

THE CARDIAC BETA-ADRENERGIC RECEPTOR BLOCKING ACTIONS  
OF PROPRANOLOL AND ITS STEREOISOMERS<sup>1,2,3</sup>

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THE introduction of the beta-adrenergic receptor blocking agent, dichloro-isoproterenol (DCI), by Powell and Slater<sup>1</sup> provided the most compelling evidence in support of the dual adrenergic receptor hypothesis of Ahlquist<sup>2</sup>. Numerous other beta-receptor antagonists have since been introduced, including pronethalol<sup>3</sup> and propranolol<sup>4,5</sup>. The competitive equilibrium nature of the adrenergic blockade produced by these agents suggests that that while they probably possess affinity for the beta-adrenergic receptor site, they are without "intrinsic activity" as beta-receptor stimulants or agonists<sup>6</sup>. Ariens has suggested that the side-chain alcoholic hydroxyl group is one of the major determinants of affinity between beta-sympathomimetics

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<sup>1</sup>The racemic mixture of propranolol will be referred to as propranolol, or as dl-propranolol. The dextrorotatory and levorotatory isomers will be referred to as d-propranolol and l-propranolol, or as dextro-propranolol and levo-propranolol, respectively.

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and beta-receptors, a suggestion which is supported by the observations that the desoxy analogs and dextrorotatory isomers of the catecholamines are relatively inactive either as beta-receptor agonists or antagonists; they presumably lack affinity for the beta-receptor surface.<sup>7,8,9</sup> It was anticipated that the same stereochemical considerations would apply to the beta-receptor antagonists; i.e., the presence of and stereochemical configuration of the side-chain alcoholic hydroxyl group is important in determining the affinity between the drug and the beta-receptors; and consequently, the ability to produce beta-adrenergic receptor blockade. Howe<sup>10</sup> has reported that the dextro-isomers of DCI and pronethalol are 40 times less potent than the corresponding levo-isomers. The present study was undertaken to compare the beta-adrenergic receptor blocking properties of the stereoisomers and racemic mixture of propranolol, and to further assess the role of the stereochemical configuration of the alcoholic hydroxyl group in the blocking activity of the beta-receptor blocking drugs.

#### Materials and Methods

Determination of Beta-Adrenergic Receptor Blockade in the Anesthetized Dog. Male, mongrel dogs (12 kg average weight) were anesthetized with pentobarbital sodium, 30 mg/kg intravenously. The cervical vagi were severed bilaterally and a Brodie-Walton strain gauge arch was sutured to the right ventricle for measurement of myocardial contractile force. Positive pressure respiration was maintained by means of a Harvard respirator. The right thoracic sympathetic chain was stimulated by means of an ABL 104A stimulator for 10 seconds at a frequency of 20 cps and at a voltage sufficient to increase the right ventricular contractile force by 25% or more (5-10 volts). Systemic blood pressure was measured from a cannulated femoral artery by means of a Statham pressure transducer. Lead II electrocardiograms were monitored. In some experiments the heart rate was monitored continuously

by means of a Grass tachometer. All recordings were made on a Grass Model 7 Polygraph.

In each experiment inotropic and chronotropic responses were obtained to calcium chloride, 5 mg/kg; isoproterenol, 0.1 and 0.4  $\mu\text{g}/\text{kg}$ ; and during stimulation of the thoracic sympathetic chain. The control responses were compared to those obtained after the administration of dl-propranolol, 0.5 mg/kg, or d-propranolol, 1-5 mg/kg. All drugs were administered intravenously. Results were expressed as a percent change from control.

Estimation of Beta-Adrenergic Receptor Blockade in Isolated Rabbit Atria. Experiments were performed on isolated left and right atrial preparations from white New Zealand male rabbits weighing between 1.4 and 2.2 kg. Atria were suspended in an organ bath containing oxygenated bathing solution maintained at 30°C. The vertically-suspended atria were attached to a Grass FT-03 force displacement transducer and the resting tension adjusted to 5 g in the electrically-driven left atria, and 2 g in the spontaneously-beating right atria. The left atria were stimulated at a frequency of 120/min by means of a Grass SD-5 stimulator at a voltage 50% higher than threshold. Isometric contractile force was recorded on a Grass Model 7 Polygraph. The bathing solution employed in these experiments was of the following composition (g/liter): NaCl, 7.0; NaHCO<sub>3</sub>, 2.1; KCl, 0.42; CaCl<sub>2</sub>, 0.24; MgCl<sub>2</sub>, 0.20; and anhydrous dextrose, 1.8. The solution was oxygenated and maintained at pH 7.4 by gassing with a 95% O<sub>2</sub> - 5% CO<sub>2</sub> mixture via a sintered disc placed directly under the tissue.

Control responses to isoproterenol,  $2 \times 10^{-7}$  M for chronotropic and  $4 \times 10^{-7}$  M for inotropic experiments, and to calcium chloride (inotropic experiments only),  $2.9 \times 10^{-3}$  M, were recorded following the one hour equilibration period. Single concentrations of either dextro- or levo-propranolol were added to the bath, followed by the challenging dose of isoproterenol. The concentrations of d-propranolol and of l-propranolol required to produce a 50% attenuation of the maximum positive inotropic and chronotropic responses

to isoproterenol were determined. The concentration of antagonist required was estimated when necessary by interpolating graphically from dose-percent inhibition curves representing several concentrations of dextro- or levo- propranolol.

Drugs. The following drugs were used: dl-isopropylnorepinephrine HCl (isoproterenol), which was prepared daily from commercial stock solutions. Propranolol was used as the hydrochloride salt and its dextro- and levo- isomers as the bases, which were dissolved in saline through the addition of hydrochloric acid. All drugs were dissolved in 0.9% saline.

#### Results

The Effects of Propranolol and d-Propranolol on the Positive Inotropic Effects of Adrenergic Stimuli in the Dog. The positive inotropic response to the intravenous administration of isoproterenol, 0.1 and 0.4  $\mu\text{g}/\text{kg}$ , and to electrical stimulation of the right thoracic sympathetic chain was antagonized completely by dl-propranolol, 0.5 mg/kg, except in one animal in which the response was 6% of control. In some experiments the inotropic responses were abolished by doses of dl-propranolol as low as 0.25 mg/kg, even though all animals were routinely given the 0.5 mg/kg dose of dl-propranolol. Five animals were studied. Figure 1 illustrates a typical experiment showing inhibition of the positive inotropic effects of isoproterenol and sympathetic stimulation. The specificity of the blockade is indicated by a failure of dl-propranolol to prevent the positive inotropic response to  $\text{CaCl}_2$ .

In contrast, d-propranolol, 5 mg/kg, produced only a 31.4% inhibition of the maximal response to stimulation of the sympathetic chain, and a 33.2% reduction of the response to isoproterenol, 0.1  $\mu\text{g}/\text{kg}$ . The maximum inotropic response to calcium chloride, 5 mg/kg, was reduced by 16.4%. These differences were not statistically significant from control values. dl-Propranolol failed to alter the maximum inotropic response to calcium chloride. Figure 2 shows a typical experiment illustrating the lack of specific beta-adrenergic

receptor blockade by d-propranolol.

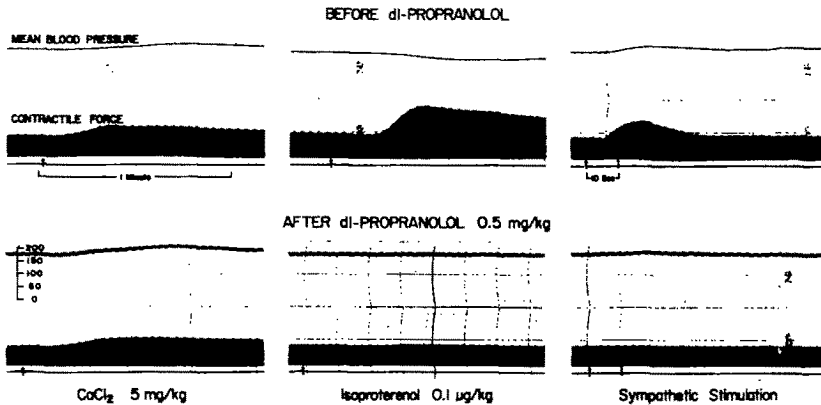


FIG. 1

Effects of dl-propranolol on the positive inotropic and chronotropic responses to adrenergic stimuli and to calcium chloride in the anesthetized, vagotomized dog. Upper portion of each record shows mean systemic blood pressure. Lower portion of record shows the right ventricular contractile force. Heart rate was determined from the record of contractile force. Paper speed was 2.5 mm/sec. Upper row shows control responses of cardiac force and blood pressure to the intravenous injections of calcium chloride and isoproterenol, and to sympathetic stimulation. Lower row of records shows the effects of the same procedures following dl-propranolol (0.5 mg/kg). Arrows indicate point at which drugs were injected and period of sympathetic stimulation.

The Effects of Propranolol and d-Propranolol on the Positive Chronotropic Effects of Adrenergic Stimuli in the Dog. The positive chronotropic responses to isoproterenol, 0.1 µg/kg and 0.4 µg/kg, and to sympathetic stimulation were measured before and following the administration of dl-propranolol, 0.5 mg/kg, of d-propranolol, 5 mg/kg. In five animals, dl-propranolol reduced the chronotropic response to sympathetic stimulation by 87.4%, completely antagonized the cardioacceleration produced by isoproterenol, 0.1 µg/kg, and 0.4

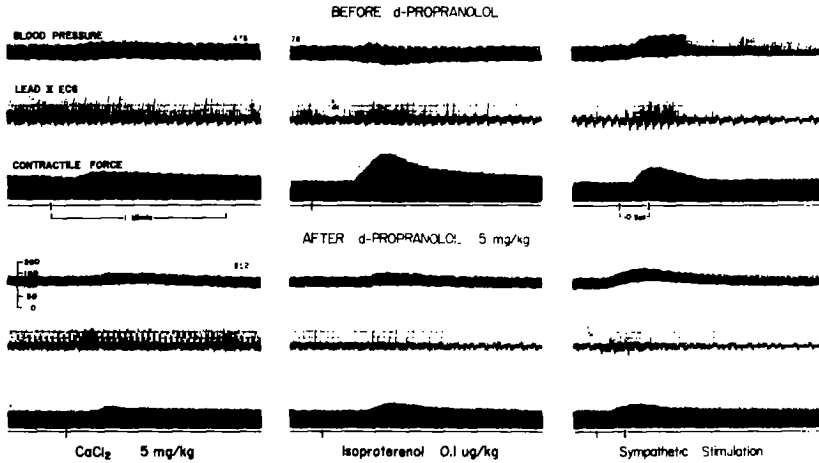


FIG. 2

Effects of *d*-propranolol on the cardiac and blood pressure responses to adrenergic stimuli and to calcium chloride in the anesthetized, vagotomized dog. Upper portion of each record shows systemic blood pressure. Middle portion shows Lead II electrocardiogram. Lower portion of each record shows right ventricular contractile force as measured with a strain gauge. Heart rate was determined from the record of contractile force. Paper speed was 2.5 mm/sec. Upper row of tracings shows control responses of heart and blood pressure to intravenous injections of calcium chloride and isoproterenol, and to electrical stimulation of the right sympathetic chain. Lower row of tracings shows the effects of the same procedures following the intravenous administration of *d*-propranolol (5 mg/kg). Arrows indicate point at which drugs were injected and period of sympathetic stimulation.

$\mu\text{g}/\text{kg}$ , in 4 of 5 animals. The positive chronotropic response to isoproterenol, 0.4  $\mu\text{g}/\text{kg}$ , was blocked in the fifth animal to the extent of 86.0%. In contrast, *d*-propranolol, 5 mg/kg, inhibited the chronotropic responses to sympathetic stimulation and isoproterenol, 0.1  $\mu\text{g}/\text{kg}$ , by 33.7% and 48.1%, respectively. Typical experiments are illustrated in Figures 1 and 2.

The Effects of Propranolol and *d*-Propranolol on the Pressor or Depressor Responses to Adrenergic Stimuli in the Dog. *dl*-Propranolol, 0.5 mg/

kg, reduced the maximum pressor response to sympathetic stimulation by 47.4%. The depressor responses to isoproterenol, 0.1  $\mu\text{g}/\text{kg}$  and 0.4  $\mu\text{g}/\text{kg}$ , were converted to pressor responses by dl-propranolol, 5 mg/kg, produced a slight, but insignificant reduction of the pressor response to sympathetic stimulation. As with dl-propranolol, d-propranolol converted the depressor response to isoproterenol, 0.1  $\mu\text{g}/\text{kg}$ , to a pressor response.

The Effects of l-Propranolol, d-Propranolol, and dl-Propranolol on the Positive Inotropic Response to Isoproterenol in Isolated Rabbit Atria. The increase in contractile force produced in electrically-driven left atrial preparations by the addition to the bath medium of isoproterenol,  $4 \times 10^{-7}$  M, was measured both before and following increasing concentrations of propranolol or one of its stereoisomers. The mean concentration of l-propranolol required to produce a 50% attenuation of the maximum response to isoproterenol was  $3.8 \times 10^{-7}$  M, a concentration which failed to alter the inotropic response to calcium chloride,  $2.9 \times 10^{-3}$  M, or to produce a depression of the resting contractile force. The results represent the mean of 6 experiments. In contrast, a mean concentration of  $2.8 \times 10^{-5}$  M of d-propranolol failed to antagonize the maximum inotropic response to isoproterenol in 5 atria. This concentration of d-propranolol depressed the resting contractile force by 38.5%. Further increases in concentration were accompanied by failure of the atrial preparation. The inotropic response to isoproterenol was partially attenuated in 2 of the 5 atria. but the inotropic response to calcium chloride was reduced an equal amount in the same two atria. Figure 3 illustrates a typical experiment.

dl-Propranolol produced a 50% attenuation of the maximum inotropic response to isoproterenol in a mean concentration of  $7.4 \times 10^{-7}$  M, which is nearly two times the amount of l-propranolol required. The inotropic response to calcium chloride was increased slightly, and the resting contractile force was depressed by about 6%.

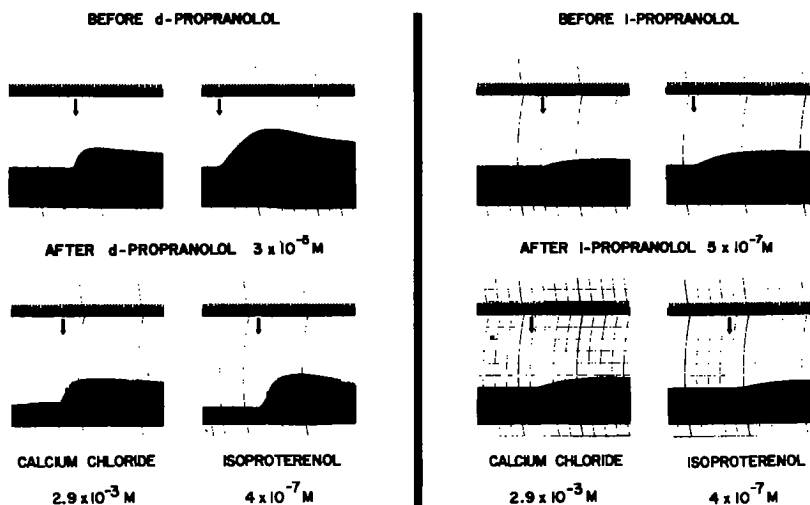


FIG. 3

Effects of *d*-propranolol (left) and *l*-propranolol (right) on the inotropic responses of electrically-driven left rabbit atria to isoproterenol and calcium chloride. Each tracing is a record of isometric contractile force as measured by a force displacement transducer. Paper speed was 0.25 mm/sec. Time markings intervals equal 5 sec. Upper row of tracings, on both left and right sides, show the control responses to calcium chloride and to isoproterenol. Lower set of tracings on the left show the effects of the same procedures in the presence of *d*-propranolol ( $3 \times 10^{-5}$  M); and on the right *l*-propranolol ( $5 \times 10^{-7}$  M). Arrows indicate point at which drugs were added to bath medium.

The Effects of *l*-Propranolol, *d*-Propranolol and *dl*-Propranolol on the Positive Chronotropic Response to Isoproterenol in Isolated Rabbit Atria.

The increase in rate of spontaneously-beating right atria produced by isoproterenol,  $2 \times 10^{-7}$  M, was measured before and after the addition of increasing concentrations of propranolol or one of its stereoisomers. The mean concentration of *l*-propranolol necessary to produce a 50% attenuation of the maximum chronotropic response to isoproterenol was  $4.9 \times 10^{-7}$  M, in contrast to a concentration of  $3.1 \times 10^{-5}$  M of *d*-propranolol required to achieve the



same effect. It was not established whether the attenuation produced by d-propranolol was due to specific beta-adrenergic receptor blockade or to a nonspecific action of the isomer; however, this concentration is known to produce a high degree of depression of myocardial contractile force. Four experiments were performed with each isomer.

A mean concentration of  $8.4 \times 10^{-7}$  M of dl-propranolol was required to produce a 50% inhibition of the chronotropic response to isoproterenol.

#### Discussion

The presence of the alcoholic hydroxyl group in propranolol makes the carbon atom to which it is attached asymmetric and thus gives rise to dextro- and levo-isomers. Howe<sup>10</sup> has reported that the levo-isomers of DCI and pronethalol are 40 times more effective in producing beta-adrenergic receptor blockade than their corresponding dextro-isomers. The results of the present study support these findings and suggest an even greater difference in potency between the stereoisomers of propranolol.

These findings suggest that the stereochemical configuration of the side-chain alcoholic hydroxyl group is significant in the production of beta-receptor blocking properties at concentrations which greatly depress cardiac muscle, and by the observations of other investigators that the desoxy analogs and dextro-isomers of the catecholamines are relatively inactive as beta-sympathomimetic drugs, suggesting that these compounds lack affinity for the beta-adrenergic receptors<sup>6</sup>. A similar function for the alcoholic hydroxyl group of the beta-sympathomimetic drugs has been suggested by Ariens<sup>6</sup>.

The results of the present investigation show that d-propranolol, in concentrations 60 to 80-fold higher than the mean concentration of l-propranolol required for 50% blockade of the beta-receptors, failed to produce a significant beta-adrenergic receptor blockade in isolated rabbit atria. These high concentrations of d-propranolol produce a significant degree of myocardial depression and attenuate the inotropic response to calcium chloride,

which was employed as a nonspecific cardiac stimulant. In contrast, dl-propranolol and l-propranolol produce beta-receptor blockade in concentrations which do not significantly depress the resting contractile force or attenuate the inotropic response to calcium chloride. In addition, the present results show that dl-propranolol is approximately one-half as effective as the l-isomer in producing beta-receptor inhibition, an observation which is in agreement with the suggestion that the d-isomer lacks affinity for the beta-receptor. The present study includes similar findings in the dog.

In both the isolated rabbit atrium and the dog heart, the positive inotropic effects of beta-adrenergic stimulation were antagonized at a lower concentration of propranolol than that required to antagonize the positive chronotropic effects. No significant differences were noted in the doses of propranolol required to antagonize the pharmacological and neurogenic beta-adrenergic stimuli.

The results of the present investigation suggest that the side-chain alcoholic hydroxyl group of l-propranolol exists in a stereochemical configuration which facilitates the interaction of the drug with the beta-adrenergic receptor (affinity); whereas, the alcoholic hydroxyl group of d-propranolol is oriented in such a way that it is unable to facilitate the drug-receptor affinity. These results are in accord with the recently published observations of Howe and Shanks<sup>11</sup> who reported dextro-propranolol to have about one-sixtieth the activity of racemic propranolol in blocking beta-receptor activation. They likewise noted some beta-blocking activity after high doses of dextro-propranolol and suggested this may be due to contamination of the dextro-isomer with levo-isomer or it may be due to a low specific activity of dextro-propranolol. The latter view is supported by the observation that 1- (3-isopropylaminopropoxy) naphthalene, which lacks the side-chain hydroxyl group of propranolol, is about as active as dextro propranolol in blocking isoproterenol-induced tachycardia.

### Summary

The ability of propranolol and its dextrorotatory and levorotatory isomers to produce beta-adrenergic receptor blockade was studied in anesthetized dogs and in isolated rabbit atria. l-Propranolol, in mean concentrations of  $3.8 \times 10^{-7}$  M, and  $4.9 \times 10^{-7}$  M, produced a 50% attenuation of the positive inotropic and chronotropic effects, respectively, of isoproterenol on isolated rabbit atria. The corresponding mean concentrations of dl-propranolol required were  $7.4 \times 10^{-7}$  M and  $8.4 \times 10^{-7}$  M. d-Propranolol failed to attenuate the responses to isoproterenol in concentrations 60-80 times higher than the required concentration of l-propranolol. In the anesthetized dog, dl-propranolol, 0.5 mg/kg, produced complete inhibition of the responses to isoproterenol and sympathetic stimulation. In contrast, no specific inhibition was observed following d-propranolol, 5 mg/kg. These findings provide additional support for the thesis that the stereochemical configuration of the side-chain alcoholic hydroxyl group is important in determining whether or not a drug of this class possesses affinity for the beta-adrenergic receptor; thus, allowing it to act as a beta-receptor blocking agent.

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