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THE EFFECTS OF BEHAVIORALLY RELEVANT DOSES OF CHLORPROMAZINE AND MOLINDONE ON CARDIAC ADENYLATE CYCLASE AND ON MYOCARDIAL CONTRACTILITY

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Abstract—Doses of chlorpromazine and of molindone that inhibit conditioned avoidance behavior of rats were tested for their abilities to inhibit cardiac adenylate cyclase. In untreated rats, basal levels of enzyme activity were 201 pmole c-AMP mg^{-1} 5 min^{-1}. Pretreatment of rats either acutely (30–100 min) or chronically (7 days) with either chlorpromazine or molindone (both 10 mg/kg, i.p.) did not alter basal levels of cardiac adenylate cyclase. *In vitro*, both drugs inhibited the enzyme at concentrations greater than 10^{-4} M. At concentrations below 10^{-4} M, neither drug appreciably inhibited basal levels or β-agonist-induced changes in cardiac adenylate cyclase. In related experiments, pithed rats were treated with various doses of chlorpromazine or molindone. Neither drug, at behaviorally relevant doses, depressed cardiac contractility or inhibited β-agonist-induced changes in contractility. At toxic doses, both drugs tended to depress contractility.

There currently exist a large number of clinically useful antipsychotic drugs. These agents are reasonably similar in terms of efficacy, but they differ strikingly in terms of their abilities to evoke adverse side effects or to participate in adverse drug interactions. In the context of adverse side effects, chlorpromazine (Cpz) is known to exert a variety of actions on mammalian heart. One of the more important actions is that of Cpz-induced changes in the electrocardiogram. These changes have been reported for human patients receiving therapeutic doses of the drug (e.g. Refs. 1 and 2). Even though it is well established that Cpz can alter electrical activity of the heart, little is known about the ability of Cpz to alter mechanical activity of the heart. Accordingly, one of the purposes of the present study is to assess the effects of Cpz on basal and on β-agonist-induced changes in myocardial contractility. In view of the presumed link between cardiac contractility and adenylate cyclase activity, an additional purpose of the study is to assess the effects of Cpz on β-sensitive adenylate cyclase.

In the context of adverse drug interactions, it has been reported that Cpz can interact adversely with the antihypertensive agent guanethidine [3]. Indeed, all three major classes of antipsychotic drugs (phenothiazines, thioxanthenes and butyrophenones) can antagonize guanethidine [3]. By contrast, molindone (Mld) is a relatively new antipsychotic drug that does not belong to the major classes mentioned above, and it does not interact with guanethidine [4]. Since Mld may be a favorable drug for use in psychiatric patients with concomitant hypertension, a question naturally arises as to whether Mld could be used in psychiatric patients with concomitant heart disease. The effects of Mld on myocardial contractility and on cardiac adenylate cyclase have not previously been reported. Therefore, a final purpose of this study is to examine the effects of Mld on mammalian heart.

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MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing between 200 and 300 g were used in these studies. Animals were subjected to a variety of biochemical, cardiovascular or behavioral manipulations as described below.

Adenylate cyclase activity. Enzyme activity was estimated by measuring the rate of conversion of radioactive adenosine triphosphate {[^{32}P]ATP, 7 Ci/m-mole, New England Nuclear, Boston, MA} to the corresponding cyclic monophosphate (cAMP). The technique used was that of Krishna, Weiss and Brodie [5]. In brief, the procedure was as follows. Animals were decapitated and their hearts were rapidly excised. Tissues were rinsed, weighed, minced and then homogenized in ice cold Tris–HCl buffer (50 mM, pH 7.5) containing $MgSO_4$ (3.0 mM). An aliquot of the tissue homogenate was added to a reaction mixture to produce a final concentration (protein) of approximately 10 mg/ml. The reaction mixture was the same as that described by Krishna *et al.*, except for two modifications described by Vulliemoz, Verosky and Triner [6]. These modifications were: (1) rather than adding theophylline to inhibit phosphodiesterase, cold cAMP (3.0 mM) was added to produce a "sparing action", and (2) phosphoenolpyruvate and pyruvate kinase were added as an ATP-generating system. The reaction mixture was incubated at 35° for 5 min, after which the reaction was terminated by a boiling water

bath (5 min). The reaction mixture was centrifuged (5,000 rpm, 15 min), and the debris obtained after centrifugation was assayed for protein [7]. The supernatant was subjected to column chromatography and barium-zinc precipitation for isolation of [^{32}P]cAMP. Levels of the cyclic nucleotide were measured by liquid scintillation spectrometry, and results were corrected for recovery. Enzyme activity, unless otherwise indicated, refers to pmole cAMP generated per mg protein per 5 min. To facilitate comparisons, data are presented as percent increase (agonist) or percent decrease (antagonist) in basal levels of enzyme activity.

Pithing. In previous studies [8], pithing has been done as described by Shipley and Tilden [9]. According to their technique, a pithing rod is pased through the orbit and the cranium into the spinal column. However, in the present study the reverse technique was used. That is, a pithing rod was inserted into the base of the spinal column, and it was passed forward through the foramen magnum into the cranium. The latter technique was easier to accomplish. Regardless of which technique was used, animals were anesthetized (pentobarbital sodium, 50 mg/kg) during pithing.

Cardiac contractility and heart rate. Cardiovascular responses were studied in pithed animals. Cardiac contractility was monitored by using a derivative computer. A cannula (P.E. 50) was inserted into the left carotid artery and driven to the left ventricle. The cannula was attached to a transducer (Hewlett Packard 1280B) and amplifier (Hewlett Packard 8805C) that was connected to the derivative computer (Hewlett Packard 8814A). This arrangement provided a continuous record (Hewlett Packard 7754A thermal recording system) of the rate of change of pressure (dP/dt) from diastole (relaxation) to systole (contraction). Although contractility varied somewhat from animal to animal, it was highly stable within animals (see Results).

Heart rate was monitored with two subdermal electrodes (active leads) in the chest wall and one subdermal electrode (reference lead) in a hind limb. Heart rate recordings were obtained by coupling a rate computer (Hewlett Packard 8812A) with a bioelectric amplifier (Hewlett Packard 8811A), both of which were connected to the recording system.

During experiments on cardiac contractility, animals were pretreated with an α-adrenergic blocking agent (phenoxybenzamine, 10 mg/kg, i.v., 30 min). This pretreatment ensured that changes in cardiac contractility would not be secondary to drug-induced (alpha agonist or antagonist) changes in blood pressure.

Conditioned avoidance behavior. Animals were trained to preform in a two-way shuttlebox conditioned avoidance paradigm. A description of the apparatus and the details of the procedure have been presented elsewhere [4]. In the present study, animals received 100 trials per day. Each trial was composed of an avoidance interval (15 sec), an escape interval (15 sec), and an intertrial interval (30 sec or longer). The elapsed time from trial onset to trial onset was 60 sec. Therefore, if an animal performed either an avoidance response or an escape response, there was automatic compensation in the intertrial interval. The phenomenon of major interest was rate on onset of antipsychotic drug effect. Therefore, performance (number of avoidance responses) was analyzed per block of 10 trials (elapsed time = 10 min).

Drugs. The drugs used were: chlorpromazine hydrochloride, phenoxybenzamine hydrochloride, and reserpine phosphate (gifts from Smith Kline and French Laboratories, Philadelphia, PA); molindone hydrochloride (gift from Endo Laboratories, Garden City, NY); l-norepinephrine bitartrate (Winthrop Laboratories, New York, NY); isoproterenol hydrochloride, *dl*-propranolol hydrochloride, and carbamylcholine chloride (Sigma Chemical Co., St. Louis, MO); and acetylcholine chloride (Calbiochem, Los Angeles, CA).

Drug dosages are expressed in terms of the free base. For i.p. injections, the volume of fluid administered varied with body weight (1 ml/kg). For i.v. injections, a bolus of 0.05 ml was used regardless of drug dosage or animal weight. Intravenous injections were routinely given through the superior vena cava.

Data. Individual data points on each figure represent the mean ± S.D. Statements on statistical analyses (P values) refer to use of the Student's *t*-test.

RESULTS

Adenylate cyclase activity in rat myocardium. Entire hearts ($n = 10$) were assayed for adenylate cyclase as described in Methods. The basal level of enzyme activity was 201 ± 12 pmoles cAMP mg^{-1} $5\,min^{-1}$. Comparable values were obtained when left ($n = 5$) and right ($n = 5$) hearts were assayed separately. Regardless of tissue (entire heart, left heart or right heart), enzyme activity was linear with time (2–20 min) and with protein (5–50 mg).

Effect of agonists on adenylate cyclase activity. Various concentrations of isoproterenol and norepinephrine were tested for their abilities to stimulate adenylate cyclase activity (Fig. 1). Both agonists were active within the concentration range of 10^{-7} to 10^{-3} M. The respective ED_{50}'s were: isoproterenol,

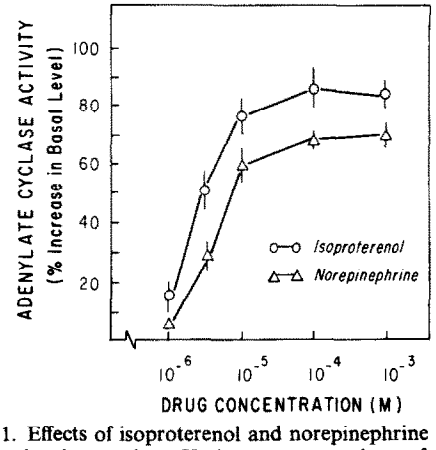

Fig. 1. Effects of isoproterenol and norepinephrine on cardiac adenylate cyclase. Various concentrations of agonist were tested for the ability to stimulate enzyme activity. Both agonists were effective, the respective ED_{50}'s being: isoproterenol, 2×10^{-6} M; norepinephrine, 4×10^{-6} M. Each data point on the figure represents the mean ± S.D. of at least 10 observations.

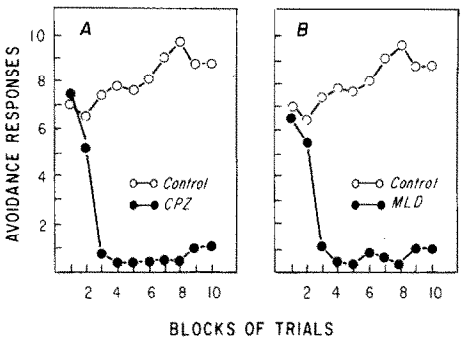

Fig. 2. Effects of chlorpromazine (part A) and molindone (part B) on conditioned avoidance behavior. Twenty rats were trained to perform an avoidance task. When training was complete, rats received 100 trials per day for 5 days (control), and data were analyzed per block of 10 trials (10 min). At the end of the control period, animals were randomly assigned to the chlorpromazine or molindone group ($n = 10$). Once again, animals received 100 trials per day for 5 days, the trials beginning immediately after drug administration (10 mg/kg, i.p.). For both the control period and the drug period, all responses during all 5 days for all animals in a particular group (control, chlorpromazine, or molindone) were averaged. Note that trained animals performed well when no drug was administered, but animals lost the ability to perform avoidance responses after drug administration. For both drugs, inhibition of avoidance behavior was nearly complete by 30 min, i.e., 3 blocks of 10 trials.

2×10^{-6} M; norepinephrine, 4×10^{-6} M. Isoproterenol produced a maximum stimulation (increase in basal enzyme activity) of 86 ± 6%; norepinephrine produced a maximum stimulation of 67 ± 5%. The difference between the drug-induced maxima is significant ($P < 0.05$).

Effect of Cpz and of Mld on conditioned avoidance behavior. Cpz and Mld exert inhibitory effects on conditioned avoidance behavior within the dosage range of 0.3–10 mg/kg (i.p.). The respective ID_{50}'s are: Cpz, *ca.* 2 mg/kg; Mld, *ca.* 0.6 mg/kg [4]. To determine the maximum rate of onset of neuroleptic drug effect, a large dose (10 mg/kg, i.p.) of either Cpz or Mld was administered to rats that had been trained to perform a conditioned avoidance response. The data are presented in Fig. 2. For both drugs, conditioned avoidance behavior was significantly inhibited within 20 min, and profoundly inhibited within 30 min. At this large dose, both drugs produced sustained inhibition (> 100 min) of conditioned avoidance behavior.

Effect of Cpz and of Mld on basal adenylate cyclase activity. A large dose of Cpz or Mld (10 mg/kg, i.p.) was administered acutely to rats (group $n = 4$). At various times (10 min intervals) after drug administration (30–100 min), groups of animals were sacrificed and hearts were assayed for enzyme activity. At no time did either drug produce a statistically significant reduction in cardiac adenylate cyclase activity. In related experiments, rats (group $n = 5$) received daily injections of Cpz or Mld (10 mg/kg, i.p., 7 days). Groups of animals were sacrificed either 1 hr or 24 hr after the final drug administration. Once again, neither drug produced a significant reduction in enzyme activity.

Various concentrations (Fig. 3) of Cpz or Mld were incubated with homogenates of heart obtained from animals that had not previously been treated with the drugs. At concentrations below 10^{-4} M, neither drug exerted an appreciable effect on adenylate cyclase. At higher concentrations, especially for Cpz, there was a reduction in basal levels of enzyme activity.

Effect of Cpz and of Mld on agonist-induced changes in adenylate cyclase activity. Homogenates of heart were incubated with various concentrations of norepinephrine plus either Cpz or Mld (10^{-6} to 3×10^{-5} M). The highest concentration of neuroleptic tested (3×10^{-5} M) was that which appeared not to alter basal levels of enzyme activity (see Fig. 3). At this concentration, neither Cpz nor Mld significantly inhibited the ability of norepinephrine to stimulate β-sensitive adenylate cyclase in heart (Fig. 4).

In a separate series of experiments, higher concentrations of Cpz or Mld (3×10^{-4} M to 10^{-3} M) were

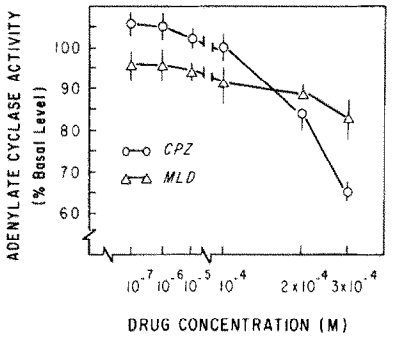

Fig. 3. Effects of chlorpromazine and molindone on cardiac adenylate cyclase activity. Various concentrations of each drug were tested for the ability to inhibit basal enzyme activity. At concentrations below 10^{-4} M, neither drug inhibited adenylate cyclase; at concentrations above 10^{-4} M, both drugs inhibited the enzyme. Inhibition produced by chlorpromazine was particularly notable. Each data point on the figure represents the mean ± S.D. of at least 12 observations.

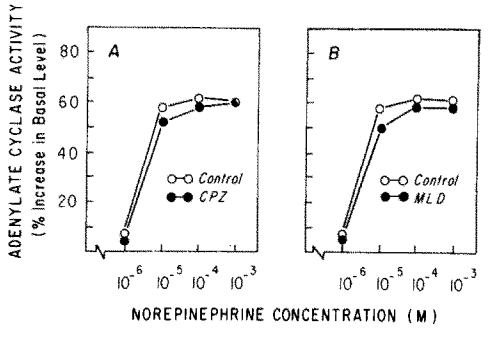

Fig. 4. The effects of chlorpromazine (part A) and molindone (part B) on β-agonist-induced changes in cardiac adenylate cyclase. Various concentrations of norepinephrine were added to tissue homogenates in the absence (control) or presence of a neuroleptic (3×10^{-5} M). Neither chlorpromazine nor molindone inhibited the ability of norepinephrine to stimulate adenylate cyclase. Each data point on the figure represents the mean ± S.D. of at least 5 observations.

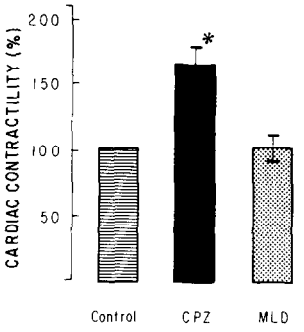

Fig. 5. The effects of chlorpromazine and molindone on cardiac contractility. Rats (group $n = 8$ or more) were pithed, after which contractility was monitored until it had stabilized, i.e., less than 5% change for at least 30 min. Rats then received either chlorpromazine or molindone (1 mg/kg, i.v.). Changes (%) in contractility after drug (10 min) were compared to basal contractility before drug (control). Note that only chlorpromazine (*) produced a significant ($P < 0.01$) increase in contractility.

used. In these experiments, both drugs impaired the ability of norepinephrine to stimulate adenylate cyclase. When the data were analyzed according to the techniques of Lineweaver and Burk [10] and of Dixon [11], it was clear that the drugs were not acting simply as competitive inhibitors of the β-receptor. The inhibition was mainly noncompetitive and probably represents, as mentioned earlier, the ability of high concentrations of Cpz and Mld to depress basal levels of enzyme activity.

Effect of Cpz and of Mld on basal cardiac contractility. Rats were pithed, prepared for monitoring of cardiac responses, and given approximately 30 min to stabilize. Although cardiac contractility varied among animals, it was highly stable within animals. Accordingly, each animal served as its own control in each experiment.

Pithed animals (group $n = 8$ or more) were used to evaluate the effects of Cpz or Mld on cardiac contractility and on heart rate. When administered i.p. at doses up to 10 mg/kg, neither drug significantly influenced contractility or rate. When administered i.v. at a dose of 1 mg/kg, Cpz significantly increased contractility (*ca.* 60%, see Fig. 5) and rate (*ca.* 60 beats/min). The cardiac effects of Cpz were presumably secondary to effects (evoked release, blockade of uptake) on biogenic amines, because the responses were abolished by pretreatment with reserpine (10 mg/kg, i.p., 12 hr) or with propranolol (5 mg/kg, i.v., 30 min). In contrast to Cpz, Mld at the same dose (1 mg/kg, i.v.) did not alter cardiac contractility or heart rate. When administered i.v. at a dose of 10 mg/kg, both drugs evoked irregularities, mainly depression, in contractility and rate.

Effect of Cpz and of Mld on agonist-induced changes in cardiac contractility. Rats were pithed and prepared for recording of cardiac contractility. For each rat, a dose of norepinephrine (*ca.* 10 μg/kg, i.v.) was determined that produced an increase in basal contractility of approximately 50%. Various groups of rats then received various doses of Cpz or Mld either i.p. or i.v. Norepinephrine was tested again at several times (30–100 min) after administration of a neuroleptic. At doses that did not alter basal contractility, Cpz and Mld did not inhibit β-agonist-induced changes in cardiac contractility.

DISCUSSION

Methodology. An accurate assessment of the myocardial effects of Cpz or of Mld is difficult to achieve. For reasons that are obvious, it is not practical to make direct measurements of the effects of these drugs on mechanical activity of human heart *in vivo*. To date, there are no published studies on the effects of these drugs on mechanical activity of human heart *in vitro*. Most investigators have elected to study the effects of Cpz on isolated tissues derived from laboratory animals (see Refs. 12–14; there are no published data on Mld). In studies of this kind, it has been found that high doses of Cpz depress either basal contractility or β-agonist-induced changes in contractility. In each of the studies, the authors have noted that the concentrations of Cpz which altered contractility were probably higher than the concentrations of Cpz that one might expect in the plasma of patients under treatment with the drug. This conclusion, although true, is subject to certain limitations. Firstly, it has never been demonstrated that adverse cardiac effects of phenothiazines are closely correlated with plasma levels of drug. Secondly, it has often been claimed that the pharmacological activity of Cpz is due, at least in part, to its metabolites. And thirdly, no attempt has ever been made to show that the concentration of drug being tested in a tissue bath bears any relationship to the dose of drug that affects behavior of an animal from whom cardiac tissue was obtained.

An effort has been made to overcome each of the limitations ennumerated above. Initially, a determination was made of the doses of Cpz and of Mld that affect behavior [4]. This was followed by a determination of the maximum rate of onset and duration of behavioral effects of the drugs (text Fig. 2). To obviate the question of whether drug concentrations that might be tested *in vitro* were relevant to the whole animal, experiments on contractility were performed *in vivo*. To answer the question of whether parent compounds or metabolites were involved, both acute and chronic drug regimens were used.

Some concern might arise regarding the use of pithed rather than intact animals. However, there is justification for choosing the pithed rat preparation. Cpz impairs α-adrenergic transmission, and in so doing it affects blood pressure of intact animals. Changes in blood pressure can influence measurements of cardiac contractility. The advantage of the pithed animal is that it is maximally, or near maximally, vasodilated; therefore, α-adrenergic antagonists can exert little influence on blood pressure. Another consideration is that Cpz acts at several sites to impede the baroceptor reflex of intact animals. This problem cannot arise in pithed animals, owing to the absence of central reflexes.

Experimental findings. In the context of the present study, a negative finding is a favorable outcome. That is, it would be desirable to establish with some certainty that neither Cpz nor Mld, at behaviorally relevant doses, inhibits basal levels or β-agonist-induced

changes in myocardial contractility or cardiac adenylate cyclase. Such an outcome appears to have been obtained. It was found that Cpz and Mld, when administered i.p. at doses that inhibit avoidance behavior, do not depress contractility and do not diminish enzyme activity. Moreover, the drugs do not inhibit β-adrenergic responses.

To make the aforementioned conclusions tenable, two observations must be explained. One of these observations was that, when administered i.v. at high doses, the drugs did alter contractility. However, the concept of a "behaviorally relevant dose" refers not only to amount and route of drug administration; it refers also to certain elements of drug kinetics. For Cpz and other neuroleptics, the rate of redistribution out of blood is extremely rapid; it is much more rapid than the rate of peritoneal absorption. Because the rate of redistribution is so rapid and so extensive (brain to plasma ratio > 30; ref. 15), an i.p. injection could never produce plasma levels of drug comparable to those obtained immediately after an i.v. bolus. As a result, the amount of drug perfusing the heart after i.p. injection can never equal the amount perfusing the heart immediately after i.v. injection. Taking into account the appropriate pharmacokinetics, one would conclude that: (a) behaviorally relevant doses of Cpz and Mld do not impair mechanical activity of the heart, but (b) excessive or toxic doses may impair mechanical activity.

Another observation that requires explanation pertains to adenylate cyclase activity. It has been known for many years that norepinephrine will stimulate cardiac adenylate cyclase [16, 17]. In fact, it is generally acknowledged that there is a link between increases in enzyme activity and increases in contractility, although the link is not as clear as was once envisioned (compare Ref. 18 with Ref. 19). In any event, high concentrations of Cpz did have a pronounced effect on adenylate cyclase *in vitro*. A question arises as to whether these data are pertinent to the *in vivo* situation. In all likelihood, the pertinence is minimal. The concentrations of Cpz that depressed basal levels of enzyme activity were 10^{-4} M or greater. These concentrations would be difficult to maintain *in vivo*. After intravenous administration of 10 mg/kg Cpz, only the instantaneous concentration would be in the range of 10^{-4} M. Within minutes, redistribution would cause plasma levels of drug to fall to the μM range. Once again, it may be judicious to conclude that: (a) behaviorally relevant doses of Cpz and Mld do not inhibit cardiac adenylate cyclase, but (b) excessive or toxic doses may inhibit the enzyme.

Implications. The stated goals of this study were to assess the cardiac effects of Cpz and Mld. As discussed above, neither drug appears likely to exert deleterious effects, insofar as contractility and adenylate cyclase are concerned. These data are encouraging, but certainly preliminary. Future work must demonstrate that the drugs do not have untoward effects on diseased heart. Moreover, the findings on laboratory animals must be reproduced on humans or on tissues derived from humans. Experiments along the latter line are presently underway.

There is one final, albeit unfortunate, matter of note. Whether intentionally (e.g. suicide) or unintentionally (e.g. hepatic shutdown), patients occasionally are exposed to extraordinary concentrations of drug. Under such circumstances, either Cpz or Mld might adversely affect the heart. The present report should not be construed as evidence that Cpz or Mld is inocuous in relation to cardiac function.

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