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Hydroxylated Chlorpromazine Metabolites: Positive Inotropic Action and the Release of Catecholamines

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SUMMARY

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The mechanism of the positive inotropic action of the 7-mono- and 7,8-dihydroxylated metabolites of chlorpromazine was studied in isolated guinea pig hearts. In electrically driven left atrial preparations, these metabolites as well as 3,7,8-trihydroxychlorpromazine produced positive inotropic effects, which were prevented by prior treatment with either reserpine or propranolol. Although 7,8-dihydroxychlorpromazine is a potent inhibitor of $(Na^+ + K^+)$-ATPase *in vitro*, it failed to affect sodium pump activity in Langendorff preparations at the time of the positive inotropic effect, indicating that this compound cannot gain access to the site of inhibition on the sodium pump during the relatively short perfusion period. 7,8-Dihydroxychlorpromazine altered the transmembrane action potential configuration. These changes were similar to those produced by catecholamines. 7,8-Dihydroxychlorpromazine also increased the cyclic 3',5'-AMP concentration in atrial muscles, an effect that appeared to be related to the positive inotropic action. Both these effects were prevented by prior treatment with reserpine. In electrically driven left atrial preparations, 7,8-dihydroxychlorpromazine released previously loaded [^{3}H]metaraminol without affecting its uptake. It is concluded the hydroxylated metabolites of chlorpromazine release catecholamines from cardiac sympathetic nerve terminals and increase myocardial contractile force.

INTRODUCTION

Chlorpromazine and related phenothiazine derivatives undergo extensive xenobiotic biotransformations. Among the many reported metabolites of chlorpromazine is 7,8-dihydroxychlorpromazine. This highly reactive metabolite has been identified in schizophrenic patients receiving chronic chlorpromazine treatment (1) and has been demonstrated to possess biological activities (2-5). Our previous study has shown that 7,8-dihydroxylated metabolites of 2-chlorophenothiazine tranquilizers and 7,8-dioxychlorpromazine are potent inhibitors of $(Na^+ + K^+)$-ATPase *in vitro* (6). The inhibition of $(Na^+ + K^+)$-ATPase seems to involve sulfhydryl group interactions. 7,8-Dihydroxychlorpromazine also produces a positive inotropic effect in isolated guinea pig hearts (6). This action, however, does not appear to be

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related to the ability of this compound to inhibit ($Na^+ + K^+$)-ATPase (7), although most ($Na^+ + K^+$)-ATPase inhibitors produce positive inotropic effects related to their actions on the sodium pump activity (8).

The present study was initiated to elucidate the mechanism of the positive inotropic action of a mono- and a dihydroxylated chlorpromazine metabolite and also a trihydroxylated derivative of chlorpromazine. The results in isolated guinea pig hearts indicate that the positive inotropic action of 7,8-dihydroxychlorpromazine is due to the release of catecholamines from sympathetic nerve terminals.

METHODS

Guinea pigs of either sex, weighing 400–500 g, were stunned by cervical dislocation and their hearts were removed immediately. Left atrial preparations were suspended vertically in aerated (95% O_2–5% CO_2) Krebs-Henseleit solution of the following millimolar composition: NaCl, 118.0; $NaHCO_3$, 27.2; KCl, 4.8; $MgSO_4$, 1.2; KH_2PO_4, 1.0; $CaCl_2$, 1.2; and glucose, 11.1 (pH 7.4), at 30°. Preparations were electrically stimulated with 1-Hz rectangular pulses of 4-msec duration and a voltage 10% above the threshold, using platinum field electrodes and a Grass S-48 stimulator coupled with a Grass SIU-5 stimulus isolation unit. Diastolic tension was adjusted to 1.0 g at least 10 min before the addition of agents. Under these conditions average control contractile force was 0.7–0.8 g. Langendorff preparations were perfused with the solution described above with a constant flow rate of 4 ml/min at 30° and electrically stimulated with 1.5-Hz stimulations of the above parameters. Isometric contractile force was recorded with a Grass force-displacement transducer, model FT-03C, and a polygraph recorder. Experiments were started after a 45–60-min equilibration period.

Reserpine, when administered, was injected subcutaneously (5 mg/kg) 24 hr before death. Propranolol treatment was performed by adding 10 μM (±)-propranolol to the incubation mixture for atrial preparations 20 min before the addition of a test drug. This concentration of propranolol shifted the dose-response curve for tyramine to the right more than an order of magnitude under the present experimental conditions. The inotropic response to test drugs was calculated as the percentage change in contractile force compared with that observed immediately before the addition of the test drug.

The sodium pump activity of ventricular slices was estimated from ouabain-sensitive ^{86}Rb uptake as described by Ku *et al.* (9). Ventricular slices were prepared from drug- or NaCl-perfused Langendorff preparations using a Stadie-Riggs tissue slicer. Slices were immediately incubated at 2–4° for 2 min in a potassium-free Krebs-Henseleit solution and subequently transferred to a warm (37°) incubation mixture. The composition of the latter incubation medium was similar to the Krebs-Henseleit solution described above, except that KCl was replaced with 2 mM RbCl containing tracer amounts of ^{86}Rb (New England Nuclear). After an 8-min incubation at 37°, slices were rinsed with similar solutions without $^{86}RbCl$, blotted, weighed, and assayed for the radioactivity of ^{86}Rb, using a γ-scintillation spectrometer (Packard, model 578). Nonspecific ^{86}Rb uptake, observed in the presence of 0.3 mM ouabain (the concentration sufficient to inhibit sodium pump activity in this species), was subtracted from the total ^{86}Rb uptake observed in the absence of ouabain to calculate that portion of ^{86}Rb uptake related to the sodium pump activity.

For transmembrane potential studies, left atrial preparations were obtained from reserpine-treated guinea pigs. These preparations were suspended horizontally and driven electrically as described above. Glass capillary microelectrodes, filled with 3 M KCl and possessing a tip resistance of approximately 30 megohms, were used for intracellular potential recordings. The microelectrode was suspended at the end of a flexible silver wire connected to a negative-capacity electrometer amplifier (W.H. Associates, Seattle). Transmembrane potential and isometric contractile force were displayed simultaneously on a storage oscilloscope and recorded with a Grass C4R kymograph camera. Resting potential and the maximal upstroke velocity, overshoot,

and duration of the action potential were analyzed with a computer (Digital Equipment, model Lab 8/e).

Uptake and release of catecholamines from sympathetic nerve terminals were estimated using ^{3}H-labeled metaraminol instead of norepinephrine, because these two compounds have similar characteristics at adrenergic neurons but metaraminol is not metabolized by monoamine oxidase or catechol *O*-methyltransferase (10, 11). [^{3}H]Metaraminol uptake was studied in electrically driven left atrial preparations. After a 45-min equilibration period, the test drug was added to the incubation medium and [^{3}H]metaraminol was added 5 min later. Atrial preparations were electrically stimulated for an additional 30 min. Then the atria were removed, rinsed, blotted, weighed, dissolved in 1.0 ml of a sample solubilizer (Soluene, Packard Instrument Company) in a scintillation vial at 60° for 3 hr, and assayed for radioactivity by liquid scintillation counting.

In [^{3}H]metaraminol release studies, 100 nM [^{3}H]metaraminol was added after a 45-min equilibration period to the incubation medium for left atrial preparations. After 30 min of loading an atrial preparation under continuous electrical stimulation, the preparation, attached to electrodes, was transferred to warm Krebs-Henseleit solution saturated with a 95% O_2–5% CO_2 gas mixture, and then successively transferred to a series of six tissue baths, each containing 5 ml of the above medium, at 10-min intervals. A 1.0-ml aliquot from each bath was assayed for radioactivity by liquid scintillation counting. Counting efficiency (approximately 30%) was monitored by the external standard channel ratio method.

Cyclic 3′,5′-AMP was estimated with a competitive binding assay, using skeletal muscle protein kinase (12). In these experiments, electrically driven left atrial preparations were incubated with 7,8-dihydroxychlorpromazine until the maximal inotropic effect was observed, or with a drug-free solution for a comparable period of time. Then the atria were quickly frozen between a pair of stainless steel blocks cooled with liquid nitrogen, and stored in liquid nitrogen until the cyclic AMP assay. Frozen atria were homogenized directly in 5 ml of ice-cold 6% trichloracetic acid within a few seconds. The homogenate was centrifuged at 3000 rpm for 15 min, and the resultant supernatant was extracted from the trichloracetic acid three times with ethyl ether saturated with distilled water. The aqueous phase was evaporated at 60° under a nitrogen stream, and the residue was dissolved in 50 mM Tris-HCl buffer (13). The concentration of cyclic AMP was assayed using a cyclic AMP assay kit (Amersham/Searle, TRK 432).

Statistical analysis was performed by means of Student's *t*-test. The criterion of statistical significance was a p value less than 0.05.

RESULTS

Contractile force studies. The positive inotropic actions of 7-monohydroxy-, 7,8-dihydroxy-, and 3,7,8-trihydroxychlorpromazine were studied in electrically driven left atrial preparations of guinea pig hearts. After a 45-min equilibration period, the contractile force of control preparations was stable for the entire experimental period (90 min). 7-Monohydroxychlorpromazine (final concentration, 20 μM) produced a gradual increase in contractile force, which reached a peak at 45 min (Fig. 1). This concentration of hydroxylated chlorpromazine derivatives was selected because our previous study (6) had shown that such a concentration produces a moderate positive inotropic effect. The dose-response relationships for 7,8-dihydroxychlorpromazine and relative magnitudes of the inotropic effects of other structurally related compounds have been reported previously (6). Addition of (±)-propranolol (final concentration, 10 μM) after the 45-min equilibration period caused a gradual decrease in contractile force, which stabilized 20 min later at approximately 20% below the level observed before the addition of propranolol. When 7-monohydroxychlorpromazine (final concentration, 20 μM) was added at this time, only a small, transient increase in contractile force was observed, and the

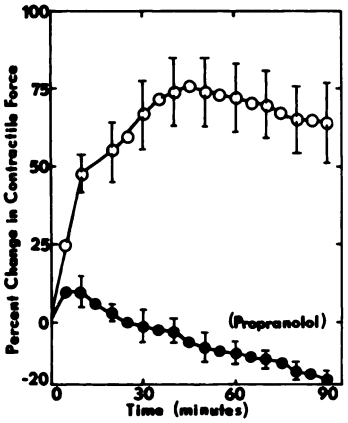

FIG. 1. *Effects of 7-monohydroxychlorpromazine on contractile force of guinea pig atrial preparations*

Left atrial preparations were electrically driven at 1 Hz. After an equilibration period, 7-monohydroxychlorpromazine (final concentration, 20 μM) was added to the medium at zero time. ●——●, (±)-Propranolol (final concentration, 10 μM) was added to the medium 20 min before the addition of 7-monohydroxychlorpromazine. At zero time (20 min after the addition of propranolol), contractile force was approximately 20% lower than the level observed before the addition of propranolol. In these preparations the response to 7-monohydroxychlorpromazine was monitored in the presence of 10 μM (±)-propranolol. The percentage change in contractile force induced by 7-monohydroxychlorpromazine was calculated against the value observed immediately before its addition. Each point represents the mean of five experiments. Vertical bars indicate standard errors of the mean.

contractile force decreased slowly after 20 min. Thus the positive inotropic action of 7-monohydroxychlorpromazine was almost completely abolished by the concentration of (±)-propranolol that blocks the *beta* adrenergic receptor.

7,8-Dihydroxychlorpromazine produced a pronounced positive inotropic effect in the absence of propranolol (Fig. 2). As can be seen by comparing Figs. 1 and 2, 5 μM 7,8-dihydroxychlorpromazine (final concentration) produced a greater positive inotropic effect than did 20 μM 7-monohydroxychlorpromazine (final concentration). The maximal positive inotropic effect of 7,8-dihydroxychlorpromazine was observed 35 min after the addition of this agent. Prior treatment of the atrial preparations with either reserpine or propranolol significantly decreased the magnitude of the positive inotropic effect of 7,8-dihydroxychlorpromazine (Fig. 2).

3,7,8-Trihydroxychlorpromazine also produced a positive inotropic effect in electrically driven left atrial preparations (Fig. 3). This compound appears to have a potency intermediate between those of 7-monohydroxychlorpromazine and 7,8-dihydroxychlorpromazine. The inotropic response of atrial preparations to 3,7,8-trihydroxychlorpromazine declined rather rapidly after reaching a peak at 35 min, even in the continuous presence of the agent (Fig. 3). The positive inotropic effect of 3,7,8-trihydroxychlorpromazine was also abolished by 10 μM (±)-propranolol.

The 7-monohydroxy, 7,8-dihydroxy, and 3,7,8-trihydroxy derivatives of chlorpromazine are capable of producing positive inotropic effects in isolated guinea pig atrial preparations. These effects appear to be mediated by *beta* adrenergic mechanisms. Among the three compounds tested, 7,8-dihydroxychlorpromazine was the most potent inotropic agent.

Sodium pump studies. The almost complete blockade of the positive inotropic effects of hydroxylated chlorpromazine metabolites by (±)-propranolol or reserpine treatment seems to indicate that such effects are due to stimulation of *beta* adre-

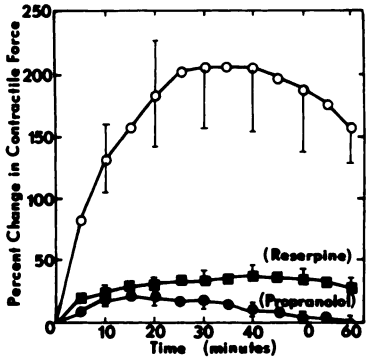

FIG. 2. *Effects of 7,8-dihydroxychlorpromazine on contractile force of guinea pig atrial preparations*

See the legend to Fig. 1. At zero time 7,8-dihydroxychlorpromazine (final concentration, 5 μM) was added to the medium. ■, atrial preparations obtained from guinea pigs treated with reserpine, 5 mg/kg, injected subcutaneously 24 hr before death.

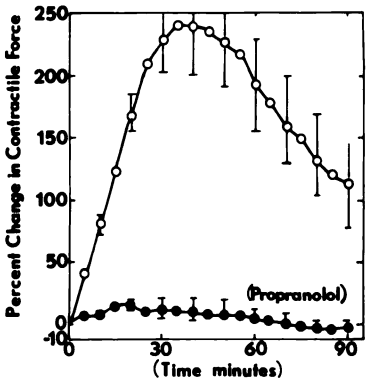

FIG. 3. *Effects of 3,7,8-trihydroxychlorpromazine on contractile force of guinea pig atrial preparations*

See the legend to Fig. 1. At zero time 3,7,8-trihydroxychlorpromazine (final concentration, 20 μM) was added to the medium.

nergic mechanisms, probably by enhancing the release of catecholamines from sympathetic nerve terminals, rather than to inhibition of sodium pump activity. 7,8-Dihydroxylated chlorpromazine, however, has been shown to be a potent (Na^+ + K^+)-ATPase inhibitor (6, 14). Therefore the action of 7,8-dihydroxychlorpromazine on transmembrane sodium pump activity was studied in Langendorff preparations at the time of its positive inotropic effect. Sodium pump activity was estimated from ouabain-sensitive ^{86}Rb uptake by sodium-loaded ventricular slices. In these studies electrically driven Langendorff preparations were perfused with 7,8-dihydroxychlorpromazine, digitoxin (as a positive control), or NaCl. Digitoxin perfusion produced marked inhibition of the ouabain-sensitive ^{86}Rb uptake (Fig. 4). The nonspecific, ouabain-insensitive ^{86}Rb uptake was unaffected, indicating that the action of digitoxin is specific for sodium pump activity. Perfusion with an inotropic concentration of 7,8-dihydroxychlorpromazine (final concentration, 5 μM), however, failed to affect both ouabain-sensitive and ouabain-insensitive ^{86}Rb uptake (Fig. 4). Thus 7,8-dihydroxychlorpromazine does not affect sodium pump activity in intact cardiac muscle cells at the time of its positive inotropic effect.

Transmembrane potential studies. The above studies indicated that the positive inotropic action of 7,8-dihydroxychlorpromazine resembles that of catecholamines rather than that of (Na^+ + K^+)-ATPase inhibitors such as digitalis. In order to study this aspect further, the action of 7,8-dihydroxychlorpromazine on transmembrane potential was examined in electrically driven left atrial preparations obtained from normal or reserpine-treated guinea pig hearts. Contractile force was monitored simultaneously.

In left atrial preparations from normal guinea pigs, 5 μM 7,8-dihydroxychlorpromazine produced a time-dependent increase in contractile force and prolonged the duration of the action potential (Fig. 5). Concomitant with an increase in dT/dt (the first derivative of developed tension), the time to peak tension development after each electrical stimulation was shortened and the rate of decline in developed tension was increased. These findings are similar to observations on the action of norepinephrine, which has been shown to

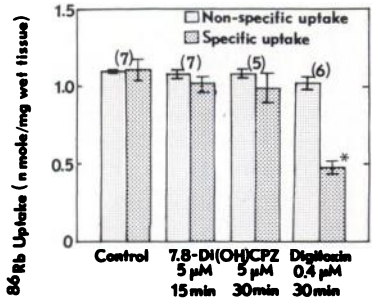

FIG. 4. *Effects of 7,8-dihydroxychlorpromazine and digitoxin on ^{86}Rb uptake by sodium-loaded ventricular slices*

Ventricular slices were obtained from Langendorff preparations of guinea pig hearts which were perfused with 5 μM 7,8-dihydroxychlorpromazine [7.8-Di(OH)CPZ] or 0.4 μM digitoxin for the time periods indicated. Control slices were obtained from Langendorff preparations perfused with a drug-free solution for comparable time period. After a brief, cold incubation for sodium loading, ^{86}Rb uptake was estimated in the presence and absence of 0.3 mM ouabain. Nonspecific ^{86}Rb uptake observed in the presence of 0.3 mM ouabain was subtracted from total uptake observed in the absence of ouabain to calculate specific (ouabain-sensitive) ^{86}Rb uptake. Numbers in parentheses indicate the number of experiments. Vertical bars indicate standard errors of the mean.

* Significantly different from control values ($p < 0.05$).

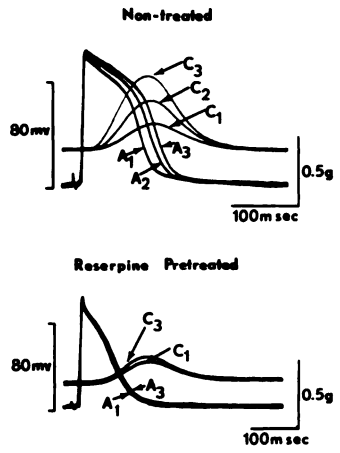

FIG. 5. *Effects of 7,8-dihydroxychlorpromazine on transmembrane potential and contractile force in guinea pig atrial preparations*

Left atrial preparations were obtained from normal or reserpine-treated guinea pigs and electrically driven at 1 Hz. Transmembrane potential (A_1, A_2, and A_3) and contractile force (C_1, C_2, and C_3) were simultaneously recorded. A_1 and C_1, before the addition of 7,8-dihydroxychlorpromazine; A_2 and C_2, 11 min after the addition of 7,8-dihydroxychlorpromazine; A_3 and C_3, 30 min after the addition of 7,8-dihydroxychlorpromazine (at this time the maximal inotropic response was observed in an atrial preparation obtained from normal heart). Typical tracing from at least six experiments in each group.

accelerate the onset of the maximum active state, increase its maximum intensity, shorten its duration, and hasten its decline (15). In reserpine-treated preparations, changes induced by 7,8-dihydroxychlorpromazine were minimal.

Computer analysis of action potential parameters confirmed these observations (Table 1). At the time of the maximal inotropic effect of 7,8-dihydroxychlorpromazine, the maximal rate of depolarization and the overshoot and duration of the action potential were significantly increased in normal atrial preparations. Reserpine treatment slightly decreased the action potential duration but failed to affect the resting membrane potential, maximal rate of depolarization, or overshoot of the action potential. In reserpine-treated preparations, however, 7,8-dihydroxychlorpromazine failed to alter significantly the maximal rate of depolarization or the overshoot of the action potential, and it prolonged the action potential only slightly. These results again suggest that the actions of 7,8-dihydroxychlorpromazine are mediated by *beta* adrenergic mechanisms.

Inhibition of catecholamine uptake. Since reserpine treatment and propranolol have similar effects on the inotropic effect of 7,8-dihydroxychlorpromazine, the action of this compound seems to involve sympathetic nerve terminals. The action of catecholamines at the neuroeffector junction can be enhanced by inhibiting the reuptake of released catecholamines. Chlorpromazine has been shown to inhibit catecholamine reuptake by sympathetic nerve terminals (10, 16). Thus the effect of 7,8-dihydroxychlorpromazine on catecholamine reuptake mechanism was studied using ^{3}H-labeled metaraminol. In these studies [^{3}H]metaraminol (final concentration, 20 nM) was added to the incubation medium, and the uptake of radioactivity by electrically driven left atrial preparations was assayed. Under the present experimental conditions, 5 μM 7,8-dihydroxychlorpromazine failed to affect [^{3}H]metaraminol uptake significantly, although 100 μM cocaine caused a marked decrease in [^{3}H]metaraminol uptake (Fig. 6). Thus the positive inotropic action of 7,8-dihydroxychlorpromazine does not appear to involve inhibition of the catecholamine uptake mechanism.

Release of catecholamines from sympathetic nerve terminals. In order to study whether 7,8-dihydroxychlorpromazine produces positive inotropic effects by releasing catecholamines from sympathetic nerve terminals, the effects of this agent on the release of loaded [^{3}H]metaraminol were studied in electrically driven left atrial preparations. Atrial preparations were first loaded with [^{3}H]metaraminol by adding it at a final concentration of 100 nM to the incubation medium. After 30 min the atrial preparation was successively transferred to six incubation baths, each containing 5.0 ml of medium, and the radioactivity released during a 10-min incubation in each bath was assayed. Atrial preparations were subjected to continuous electrical stimulation during the entire experimental period. In control

TABLE 1

Effects of 7,8-dihydroxychlorpromazine on transmembrane potential in electrically driven left atrial preparations

Each value represents the mean ± standard error of 12-20 observations (6-10 preparations).

Treatment	Resting potential	Action potential			
		Maximal rate of depolarization	Overshoot	Duration	
				20% depolarization	50% depolarization
	mV	*V/sec*	*mV*	*msec*	
Normal guinea pigs					
Control	78.8 ± 0.7	99.1 ± 4.6	22.9 ± 1.0	26.5 ± 2.1	54.2 ± 2.6
7,8-Dihydroxychlorpromazine[a]	78.8 ± 0.5	124.0 ± 6.5[b]	28.5 ± 1.1[b]	36.0 ± 2.9[b]	66.9 ± 3.3[b]
Reserpine-treated guinea pigs					
Control	77.7 ± 0.8	102.0 ± 3.4	24.1 ± 1.0	18.2 ± 0.9	44.2 ± 1.0
7,8-Dihydroxychlorpromazine[c]	78.6 ± 0.8	109.0 ± 6.6	23.1 ± 2.0	20.5 ± 1.5	48.5 ± 1.7[b]

[a] Observations were made at the time of the maximal inotropic response (30-40 min after the addition of 7,8-dihydroxychlorpromazine).

[b] Significantly different from control values ($p < 0.05$).

[c] Observations were made 30 min after the addition of 7,8-dihydroxychlorpromazine.

preparations the amount of [^{3}H]metaraminol released during each 10-min period decreased with time (Fig. 7). Tyramine (final concentration, 10 μM), a known releaser of catecholamines, caused a pronounced increase in the amount of [^{3}H]metaraminol released. An inotropic concentration of 7,8-dihydroxychlorpromazine (final concentration, 5 μM) also accelerated the release of [^{3}H]metaraminol. Since [^{3}H]metaraminol uptake studies indicated that 7,8-dihydroxychlorpromazine does not inhibit the uptake of this compound, 7,8-dihydroxychlorpromazine seems to release catecholamines from sympathetic nerve terminals.

Cyclic AMP concentrations. If the positive inotropic action of 7,8-dihydroxychlorpromazine involves the release of catecholamines from sympathetic nerve terminals, this compound should increase the concentration of cyclic AMP in the myocardium at the time of its positive inotropic effect (17-19). Thus the concentration of cyclic AMP was assayed in atrial preparations. After the addition of 7,8-dihydroxychlorpromazine (final concentration, 5 μM), the contractile force of the electrically driven left atrial preparation increased gradually, reaching a peak at approximately 30 min. At this time the concentration of cyclic AMP was increased significantly (Fig. 8). Reserpine treatment had no significant effect on the atrial cyclic AMP concentration, but markedly reduced the effects of 7,8-dihydroxychlorpromazine on cyclic AMP concentrations as well as on myocardial contractile force. Thus 7,8-dihydroxychlorpromazine increases the myocardial cyclic AMP concentration in conjunction with its action on myocardial contractility.

DISCUSSION

The present study confirms our previous findings that 7,8-dihydroxychlorpromazine produces a positive inotropic effect in electrically driven left atrial preparations of guinea pig hearts (6, 7) and extends them to two other hydroxylated derivatives of chlorpromazine, 7-monohydroxy- and 3,7,8-trihydroxychlorpromazine. The positive inotropic effects of all three compounds were markedly reduced by prior treatment with reserpine or propranolol, indicating that such effects are probably related to the action of these agents on sympathetic nerve terminals. Of the three hydroxylated compounds tested, the 7,8-dihydroxychlorpromazine metabolite was

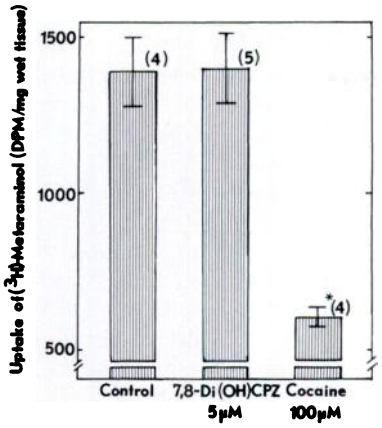

FIG. 6. *Effects of 7,8-dihydroxychlorpromazine and cocaine on [³H]metaraminol uptake by electrically driven left atrial preparations*

Left atrial preparations were obtained from guinea pigs and electrically driven at 1 Hz. 7,8-Dihydroxychlorpromazine [7,8-Di(OH)CPZ] (final concentration, 5 μM) or cocaine (final concentration, 100 μM) was added to the medium after an equilibration period. [³H]Metaraminol (final concentration, 20 nM) was added 5 min later, and the amount of [³H]metaraminol in tissue was estimated after an additional 30-min incubation period. Control preparations were incubated with [³H]metaraminol for comparable time periods without the above agents. Numbers in parentheses indicate the number of experiments. Vertical bars indicate standard errors of the mean.

* Significantly different from control value ($p < 0.05$).

the most potent positive inotropic agent.

Many $(Na^+ + K^+)$-ATPase inhibitors produce positive inotropic effects that are related to their action on sodium pump activity (8). 7,8-Dihydroxychlorpromazine has been shown to be a potent $(Na^+ + K^+)$-ATPase inhibitor when tested *in vitro* using partially purified enzyme preparations (7). The positive inotropic action of this agent, however, does not seem to involve $(Na^+ + K^+)$-ATPase inhibition. At the time of its positive inotropic effect, 7,8-dihydroxychlorpromazine failed to cause significant inhibition of sodium pump activity, a functional correlate of $(Na^+ + K^+)$-ATPase in intact cells. Thus it appears that this highly water-soluble compound did not gain access to the inhibitory site on $(Na^+ + K^+)$-ATPase in the beating heart under the present experimental conditions. It has been shown previously that the essential sulfhydryl groups of the sodium pump are located at the inner surface of the cell membrane (20, 21) and that inhibition of $(Na^+ + K^+)$-ATPase by 7,8-dihydroxychlorpromazine involves sulfhydryl group interactions (7). Thus it appears that 7,8-dihydroxychlorpromazine cannot penetrate the cell membrane. This conclusion is consistent with the report of Palmer and Manian (5) that 7,8-dihydroxychlorpromazine inhibits adenylate cyclase in disrupted brain cells but fails to alter norepinephrine-induced cyclic AMP accumulation in rat brain slices.

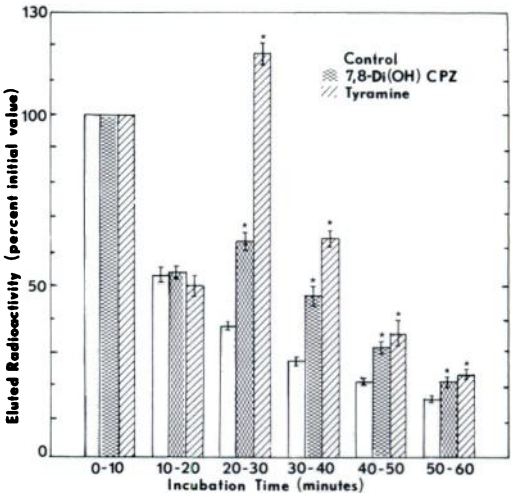

FIG. 7. *Effects of 7,8-dihydroxychlorpromazine and tyramine on release of [³H]metaraminol from guinea pig atrial preparations*

Guinea pig left atrial preparations were electrically stimulated at 1 Hz throughout the entire experimental period. After an equilibration period, 100 nM [³H]metaraminol was added to the incubation medium. After an additional 30-min incubation in the presence of [³H]metaraminol, the atrial preparation, attached to electrodes, was transferred successively to six 5-ml warm baths at 10-min intervals. The radioactivity released in the first bath (0–10 min) was set at 100%, and the radioactivity released in subsequent baths was expressed as a percentage of this value. 7,8-Dihydroxychlorpromazine [7,8-Di(OH)CPZ] (5 μM) or tyramine (10 μM), if present, was added to the third (20–30 min) to sixth (50–60 min) baths. Each point represents the mean of four to seven experiments. Vertical bars indicate standard errors of the mean.

* Significantly different from corresponding control values ($p < 0.05$).

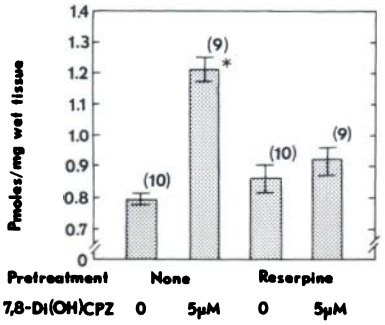

FIG. 8. *Effects of 7,8-dihydroxychlorpromazine on cyclic AMP concentrations in guinea pig atrial preparations*

Left atrial preparations were obtained from normal or reserpine-treated guinea pigs and electrically driven at 1 Hz. 7,8-Dihydroxychlorpromazine [7,8-Di(OH)CPZ] (final concentration, 5 μM) was added after an equilibration period, and the atrial preparation was quickly frozen at the time of the maximal inotropic response (approximately 35 min after drug addition). Control preparations were incubated for comparable time periods. The tissue concentration of cyclic AMP was estimated by a competitive protein binding assay. Numbers in parentheses indicate the number of experiments. Vertical bars indicate standard errors of the mean.

* Significantly different from corresponding control value ($p < 0.05$).

Since the positive inotropic effects of hydroxylated chlorpromazine metabolites were blocked by a *beta* adrenergic blocking agent, (±)-propranolol, and also by reserpine treatment, such effects seem to be mediated by the action of these agents on sympathetic nerve terminals. This contention was supported by transmembrane potential studies. A positive inotropic concentration of 7,8-dihydroxychlorpromazine prolonged the action potential and increased the action potential overshoot and the maximal rate of depolarization. It has been shown previously that catecholamines increase the overshoot and prolong the duration of action potentials (22–26). Changes in action potential configuration induced by 7,8-dihydroxychlorpromazine developed gradually, with a concomitant gradual development of the positive inotropic effect. Moreover, 7,8-dihydroxychlorpromazine failed to affect action potential configurations in reserpine-treated atrial preparations.

7,8-Dihydroxychlorpromazine significantly increased the cyclic AMP concentration in atrial muscles at the time when the positive inotropic action was observed. This increase was prevented by treatment of the animals with reserpine. These findings support the contention that the inotropic effect of 7,8-dihydroxychlorpromazine is due to its action on the sympathetic nerve terminals. Present data are not inconsistent with previous results by Palmer and Manian (5), who reported that 7,8-dihydroxychlorpromazine failed to affect the norepinephrine-induced increase in cyclic AMP concentration in rat brain slices except at very high concentrations. The present study indicates that 7,8-dihydroxychlorpromazine enhances the effects of catecholamines on myocardial cells by acting on the sympathetic nerve terminals. The catecholamine-induced increase in myocardial cyclic AMP concentration was apparently not prevented by the direct action of 7,8-dihydroxychlorpromazine on the cardiac cells. Palmer and Manian (27) also reported that 7,8-dihydroxychlorpromazine increases the cyclic AMP concentration in slices from the lateral and medial hypothalamus and cerebral cortex of rat brain in the absence of added norepinephrine.

Studies with ^{3}H-labeled metaraminol indicate that the enhancement by 7,8-dihydroxychlorpromazine of adrenergic influences on cardiac muscles is due to the release of catecholamines rather than to inhibition of the catecholamine uptake mechanisms. The parent compound, chlorpromazine, has been shown to inhibit catecholamine uptake in sympathetic nerve terminals, but does not cause the release of stored catecholamines (10, 16). Thus it appears that hydroxylated chlorpromazine has different pharmacological actions from those of the parent compound. It is not known, however, whether hydroxylated metabolites of chlorpromazine act in a similar manner in the central nervous system. Additionally, hydroxylated metabolites may produce different pharmacological actions when they are generated within cells.

In conclusion, hydroxylated derivatives

of chlorpromazine produce positive inotropic effects by releasing catecholamines from cardiac sympathetic nerve terminals.

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