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# INOTROPIC ACTION OF HYDROXYLATED CHLORPROMAZINE METABOLITES AND RELATED COMPOUNDS*

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**Abstract**—Effects of several hydroxylated chlorpromazine metabolites and related compounds on cardiac contractile force were studied using electrically stimulated left atrial preparations of guinea pig hearts. 7,8-Dihydroxychlorpromazine and 7,8-dioxochlorpromazine produced marked positive inotropic effects in $\mu$M concentrations. 7,8-Dihydroxyperphenazine and 7-hydroxychlorpromazine had relatively weak inotropic actions. 2-Hydroxydesmethylimipramine also had a weak inotropic action. Other compounds, such as chlorpromazine, 8-hydroxychlorpromazine, 2-hydroxy- or 3-hydroxy-promazine, and 2-hydroxyimipramine, failed to significantly alter cardiac contractile force. The positive inotropic effects of 7,8-dihydroxychlorpromazine or 7,8-dioxochlorpromazine were markedly reduced by pretreatment with (±)-propranolol, indicating the involvement of $\beta$-adrenergic mechanisms in the inotropic action of these agents.

Cardiovascular actions of chlorpromazine and related phenothiazine derivatives may be generally characterized as inhibitory. The mechanisms of such actions are complex since these agents act directly on the heart and blood vessels, and also indirectly on the central nervous system and peripheral autonomic systems to modify cardiovascular function [1]. These actions of chlorpromazine may be complicated further by the presence of pharmacologically active metabolites [2–6] which are produced during an extensive biotransformation of this compound [2, 7–11].

One of such active metabolites of chlorpromazine is 7,8-dihydroxychlorpromazine. This compound has been shown to inhibit isolated $Na^+$,$K^+$-ATPase [12] and adenylate cyclase [13], to prevent carbachol-induced rise in cyclic AMP concentrations in rat brain slices [14], and to release calcium ions from isolated mitochondria [3, 15]. It also produces a positive inotropic effect in the isolated heart [12]. The present study was initiated to investigate the cardiac actions of several hydroxylated chlorpromazine metabolites and structurally related compounds (Fig. 1).

## METHODS

Guinea pigs of either sex weighing 350–500 g were stunned by cervical dislocation. Their hearts were immediately removed, and left atrial preparations were suspended vertically between a pair of platinum field stimulation electrodes as described by Levy [16] in a bath containing Krebs–Henseleit solution of the following mM composition: NaCl, 118.0; $NaHCO_3$, 27.2; KCl, 4.8; $MgSO_4$, 1.2; $KH_2PO_4$, 1.0; $CaCl_2$, 2.5; and glucose, 11.1 [17]. The solution was aerated with a 95% $O_2$–5% $CO_2$ gas mixture which produced the pH value of 7.4 and was maintained at 30 ± 0.1°. Atrial preparations were electrically stimulated at 1 Hz with square-wave pulses of 3-msec duration at a voltage not exceeding 15 per cent above threshold. Isometric contractile force was recorded with a force displacement transducer (Grass Instruments Co., Quincy, MA; model FT-03C) attached to a Polygraph recorder. Resting tension was adjusted to 1.0 g. After a 60-min equilibration period, the effect of one concentration of a test drug was studied in each preparation.

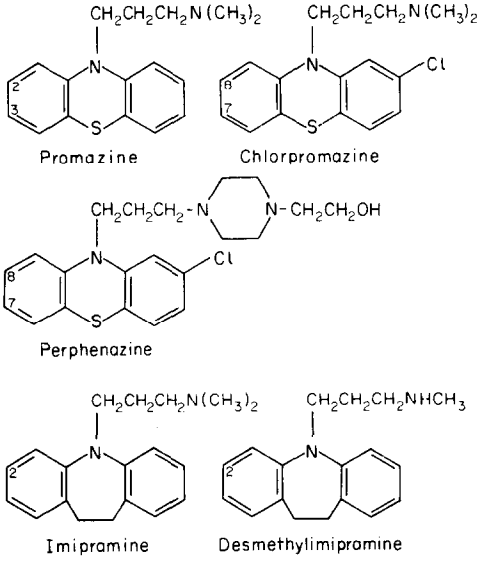


Fig. 1. Chemical structure of parent compounds. Positions on the tricyclic ring structure which may be modified are numbered.

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In one series of experiments, (±)-propranolol was added to the medium (final concn, 10 μM) 20 min before the addition of the test drug. Preliminary experiments indicated that this concentration of propranolol shifts the dose–response curve for isoproterenol to the right by approximately an order of magnitude under these conditions. Changes in isometric contractile force produced by the test drug were expressed as per cent of the contractile force observed immediately prior to drug addition.

When 7,8-dihydroxychlorpromazine and 7,8-dioxochlorpromazine (Fig. 1) were dissolved in distilled water, they formed dark red solutions. Monohydroxy-derivatives of chlorpromazine or promazine, 7,8-dihydroxyperphenazine, 2-hydroxyimipramine and 2-hydroxydesmethylimipramine were dissolved in slightly acidic medium and formed clear colorless solutions.

Chlorpromazine was a gift from Dr. Harry Green of Smith, Kline & French Laboratories, Philadelphia, PA. Hydroxylated derivatives of phenothiazines and tricylic antidepressants were obtained from Psychopharmacology Research Branch, National Institute of Mental Health, Rockville, MD. Other chemicals were of reagent grade. Statistical significance of the data was analyzed by Student's *t*-test.

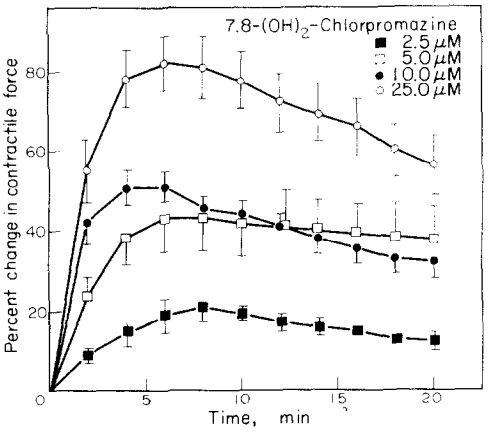


Fig. 2. Effects of various concentrations of 7,8-dihydroxychlorpromazine on the cardiac contractile force. Left atrial preparations of guinea pig hearts were electrically stimulated at 1 Hz. After a 60-min equilibration period at 30°, a test drug was added to the medium at time zero. The changes in the isometric contractile force are expressed as a percentage of the value observed immediately before the addition of the drug. A vertical line indicates standard error of the mean. Average contractile force before the addition of drug was 0.85 g. Each point represents the mean of six experiments.

## RESULTS

After a 60-min equilibration period, contractile force of electrically stimulated guinea pig left atrial preparations was stable for at least 120 min. Under the present experimental conditions, chlorpromazine (final concn, 10 or 100 μM) failed to affect cardiac contractile force during a 20-min observation period (data not shown). The addition of 7-monohydroxychlorpromazine (final concn, 5 μM) produced a slight, gradual increase in contractile force (Table 1). The positive inotropic effect of 8-monohydroxychlorpromazine was smaller than that of 7-monohydroxychlorpromazine. Monohydroxylated metabolites of promazine; 3-hydroxypromazine or 2-hydroxypromazine failed to produce a significant change in cardiac contractility at 5 μM concentrations. At this same concentration (5 μM), 7,8-dihydroxychlorpromazine and 7,8-dioxochlorpromazine produced a marked increase in cardiac contractile force which developed rather rapidly, reaching the maximum at approximately 7 min (Table 1). 7,8-Dihydroxyperphenazine (5 μM), however, produced only a slight positive inotropic effect which developed relatively rapidly (Table 1).

The positive inotropic action of 7,8-dihydroxychlorpromazine was dependent on the concentration of this agent over the range of 2.5 to 25 μM (Fig. 2). The action of 7,8-dihydroxychlorpromazine on

Table 1. Inotropic effects of hydroxylated derivatives of phenothiazines and tricyclic antidepressants*

| Compound | No. of experiments | Control force | Inotropic response† (per cent changes in contractile force) 4 min | Maximal change | 20 min |
|---|---|---|---|---|---|
| 7-Hydroxychlorpromazine | 4 | 0.74 ± 0.29 | 6.8 ± 1.0‡ | 19.0 ± 3.2‡ (20 min)§ | 19.0 ± 3.2‡ |
| 8-Hydroxychlorpromazine | 4 | 0.59 ± 0.11 | 5.9 ± 0.8‡ | 8.4 ± 1.8* (20 min) | 8.4 ± 1.8‡ |
| 3-Hydroxypromazine | 4 | 0.88 ± 0.21 | 3.6 ± 4.4 | 3.9 ± 6.1 (10 min) | 3.0 ± 6.0 |
| 2-Hydroxypromazine | 4 | 0.79 ± 0.11 | −1.3 ± 0.5 | −6.4 ± 2.1 (16 min) | −5.4 ± 2.2 |
| 7,8-Dihydroxychlorpromazine | 5 | 0.91 ± 0.14 | 38.7 ± 7.4‡ | 43.4 ± 8.5* (8 min) | 37.6 ± 8.7‡ |
| 7,8-Dioxochlorpromazine | 6 | 0.82 ± 0.12 | 34.9 ± 5.7‡ | 36.9 ± 6.9‡ (6 min) | 23.1 ± 9.6 |
| 7,8-Dihydroxyperphenazine | 5 | 0.73 ± 0.12 | 9.4 ± 4.9 | 16.2 ± 8.0 (14 min) | 14.0 ± 8.7 |
| 2-Hydroxyimipramine | 4 | 0.88 ± 0.28 | 0.6 ± 3.4 | −10.5 ± 6.2 (20 min) | −10.5 ± 6.2 |
| 2-Hydroxydes-methylimipramine | 4 | 0.85 ± 0.15 | 23.8 ± 7.7‡ | 23.8 ± 7.7‡ (4 min) | 20.3 ± 10.1 |

* Left atrial preparations of guinea pig hearts were electrically stimulated at 1 Hz. After a 60-min equilibration period at 30°, a test drug (final concn, 5 μM) was added to the medium. The changes in the isometric contractile force are expressed as a percentage of the value observed immediately before the addition of drug. Values are mean ± S. E. M.

† Per cent changes in contractile force after drug addition. Negative values indicate decrease in contractile force.

‡ Significantly different from zero ($P < 0.05$).

§ Time when the maximal change in contractile force was observed.

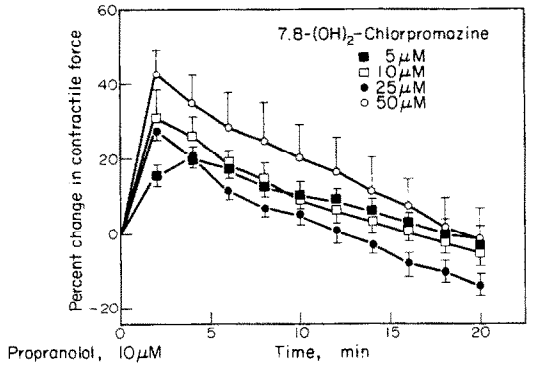


Fig. 3. Effects of various concentrations of 7,8-dihydroxychlorpromazine on the cardiac contractile force in the presence of propranolol. See legend to Fig. 2. In these experiments, (±)-propranolol (final concn, 10 μM) was added to the medium after the equilibration period and the test drug was added 20 min later (time zero) when the contractile force was stabilized at a level approximately 20 per cent lower than that observed before the addition of propranolol. Changes in cardiac contractile force induced by 7,8-dihydroxychlorpromazine are expressed as a percentage of the contractile force observed immediately before the addition of 7,8-dihydroxychlorpromazine. Average contractile force before the addition of 7,8-dihydroxychlorpromazine was 0.76 g. Each point represents the mean of six experiments.

contractile force appeared to be transient: an initial rapid increase in cardiac contractile force was followed by a gradual decrease in the contractile force. After a 60-min incubation, however, the cardiac contractile force in the presence of 25 μM 7,8-dihydroxychlorpromazine was greater than that observed before the addition of this agent (data not shown). It should be noted that 7,8-dihydroxychlorpromazine did not induce arrhythmias at these concentrations.

Addition of (±)-propranolol (final concn, 10 μM) after the equilibration period produced a gradual decrease in cardiac contractile force which stabilized approximately 20 min after addition. At this time, cardiac contractile force was approximately 20 per cent lower than that observed prior to propranolol addition. In the presence of propranolol, 7,8-dihydroxy-

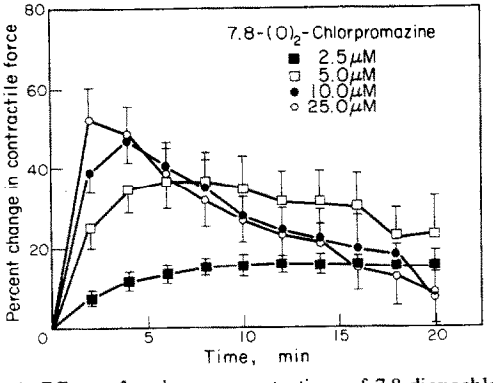


Fig. 4. Effects of various concentrations of 7,8-dioxochlorpromazine on the cardiac contractile force. See legend to Fig. 2. 7,8-Dioxochlorpromazine was added to the incubation medium in the absence of propranolol. Average control contractile force was 0.82 g. Each point represents the mean of six experiments.

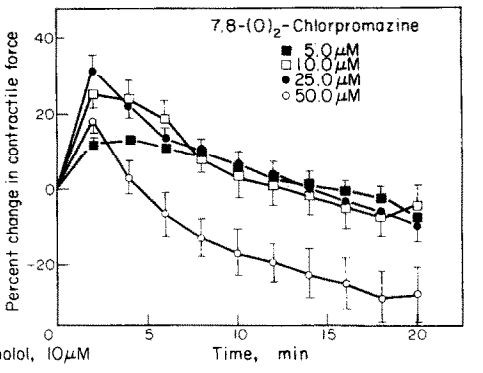


Fig. 5. Effects of various concentrations of 7,8-dioxochlorpromazine on the cardiac contractile force in the presence of propranolol. See legend to Fig. 5. 7,8-Dioxochlorpromazine was added to the incubation medium in the presence of propranolol. Average contractile force before the addition of 7,8-dioxochlorpromazine was 0.76 g. Each point represents the mean of six experiments.

chlorpromazine produced a weak, transient positive inotropic effect, reaching a peak at 2–3 min after drug addition (Fig. 3). After this time, cardiac contractile force decreased significantly.

The positive inotropic action of 7,8-dioxochlorpromazine in the absence of propranolol was also dose dependent and biphasic (Fig. 4). With higher concentrations of 7,8-dioxochlorpromazine, the negative inotropic effect, which was observed after the initial transient positive inotropic effect, was more pronounced than that produced by 7,8-dihydroxychlorpromazine. Consistent with this observation, high concentrations of 7,8-dioxochlorpromazine produced marked negative inotropic effects in the presence of propranolol (Fig. 5). In the presence of propranolol, the positive inotropic effect of 7,8-dioxochlorpromazine was transient and markedly reduced compared to that observed in the absence of propranolol (compare Figs. 4 and 5).

Because of the structural similarities (Fig. 1), inotropic effects of 2-hydroxyimipramine and 2-hydroxydesmethylimipramine were also studied. In the absence of propranolol, 2-hydroxyimipramine failed to affect the isometric contractile force of quinea pig left atrial preparations whereas 2-hydroxydesmethylimipramine produced a sustained positive inotropic effect which developed rather rapidly reaching the steady level within 5 min (Table 1).

## DISCUSSION

7,8-Dihydroxychlorpromazine and 7,8-dioxochlorpromazine were capable of producing concentration-dependent positive inotropic effects in electrically stimulated left atrial preparations of guinea pig hearts. The inotropic responses to 5 μM monohydroxychlorpromazines or 7,8-dihydroxyperphenazine were markedly smaller than those produced by the same concentration of either 7,8-dihydroxychlorpromazine or 7,8-dioxochlorpromazine. Monohydroxypromazines failed to produce a significant positive inotropic effect at the above concentration.

Superficially, potencies of these compounds to produce positive inotropic effects are related to their in-

hibitory effects on isolated $Na^+,K^+$-ATPase. For example, 7,8-dihydroxychlorpromazine is the most potent $Na^+,K^+$-ATPase inhibitor among the above compounds [12, 18]. The concentration of 7,8-dioxochlorpromazine to cause a 50 per cent inhibition of $Na^+,K^+$-ATPase *in vitro* is slightly higher than that of 7,8-dihydroxychlorpromazine, whereas that of 7,8-dihydroxyperphenazine needed to cause a 50 per cent inhibition of the isolated $Na^+,K^+$-ATPase is almost twice the concentration of 7,8-dihydroxychlorpromazine necessary to produce a comparable effect. Monohydroxylated metabolites were relatively ineffective inhibitors of $Na^+,K^+$-ATPase unless activated by ultraviolet irradiation [12]. The relationship, however, between $Na^+,K^+$-ATPase inhibition and positive inotropy appears to be coincidental for the following reason. A number of $Na^+,K^+$-ATPase inhibitors produce positive inotropic effects [19]. The inotropic effects of other $Na^+,K^+$-ATPase inhibitors are unaffected by a concentration of propranolol which effectively blocks $\beta$-adrenergic receptors. In contrast, the positive inotropic effect of 7,8-dihydroxychlorpromazine or 7,8-dioxochlorpromazine was markedly reduced by propranolol. Thus, it appears that the positive inotropic effect of the phenothiazine metabolites involves $\beta$-adrenergic mechanisms, either by their direct actions on the $\beta$-adrenergic receptors or by indirect actions on the sympathetic nerve terminals.

It should be noted that 7,8-dihydroxychlorpromazine, the most potent inotropic compound among this group, has two vicinal hydroxyl groups on the benzene ring and thus somewhat resembles the structure of catecholamines. Differences in the inotropic action of 7,8-dihydroxychlorpromazine and 7,8-dihydroxyperphenazine, and those of 2-hydroxyimipramine and 2-hydroxydesmethylimipramine suggest the importance of the side chain containing a nitrogen atom and/or of the electronegative chloride on the molecule. Whether such a structural resemblance to the catecholamines is important for the inotropic action of these compounds is yet to be determined.

In the present study, 2-hydroxydesmethylimipramine produced a sustained positive inotropic effect. This observation is in contrast to a previous report by Jandhyala *et al.* [20] in which 2-hydroxyimipramine and 2-hydroxydesmethylimipramine have been shown to decrease cardiac contractility in the anesthetized dogs. These results indicate the complex nature of the cardiovascular effects of the metabolites of tricylcic antidepressants.

In conclusion, several hydroxylated phenothiazine derivatives and tricyclic antidepressants, including those which have been shown to be metabolites of these agents, produce positive inotropic effects in isolated guinea pig hearts in contrast to the cardiodepressant actions of parent compounds. Such actions may contribute to the complexity of the cardiovascular effects of phenothiazines and tricyclic anti-depressants.

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