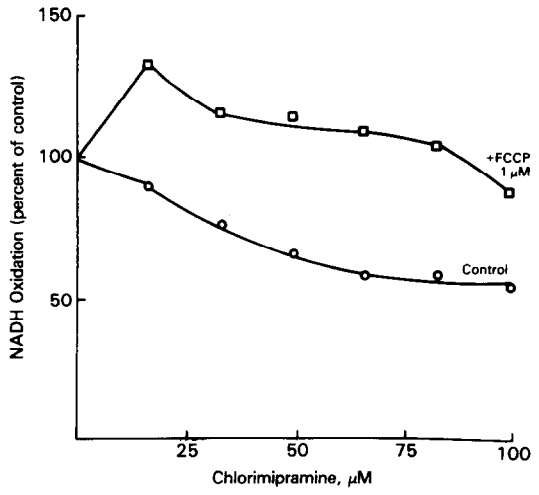

Fig. 6. Inhibition of NADH oxidation by chlorimipramine. The experimental conditions were as described in the legend to Fig. 4.

Table 2. Effects of tricyclic antidepressants on ATP hydrolysis in intact rat liver mitochondria

Concentration (μM)	P_i released from ATP (nmoles/min/mg protein)	
	Imipramine	Chlorimipramine
None (control)	11 ± 2	11 ± 2
50	24 ± 2	15 ± 2
100	40 ± 3	31 ± 1
200	51 ± 4	40 ± 5
500	77 ± 6	23 ± 2

The reaction mixture contained 60 mM Tris–Cl buffer (pH 7.4), 6 mM ATP, 0.1 ml of mitochondria (2 mg protein), other additions as indicated, and sufficient 0.25 M sucrose to make a final volume of 1.0 ml. Incubation time, 10 min; temperature, 30°. Values are mean ± S.E.M., N = 5.

mitochondrial ATPase. A similar phenomenon was observed with pentachlorophenol [14], a classical uncoupler of oxidative phosphorylation.

Beef heart submitochondrial preparations. Imipramine and chlorimipramine inhibited the ATPase activity of submitochondrial particles; 50% inhibition was observed at concentrations of 100 and 45 μM respectively (Fig. 7, A and B). Inhibition by imipramine was partially reversed by the subsequent addition of FCCP, while that caused by chlorimipramine was unaffected by addition of the uncoupler. With imipramine at 66 μM, addition of 1 μM FCCP decreased the inhibited ATPase activity from 33% of the control value to 15%. In the presence of 66 μM chlorimipramine, addition of 1 μM FCCP had no effect on the inhibited ATPase activity (i.e. it remained at 20% of the control level).

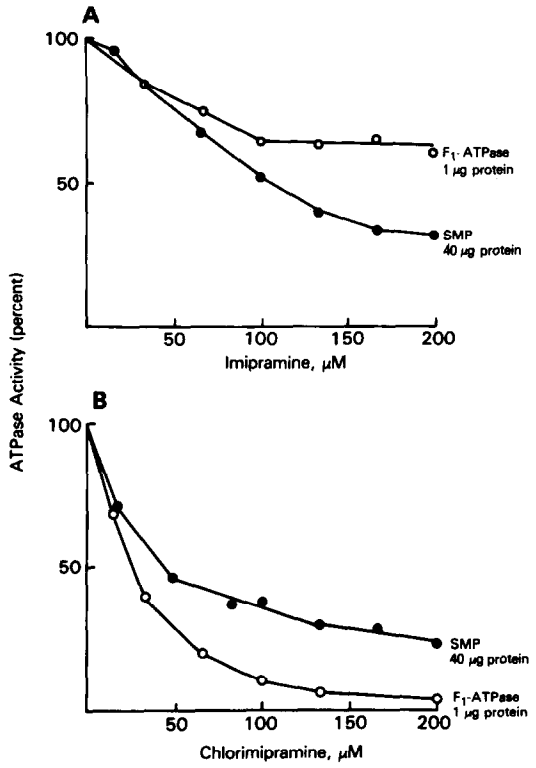

Fig. 7. Effects of tricyclic antidepressants on ATPase activities of beef heart submitochondrial particles and F_1-ATPase. The assay medium consisted of 30 mM Tris-acetate (pH 7.6), 25 mM potassium acetate, 3 mM magnesium acetate, 3 mM ATP, 10 mM phosphoenolpyruvate, 1.67 μM rotenone, 0.2 mM NADH, 25 units of lactate dehydrogenase and 55 units of pyruvate kinase, plus other additions as indicated, in a final volume of 3.0 ml. Decreases in absorbance at 340 nm were followed at 30° after the addition of either SMP or the soluble F_1-ATPase.

Table 3. Inhibitory effects of tricyclic antidepressants on electron transport and ATPase

Compound	IC_{50}* (μM)		
	NADH oxidase	ATPase	
		SMP	F_1
Imipramine	150	100	200
Chlorimipramine	100	45	27

* Concentration required for 50% inhibition.

F_1-ATPase. Both drugs also inhibited beef heart soluble F_1-ATPase. The enzyme was more sensitive to chlorimipramine than to imipramine (Fig. 7, A and B). Chlorimipramine at 27 μM inhibited the soluble ATPase by 50%, and at 200 μM by 92% (Fig. 7B). Imipramine, in contrast, produced its maximal inhibition (35%) at 100 μM; no further inhibition was observed at higher concentrations (Fig. 7A).

A summary of these effects on electron transport and ATPase activities of the beef heart mitochondrial preparations is provided in Table 3.

Chromaffin granules and ghosts. Although both drugs inhibited the ATPase activities of chromaffin granule preparations (Table 4), much higher concentrations were required than caused comparable inhibition of the SMP or soluble F_1-ATPase preparations. As with the other ATPase studied, chlorimipramine was the more effective of the two drugs, but the differences were less evident than those observed with mitochondria. For example, chlorimipramine, on a molar basis, was only 1.2

Table 4. Effect of tricyclic antidepressants on the ATPase activity of chromaffin granules

Compound	IC_{50} (μM)	
	Intact granules	Ghosts
Imipramine	500	>1000
Chlorimipramine	425	600
Quercetin	41	19

The composition of the assay medium was the same as that detailed in the legend to Fig. 7, plus additions as indicated. Temperature, 24°. The protein content of the intact granules was 800 μg/assay, and that of the ghosts, 475 μg. Approximately 75% of the granule proteins was released when the granules were lysed to form ghosts. Specific activities were: granules = 30, and ghosts = 15.5 nmoles ATP hydrolyzed/min/mg protein.

times more potent than imipramine as an inhibitor of chromaffin granule ATPase.

Inhibition of the ATPase activity of ghosts was considerably less than that of granules. In contrast, quercetin, a known inhibition of chromaffin granule ATPase [22], was more effective in inhibiting the ATPase activity of ghosts than of granules (Table 4).

DISCUSSION

The results reported here provide evidence that the tricyclic antidepressant drugs, imipramine and chlorimipramine, exhibit some characteristics of reserpine and classical uncouplers of oxidative phosphorylation. The tricyclic compounds released respiratory control, impeded synthesis of ATP, and enhanced the ATPase activity of isolated, intact rat liver mitochondria (Figs. 1 and 2 and Table 2). The two drugs had several properties that are distinct from those of classical uncouplers, however. First, they had a weak ability to collapse the proton gradient of intact mitochondria (Fig. 3). Second, both compounds inhibited NADH oxidation by submitochondrial particles (Figs. 4–6). Inhibition by the drugs was reversed by FCCP (Fig. 4), supporting the concept derived from the ATPase studies that the tricyclic compounds have an "oligomycin-like" effect on the inner mitochondrial membrane. Finally, the drugs inhibited soluble F_1-ATPase and, in addition, acted in an "oligomycin-like" fashion to inhibit membrane-bound ATPase.

Chlorimipramine on a molar basis was almost always more effective than imipramine in altering the enzymatic activities measured. Nevertheless, there were qualitative differences between the actions of the two drugs. Inhibition by imipramine of the membrane-bound ATPase was partially reversed in submitochondrial particles by FCCP, but the uncoupler did not appear to reactivate the ATPase activity of the chlorimipramine-inhibited enzyme in these particles. Marked inhibition of NADH oxidation in the presence of FCCP occurred with higher (200 μM) concentrations of imipramine, showing that this compound had a direct inhibitory effect on the electron transport chain. In contrast, chlorimipramine did not diminish the increased respiration stimulated by FCCP, and thus appears not to have inhibited the electron transport chain.

The effect of the tricyclic antidepressants to inhibit the ATPase activity of chromaffin granules and ghosts was qualitatively similar to that observed with beef heart mitochondrial preparations. Unlike quercetin, however, imipramine and chlorimipramine were much less effective in suppressing the hydrolysis of ATP. This is surprising in view of the similarity of the chromaffin granule ATPase and the mitochondrial oligomycin-sensitive ATPase complex [22].

The mechanism by which these drugs uncouple oxidative phosphorylation is unknown. Their weak protonophoric activity precludes their acting as classical weak acid uncouplers (e.g. CCCP), which cause uncoupling by promoting proton translocation [19]. These drugs, being hydrophobic amines, may mimic the weak acid uncouplers by forming lipophilic ion pairs and cause uncoupling by a mechanism similar to that proposed by Garlid and Nakashima [23].

Evidence adduced in this study demonstrates that the tricyclic antidepressants had multiple effects on mitochondrial bioenergetics. These effects are similar to the multiple sites of inhibition of mitochondrial electron transport by local anesthetics [24]. The drugs appear to be tightly bound to mitochondria (Table 1), presumably bound to mitochondrial protein (cf. Ref. 25). Hydrophobic interactions with these proteins may account for the enzyme inhibition observed with electron transport and ATPase (Table 3). Such interactions also could lead to uncoupling by altering intermembrane processes that mediate energy transfer between electron transport and ATPase as suggested for the action of general anesthetics by Rottenberg [26].

At present, there are no data as to whether or not the two drugs affect energy-linked processes *in vivo*. Their abilities to uncouple mitochondrial oxidative phosphorylation *in vitro* were not eliminated by repeated washings. Thus, it is possible that sufficient drug accumulated in neuronal mitochondria during chronic dosing to produce significant effects on energy metabolism.

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