Effects of D- and DL-Propranolol on Myocardial Adenyl Cyclase Activity[1] (37473)

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(Introduced by A. L. Sellers)

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There is considerable evidence to suggest that the positive inotropic effects of the catecholamines are related to their ability to activate adenyl cyclase activity, and thus increase tissue levels of cyclic AMP (1–4). However, other authors noted a dissociation of the positive inotropic effect of norepinephrine from the rise in cardiac cyclic AMP (5). Thus, the precise relationship between cyclic AMP, the beta receptor, and the inotropic effects of agents which presumably act at this level is still unsettled. In this regard, beta adrenergic blocking agents, such as propranolol, competitively inhibit the ability of catecholamines to activate adenyl cyclase activity. Although this antagonistic effect of beta blockers has been studied extensively (1, 3, 6), the direct effects of beta adrenergic blocking agents on the adenyl cyclase system have received little attention. Since propranolol produces myocardial depression (7–9), it was the purpose of this study to quantitatively relate its effects on cardiac adenyl cyclase activity and force. Furthermore, since the dextro isomer of propranolol has only about 1/60 the beta blocking activity of the DL mixture [Inderal (R) (10)] but produces the same myocardial depression of contractile force *in vitro* (8), this optically pure isomer was also studied. A comparison of the effects of these two drugs on adenyl cyclase activity and force development was expected to provide additional evidence regarding the relationship of cyclic AMP regulation to force development, albeit in a negative inotropic direction.

Methods. After sodium pentobarbital anesthesia (25 mg/kg intraperitoneally), the right ventricle from cats and both ventricles from guinea pigs were rapidly removed and immediately placed in ice-cold buffer (0.05 *M* Tris–HCl, pH 7.5). One heart was used for each experiment. Tissue was rapidly minced, weighed and homogenized in an ice-cold, hand-driven conical Contess tissue homogenizer. Connective tissue was removed by straining through gauze and the homogenate was centrifuged at 10,000*g* for ten minutes, at 0°. The preparation was washed twice by resuspending in 3 volumes of buffer (based on original tissue weight) and was used immediately for the adenyl cyclase assay. In another group of cats, catecholamine depletion was produced by reserpinization (3.0 mg/kg intraperitoneally, 48 and 24 hr prior to sacrifice). Although this dose schedule has been previously shown to produce catecholamine depletion, this was confirmed in the present study by placing the right ventricular papillary muscles in a muscle bath *in vitro* and recording isometric force. After stabilization of the muscles with a stimulus voltage 10% above threshold, the voltage was suddenly increased to ten times threshold. After this increase in voltage, no increase in force was noted, a characteristic finding in muscles depleted of catecholamines (14). The method employed for tissue determination of adenyl cyclase previously described by us (11), is a combination of the incubation system described in detail by Drummond and Duncan (12), and the silicic paper chromatography described by

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Woods and Waitzman (13). The assay system (in a total volume of 150 μl per vessel) consists of: 40 m*M* Tris–HCl (pH 7.5), 8 m*M* theophylline, 2 m*M* cyclic 3′5′-AMP, 5.5 m*M* KCl, 20 m*M* PEP, 15 m*M* $MgSO_4$, 0.4 m*M* ATP, 130 μg/ml of pyruvic kinase, 1 μCi of Na 8-^{14}C-ATP (30 mCi/mmole), and 30 μl of tissue suspension.

After addition of all components except the tissue, the assay tubes were equilibrated at 37° for two minutes. The reaction was started by the addition of suitably diluted tissue and incubation was carried out for ten minutes at 37°. The agents to be studied, D-propranolol[3], DL-propranolol[3] and norepinephrine[4], were added immediately prior to the tissue. The reaction was terminated by placing the tubes in a boiling water bath for three minutes. Denatured protein was removed by centrifugation at 3000 rpm for 15 min.

An aliquot (50 μl) of each clear supernatant was applied on a neutral silicic acid–glass fibro-matrix 1000 μm in thickness (chrom AR-1000, Malinckrodt Chemical Works). Ascending chromatography was accomplished in sandwich chambers (Brinkmann Instruments, Inc.) using the solvent, 2-propanol–ethyl acetate–13.6 *N* ammonium hydroxide (55:29:16).

Development time was approximately 50 min. Developed spots were visualized under shortwave uv. The cyclic AMP spots were counted after dispersion in 10 ml of scintillation mixture in a Packard liquid scintillation counter. In each experiment, control adenyl cyclase activity and that following 10^{-6} *M* norepinephrine were determined in triplicate. D- and DL-propranolol in concentrations of 10^{-9}–10^{-4} *M* were also studied in triplicate in each experiment. Aqueous solutions of the drugs were prepared fresh daily, immediately prior to the assay. The adenyl cyclase activity was expressed in picomoles of cyclic AMP formed/gram of heart/minute and normalized as a percent of the control values.

Results. A. Normal cats. The effects of D-, and DL-propranolol on the adenyl cyclase activity of myocardial homogenates of cats are illustrated in Fig. 1; 10^{-6} *M* norepinephrine (NE) produced an increase in adenyl cyclase activity of 41.5 ± 6.8% (SEM); (DL) propranolol depressed adenyl cyclase activity 10% at 10^{-8} *M*, 22% at 10^{-7} *M*, and 35% at 10^{-4} *M*. At all dose levels studied, D-propranolol produced about 50% as much depression as DL-propranolol. The dashed line and right-hand scale plot the identical effects of D- and DL-propranolol on isometric force developed by cat papillary muscles as previously reported by one of the authors (8). The dissociation between the effects of propranolol on force and adenyl cyclase activity is evident.

B. Normal guinea pigs. Somewhat similar results were obtained in normal guinea pigs (Fig. 2); 10^{-6} *M* norepinephrine (NE) produced an increase in adenyl cyclase activity of 50 ± 5%. The depressive effect of DL-propranolol was generally less marked in this species than in the cat. D-Propranolol produced slightly less depression of adenyl cyclase activity than did DL-propranolol. Appropriate paired statistical analysis indicates that these differences are significant ($p < 0.05$) at most dose levels studied.

C. Reserpinized cats. The results obtained from the myocardial homogenates of reserpinized cats are illustrated in Fig. 3. Norepi-D-Propranolol had no effect on adenyl cyclase activity was reduced slightly to 34 ± 3.8%. D-propranolol had no effect on adenyl cyclase levels with the doses studied. The effects of DL-propranolol were less than those found in normal cats, but were still statistically different ($p < 0.05$) from both control and D-propranolol when analyzed by paired *t* testing at each level. The dashed line and right-hand scale again plot the effects of D- and DL-propranolol on isometric force developed by reserpinized cat papillary muscle (8).

Discussion. This study has quantitated the effects of D- and DL-propranolol on the adenyl cyclase activity of washed particles of the myocardium of cats and guinea pigs. This differs from previous studies (1, 3, 4) which have concentrated on the beta blocking effects of propranolol in response to

[3] Kindly supplied by Ayerst Laboratories as HCl powder.

[4] Kindly supplied by Sterling–Winthrop Laboratories as levophed bitartrate powder.

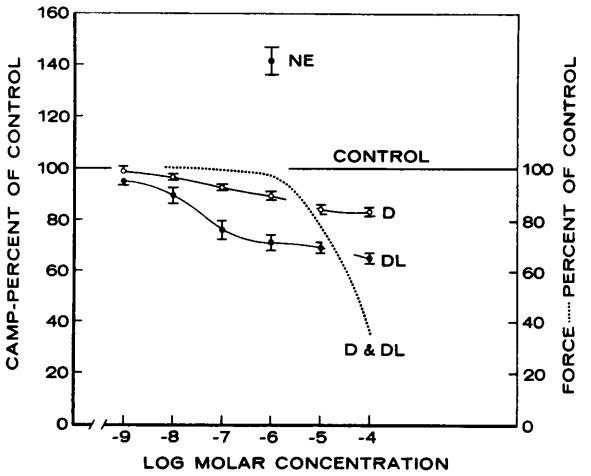

FIG. 1. Effects of norepinephrine (NE), D- and DL-propranolol on the adenyl cyclase activity of myocardial homogenates of five cats. The control level of adenyl cyclase activity was 6050 ± 420 (SEM) pmoles of cyclic AMP formed/g of heart/min. Triplicate samples were averaged for each experiment. The results are expressed as the mean ± SEM for all hearts. The difference between D and DL propranolol was significant at every dose level ($p < 0.05$) except 10^{-6} *M*. The dashed line and right-hand scale plot the identical depressive effects of D- and DL-propranolol on isometric force of cat right ventricular papillary muscles as previously reported by one of the authors (8).

catecholamine stimulation. Although there were slight quantitative differences between the two species studied, the same general dose-related depression of adenyl cyclase activity was observed.

It should be noted that the control adenyl cyclase level in the reserpinized animals was lower than in the normal animals. Previous studies of myocardial adenyl cyclase activity in reserpinized cats did not reveal either differences in basic levels or differences in the thyroid and norepinephrine-induced stimula-

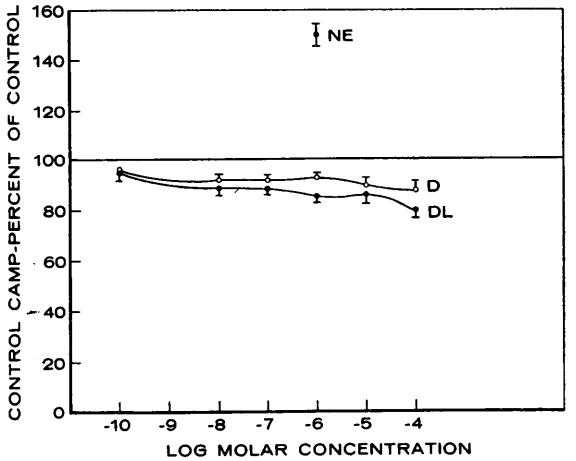

FIG. 2. Effects of norepinephrine (NE), D- and DL-propranolol on the adenyl cyclase activity of myocardial homogenates of five guinea pigs. The control level of adenyl cyclase activity was 11,200 ± 350 pmole of cyclic AMP formed/g of heart/min. Results are expressed as the mean ± SEM. The difference between D- and DL-propranolol was found statistically significant ($p < 0.05$) at dose levels of 10^{-6}, 10^{-5}, 10^{-4} *M*.

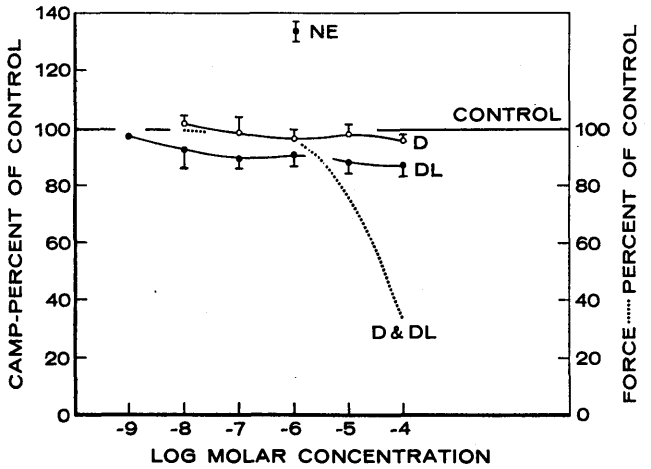

FIG. 3. Effects of norepinephrine (NE), D- and DL-propranolol on the adenyl cyclase activity of myocardial homogenates of six cats which had been previously reserpinized. The control level of adenyl cyclase activity was 3968 ± 329 pmole of cyclic AMP formed/g of heart/min. The difference between D- and DL-propranolol was statistically significant ($p < 0.05$) at each dose level, as was the difference between control values and all dose levels of 10^{-8} *M* DL-propranolol and greater. The dashed line and right-hand scale plot the identical depressive effects of D- and DL-propranolol on isometric force of reserpinized cat right ventricular papillary muscles, as in Fig. 1.

tion of the cyclase activity (15). This difference from our results, might be due to a higher dose of reserpine used in the present study. On the other hand, this dose of reserpine did not alter the force.

In this regard, the quantitative differences produced by DL-propranolol and D-propranolol in the normal cats, as compared with the reserpinized ones, should be interpreted cautiously. Our results seem to show that DL-propranolol has two separable effects. One appeared to be related to an inhibition of the intrinsic catecholamines, an effect probably due to competition with the beta receptor. The second one, a direct depressant effect on the adenyl cyclase activity, was illustrated in the reserpinized animals. However, since the level of the intrinsic catecholamines was not measured, this latter point is by inference only.

When one compares the quantitative effects of these two agents on adenyl cyclase activity with their effects on force, it is obvious that:

1. Although there was no significant difference between their effect on force, their potency of depressing the enzyme activity was different, with the DL mixture more active than the pure dextroisomer.

2. At low dose levels (10^{-8}–10^{-6} *M*), DL-propranolol reduced the adenyl cyclase activity, both in the presence and the absence of intrinsic catecholamines, but had no effect on force development. These data demonstrate an *in vitro* dissociation between the negative effects of propranolol isomers on adenyl cyclase activity and on force development. This evidence is in contrast to the close correlation between the effects of catecholamines on both enzyme activity and force development.

Although we investigated only the activity of the enzyme responsible for cAMP production, it seems reasonable to postulate that cyclic AMP content should follow the changes in the adenyl cyclase activity, since there is no known effect of propranolol on phosphodiesterase, the enzyme catalyzing cAMP degradation. The role of cyclic AMP in myocardial contractility in general and its quantitative correlation with different pathological states and various effects of drugs is still obscure (1, 4). The same state of incomplete knowledge is true concerning the

problems of identity or difference between receptors mediating biochemical effects versus receptors mediating physiological effects.

The role of cyclic AMP might be related only to a positive inotropic modification of a basal level of contractile force. Although this modification appears to occur with various positive inotropic interventions (1, 3, 4), the present study suggests that this does not occur with the negative inotropic intervention of propranolol.

Summary. The direct effects of D- and DL-propranolol (Inderal) on myocardial adenyl cyclase activity were determined on *in vitro* preparations from cats and guinea pigs. In both species, DL-propranolol depressed myocardial adenyl cyclase activity at all dose levels greater than 10^{-8} *M*. The pure dextroisomer produced less depression of the adenyl cyclase activity. In preparations from reserpinized cats, D-propranolol had no effect on myocardial adenyl cyclase activity up to 10^{-4} *M*, while the effects of DL-propranolol were slightly reduced. Since D- and DL-propranolol have identical depressive effects on myocardial contractile force, the present data demonstrate a dissociation between their effects on adenyl cyclase activity and force development.

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