

# *Acta Medica Okayama*

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*Volume 39, Issue 4*

1985

*Article 5*

AUGUST 1985

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## Abstract

The effects of tricyclic drugs (clomipramine, imipramine, chlorpromazine and promethazine) on isolated liver mitochondria of rats were examined. All the drugs tested accelerated state 4 respiration. Their stimulative potency at concentrations below 100 microM was in the order of chlorpromazine greater than clomipramine greater than imipramine, promethazine. On state 3 respiration, the chlorine containing drugs had an inhibitive effect at high concentrations, while the other drugs seemed to have a slightly stimulative effect. These drugs stimulated latent ATPase activity of mitochondria. Clomipramine and chlorpromazine inhibited 2, 4-dinitrophenol-stimulated ATPase activity in a dose-dependent fashion. Imipramine also inhibited 2, 4-dinitrophenol-stimulated ATPase activity at high concentrations. Promethazine, however, had almost no effect. All the drugs induced potassium release from mitochondrial vesicles, and their potency was in the order of clomipramine greater than chlorpromazine greater than imipramine greater than promethazine. These results suggest that clomipramine, imipramine, chlorpromazine and promethazine cause impediments in both mitochondrial respiration and ion compartmentation, and that the chlorine containing drugs are more toxic than others on the functions of the mitochondrial membrane.

**KEYWORDS:** tricyclic drugs, mitochondria, oxidative phosphorylation, potassium release, ATPase activity

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\*PMID: 2931948 [PubMed - indexed for MEDLINE]

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## EFFECT OF TRICYCLIC DRUGS ON MITOCHONDRIAL MEMBRANE

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*Received February 1, 1985*

*Abstract.* The effects of tricyclic drugs (clomipramine, imipramine, chlorpromazine and promethazine) on isolated liver mitochondria of rats were examined. All the drugs tested accelerated state 4 respiration. Their stimulative potency at concentrations below 100  $\mu$ M was in the order of chlorpromazine > clomipramine > imipramine, promethazine. On state 3 respiration, the chlorine containing drugs had an inhibitive effect at high concentrations, while the other drugs seemed to have a slightly stimulative effect. These drugs stimulated latent ATPase activity of mitochondria. Clomipramine and chlorpromazine inhibited 2, 4-dinitrophenol-stimulated ATPase activity in a dose-dependent fashion. Imipramine also inhibited 2, 4-dinitrophenol-stimulated ATPase activity at high concentrations. Promethazine, however, had almost no effect. All the drugs induced potassium release from mitochondrial vesicles, and their potency was in the order of clomipramine > chlorpromazine > imipramine > promethazine. These results suggest that clomipramine, imipramine, chlorpromazine and promethazine cause impediments in both mitochondrial respiration and ion compartmentation, and that the chlorine containing drugs are more toxic than others on the functions of the mitochondrial membrane.

*Key words:* tricyclic drugs, mitochondria, oxidative phosphorylation, potassium release, ATPase activity.

Tricyclic drugs have been widely used in the therapy of mental disorders. In the case of serious psychoses, long-term medication with high doses of these drugs can bring about mental and physical impediments. Obstructive jaundice has been reported during treatment with chlorpromazine (CPZ) and imipramine (IMP), but a dose-relation has not been made clear (1, 2). Mechanisms of hepatic damage due to tricyclic drugs have also been discussed by several authors (3-7). In this study, we examined *in vitro* the effects of clomipramine (CLP) and IMP with reference to the effects CPZ and promethazine (PMZ) on the biological membrane. The present data indicate that the tricyclic drugs tested influence oxidative phosphorylation and other functions of isolated liver mitochondria of rats.

### MATERIALS AND METHODS

*Animals and preparation of mitochondria.* Male Donryu rats weighing approximately 200 g

and fed on a laboratory stock diet were fasted overnight and sacrificed by decapitation. Liver mitochondria were prepared according to the modified (8) method of Hogeboom and Schneider (9) in a medium containing 0.25 M sucrose, 0.2 mM EDTA and 4 mM Tris-HCl buffer (pH 7.4). The isolated mitochondria were resuspended and washed twice with the above medium less EDTA. The concentration of mitochondrial protein was determined by the biuret reaction using bovine serum albumin as a standard.

*Measurement of oxygen uptake and oxidative phosphorylation.* Mitochondrial suspension was incubated in 3.5 ml of reaction medium containing 0.15 M KCl, 10 mM Tris-HCl buffer (pH 7.4), 5 mM MgCl<sub>2</sub> and 2.5 mM phosphate buffer (pH 7.4). The drugs tested were dissolved in distilled water and added to the above reaction mixture, followed 1 min later by 5 mM sodium succinate as a respiratory substrate, and then 0.3 mM ADP as a substrate for phosphorylation and 0.02 mM 2, 4-dinitrophenol (DNP) at regular intervals. The incubation was carried out at 25 °C with continuous stirring. Oxygen consumption was measured according to the method of Hagihara (10) with a galvanic type electrode (Kyusui Kagaku Co.,) connected to a recorder. The experimental results were evaluated statistically by means of Student's t-test.

*Measurement of mitochondrial ATPase activity.* Mitochondrial latent and DNP-stimulated ATPase activities were measured as follows: A reaction medium containing 0.2 M sucrose, 20 mM KCl, 3 mM MgCl<sub>2</sub> and 5 mM Tris-HCl buffer (pH 7.4) was mixed with 5 mM disodium ATP and mitochondria (approximately 2-4 mg protein) in a final volume of 2 ml. Incubation was carried out at 25 °C for 15 min. One milliliter of ice-cold perchloric acid (24 %) was added, mixed and left standing for 10 min. The supernatant solution was separated by centrifugation. An aliquot of the solution was used as material for determination of liberated inorganic phosphate according to the method of Takahashi (11).

*Measurement of K<sup>+</sup> release.* The K<sup>+</sup> concentration change of the reaction medium during incubation of mitochondria was measured at 25 °C with a K<sup>+</sup> electrode (Beckman) connected to a pH meter. The reaction medium contained 150 mM choline chloride and 10 mM Tris-HCl buffer (pH 7.5).

*Reagents.* Clomipramine HCl and imipramine HCl were donated by CIBA-GEIGY Corp., and chlorpromazine HCl and promethazine HCl were generous gifts from Shionogi. ADP and ATP were commercial products of reagent grade.

## RESULTS

*Effect of isolated mitochondria on respiratory activity.* The effects of the tricyclic drugs on the respiratory activity of isolated liver mitochondria of rats are shown in Table 1 and Fig. 1. As illustrated in Fig. 1, all the drugs tested stimulated state 4 respiration. However, there were certain differences among the mode of the stimulation of state 4 respiration by each drug, *i.e.*, state 4 respiration was enhanced by CLP and IMP with increasing concentrations up to 200 μM, while the enhancement of state 4 respiration by CPZ and PMZ was maximum at 100 μM and 150 μM, respectively. The degree of the stimulative effect of these drugs at concentrations below 100 μM on state 4 respiration was in the descending order of CPZ, CLP, and IMP and PMZ. On state 3 respiration, both IMP and PMZ seemed to have a slightly accelerative effect. On the contrary, higher concentrations (> 150 μM) of CLP and CPZ lowered state 3 respiration. Deterioration of the RCI and ADP/O ratio was caused by all the drugs tested.

TABLE 1. EFFECT OF TRICYCLIC DRUGS ON MITOCHONDRIAL RESPIRATORY ACTIVITY

Experiment 1 (2.21 mg protein/ml)					
Clomipramine ( $\mu\text{M}$ )	None	50	100	150	200
Oxygen uptake <sup>a</sup> State 3	85.0 $\pm$ 5.1	89.9 $\pm$ 4.4	82.1 $\pm$ 0.6	80.7 $\pm$ 2.9	72.5 $\pm$ 2.7*
State 4	17.7 $\pm$ 0.9	31.0 $\pm$ 0.4	35.3 $\pm$ 3.0	41.7 $\pm$ 1.9	46.2 $\pm$ 4.8
RCI <sup>b</sup>	4.83 $\pm$ 0.53	2.90 $\pm$ 0.11	2.33 $\pm$ 0.20	1.94 $\pm$ 0.15	1.58 $\pm$ 0.15
ADP/O ratio	2.01 $\pm$ 0.06	1.60 $\pm$ 0.11	1.40 $\pm$ 0.04	1.22 $\pm$ 0.06	1.05 $\pm$ 0.07
Experiment 2 (2.21 mg protein/ml)					
Imipramine ( $\mu\text{M}$ )	None	50	100	150	200
Oxygen uptake <sup>a</sup> State 3	79.5 $\pm$ 1.6	85.6 $\pm$ 1.4	82.5 $\pm$ 2.3	83.7 $\pm$ 2.2	82.9 $\pm$ 1.8
State 4	16.7 $\pm$ 0.9	24.0 $\pm$ 1.9	27.1 $\pm$ 1.3	32.1 $\pm$ 1.2	37.2 $\pm$ 2.9
RCI <sup>b</sup>	4.76 $\pm$ 0.17	3.57 $\pm$ 0.26	3.08 $\pm$ 0.23	2.61 $\pm$ 0.12	2.24 $\pm$ 0.18
ADP/O ratio	2.35 $\pm$ 0.11	1.97 $\pm$ 0.12	1.72 $\pm$ 0.12	1.58 $\pm$ 0.08	1.42 $\pm$ 0.07
Experiment 3 (1.70 mg protein/ml)					
Chlorpromazine ( $\mu\text{M}$ )	None	50	100	150	200
Oxygen uptake <sup>a</sup> State 3	78.8 $\pm$ 7.6	88.6 $\pm$ 2.0	85.2 $\pm$ 5.8	75.8 $\pm$ 4.0	64.2 $\pm$ 0.6**
State 4	15.7 $\pm$ 1.6	31.6 $\pm$ 4.1	39.4 $\pm$ 2.7	31.9 $\pm$ 2.7	26.6 $\pm$ 3.0
RCI <sup>b</sup>	5.01 $\pm$ 0.02	2.83 $\pm$ 0.31	2.17 $\pm$ 0.11	2.39 $\pm$ 0.31	2.44 $\pm$ 0.27
ADP/O ratio	1.99 $\pm$ 0.11	1.83 $\pm$ 0.07	1.25 $\pm$ 0.11	1.05 $\pm$ 0.03	0.89 $\pm$ 0.06
Experiment 4 (1.61 mg protein/ml)					
Promethazine ( $\mu\text{M}$ )	None	50	100	150	200
Oxygen uptake <sup>a</sup> State 3	105.8 $\pm$ 10.5	118.0 $\pm$ 11.1	123.3 $\pm$ 3.6	118.6 $\pm$ 2.6	117.0 $\pm$ 7.8
State 4	20.4 $\pm$ 1.0	28.2 $\pm$ 2.8	33.4 $\pm$ 4.3	40.4 $\pm$ 3.3	37.4 $\pm$ 3.1
RCI <sup>b</sup>	5.19 $\pm$ 0.48	4.20 $\pm$ 0.50	3.73 $\pm$ 0.52	2.94 $\pm$ 0.21	3.15 $\pm$ 0.44
ADP/O ratio	1.81 $\pm$ 0.25	1.47 $\pm$ 0.10	1.30 $\pm$ 0.11	1.28 $\pm$ 0.11	1.19 $\pm$ 0.12

*a*: natoms oxygen/min/mg protein.

*b*: RCI: Respiratory Control Index (State 3/State 4).

Experiments 1 (2), 3 and 4 are different in their mitochondrial source. Each value represents the mean  $\pm$  standard deviation ( $\pm$  S.D.) of three determinations. \* and \*\* are significant difference from the control (\*:  $p < 0.02$ , \*\*:  $p < 0.05$ ).

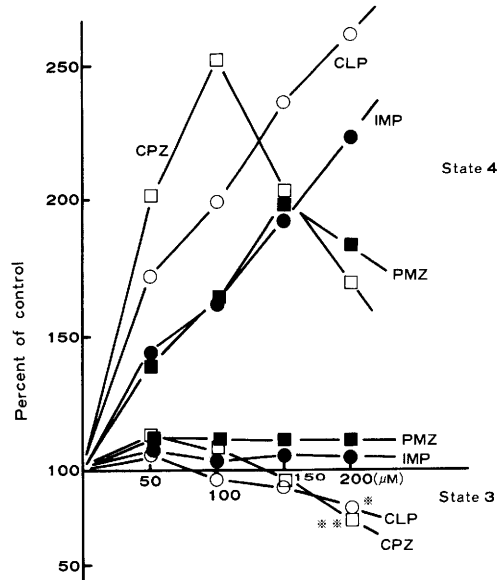


Fig. 1. Effect of tricyclic drugs on state 3 and state 4 respiration of mitochondria. The symbols \* and \*\* indicate significant difference from the control (\* :  $p < 0.02$ , \*\* :  $p < 0.05$ ), and the following abbreviations are used: CLP, clomipramine; IMP, imipramine; CPZ, chlorpromazine; PMZ, promethazine.

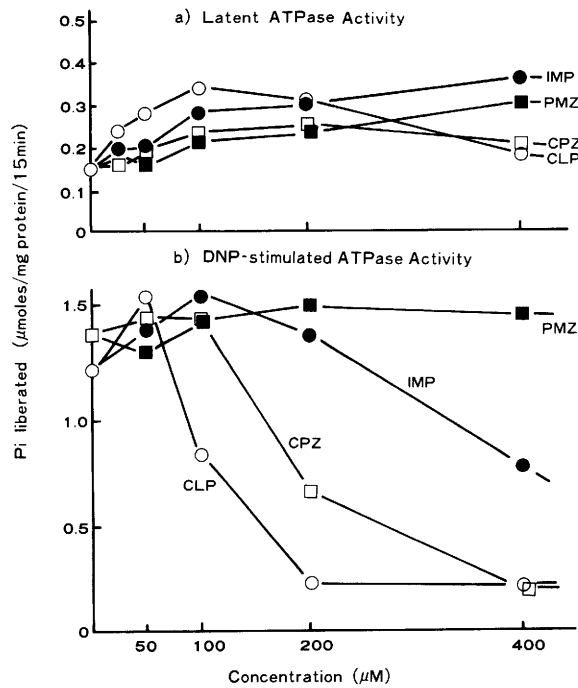


Fig. 2. Effect of tricyclic drugs on mitochondrial ATPase activity. 1.41 to 1.78 mg/ml of mitochondrial protein was used. Each point represents the mean of three determinations.

*Effect on mitochondrial ATPase activity.* In intact mitochondria, latent ATPase activity is negligible. However, it is stimulated by uncoupler, surfactant or mechanical damage to membranes. This stimulated ATPase activity is inhibited by energy transfer inhibitors such as oligomycin (12) and not affected by electron transport inhibitors such as  $\text{CN}^-$  or antimycin A. To elucidate the mode of action of these tricyclic drugs on mitochondria, the effect on ATPase activity was examined. As shown in Fig. 2, all the drugs tested stimulated latent ATPase activity, though the stimulation decreased with increasing concentrations in the cases of CLP and CPZ. DNP-stimulated ATPase activity was inhibited by CLP and CPZ at high concentrations. IMP also inhibited DNP-stimulated ATPase activity at high concentrations. However, PMZ had almost no effect.

*Effect on the membrane permeability of mitochondria.* Various substances such as certain metal ions and phosphate ions are usually maintained in intact mitochondria, but when the membrane is damaged, such substances leak from the mitochondrial vesicles. Therefore the effect of these tricyclic drugs on the  $\text{K}^+$ -compartmentation of mitochondria, which is closely related to structural and functional changes of mitochondria, was examined. As shown in Fig. 3, all of the drugs (200  $\mu\text{M}$ ) induced a rapid release of intramitochondrial  $\text{K}^+$ , the potency was in the order of  $\text{CLP} > \text{CPZ} > \text{IMP} > \text{PMZ}$ .

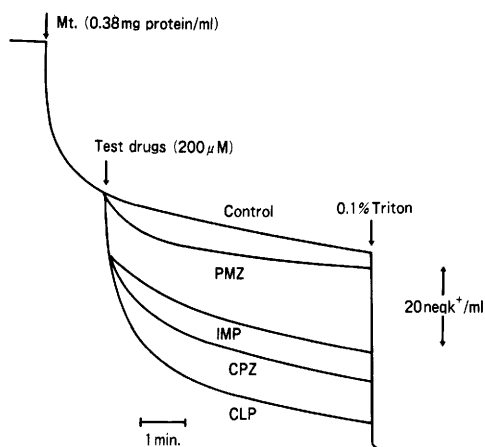


Fig. 3. Effect of tricyclic drugs on the  $\text{K}^+$ -compartmentation of rat liver mitochondria. The mitochondrial protein concentration was 0.38 mg protein/ml.

#### DISCUSSION

There have been a number of reports describing both *in vivo* and *in vitro* effects of various chemical compounds on mitochondrial oxidative phosphorylation. However, it is not so easy to classify clearly these substances according to their mode of action on mitochondria. The tricyclic drugs tested in this study affected mito-

chondrial oxidative phosphorylation and ATPase activity, though there were certain differences in the mode of action among them. Increase in state 4 respiration, decrease in RCI and ADP/O ratio, and stimulation of latent ATPase activity by these tricyclic drugs suggest their uncoupling action.

On the other hand, a high concentration ( $> 150 \mu\text{M}$ ) of CLP or CPZ inhibited state 3 respiration, while these two drugs stimulated state 4 respiration. These results may be due to the inhibitive effect of the drugs on energy transfer reactions. Matsubara and Hagihara (7) observed that phenothiazine derivatives act as uncouplers at lower concentrations, but as electron transfer inhibitors at higher concentrations.

Our data on CPZ agree with their report in that CPZ stimulates state 4 respiration and inhibits state 3 respiration. Differences between CLP and IMP, and CPZ and PMZ in their action on mitochondrial respiratory activity may be due to the presence or absence of chlorine in the molecules.

Ogata and Hasegawa (13) have reported that a chlorinated aromatic hydrocarbon,  $\alpha$ -chloronaphthalene, affects oxidative phosphorylation in isolated rat liver mitochondria and acts both as an uncoupler and energy transfer inhibitor.

According to the classification of various inhibitors of mitochondrial respiration by Ogata *et al.* (14), CLP and CPZ are classified as energy transfer inhibitors as well as uncouplers, and IMP and PMZ as uncouplers. The potassium releasing action of these tricyclic drugs on mitochondrial vesicles is related mostly to their hydrophobicity. Their order of potassium releasing potency parallels their action on DNP-stimulated ATPase activity. Ahtee and Passonen (15) described the haemolytic effect of IMP, CPZ and other phenothiazine derivatives. Haemolysis of red cells of rabbits was induced by these drugs.

Yasuhara *et al.* (4, 5) has reported that the surface activity of tricyclic antidepressants may play a role in their hepatotoxic properties. In these reports, they pointed out that the cytotoxic potency of CLP was greater than that of IMP. Matsuo also indicated that the ranking order of surface activity of tricyclic drugs was:  $\text{CLP} \geq \text{CPZ} > \text{IMP}$  (6).

Our findings concerning the effect of these drugs on mitochondrial membrane agree with these reports. Moreover, antimitochondrial antibody was detected in persons who developed jaundice after treatment with CPZ (1), and hepatic damage seemed due to the allergic action which occurred after therapy with IMP and desipramine (16).

Based on the above observations, the present data suggest that CLP, IMP, CPZ and PMZ cause impediments in both mitochondrial respiration and ion compartmentation, and that chlorine containing drugs have stronger actions than those which do not contain chlorine. As for the therapeutic use of tricyclic drugs, in addition to allergic actions, it is also necessary to take into consideration their functional effects.



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