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Inotropic and Chronotropic Effects of a Series of β-Adrenergic Blocking Drugs: Some Structure-Activity Relationships.* (31138)

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Since the discovery in 1958 by Powell and Slater(1) that dichloroisoproterenol (DCI) had significant *beta*-adrenergic receptor blocking‡ actions, many compounds have become available which have even more specific and potent *beta*-adrenolytic properties. This report deals with a study of the inotropic and chronotropic effects of six of these *beta*-adrenolytic drugs and their isomers on the isolated heart.

While all the compounds to be discussed have some degree of *beta*-adrenergic blocking action, we were impressed with the fact that there were wide differences in the effects of these compounds on the contractile and electrical properties of the heart. Therefore, the material to be presented is an attempt to correlate the chemical structural features of these compounds with (a) *beta*-blocking actions and (b) influence on cardiac contraction, rate, and electrical properties.

Methods. Inotropic studies. The effects of the compounds on the isometric force of contraction of electrically driven rabbit left atrial preparations were studied according to the methods previously described(2). Basically, isolated left atria were suspended in a muscle bath containing 50 ml Krebs-Ringer solution, and stimulated at a rate of 120 beats/min using a square wave stimulus of 5 msec pulse duration at a strength 3 times threshold voltage. The atria were equilibrated for 100 minutes under these conditions before any drugs were added to the bath. Changes in force of contraction are expressed in terms of per cent of the post equilibration force (0 time in the Figures).

Chronotropic studies. The effects of the drugs on heart rate were determined on iso-

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‡ The terms *beta*-adrenergic receptor blockade, *beta*-adrenolytic and *beta*-blockade are used interchangeably in this report.

lated, spontaneously beating right atrial preparations of the rabbit heart. The right atria were suspended in the muscle baths containing Krebs-Ringer solution(2) and allowed to equilibrate for 30-45 minutes before control or drug observations were begun. Heart rate in beats/min (BPM) was measured from the contraction recorded with a Grass force-displacement transducer.

Electrical measurements. The functional refractory period of the electrically driven left atria was determined by the method of Dawes(3) as modified by Vaughan Williams and Szekeres(4). Electrical threshold was also measured by noting the minimum voltage required to produce contraction.

Determination of beta-*blocking action.* The *beta*-adrenergic blocking actions of the drugs were determined on the electrically driven left atria according to methods described earlier (2). Briefly, the *beta*-adrenolytic action was determined by measuring the concentration of drug required to produce a 50% decrease in the maximum positive inotropic effect of a 9.4 $\times$ 10^{-8} M concentration of isoproterenol HCl. The concentration producing a 50% inhibition was determined graphically from a 2 or 3 point plot of the per cent inhibition *vs* concentration of the antagonist. In all cases, a preliminary experiment was done to ascertain whether or not the compounds studied satisfied the criteria for competitive antagonists(5). Other investigations have dealt with the question of the specificity of the *beta*-blocking action in more detail(1,6-12).

Compounds used. The following *beta*-adrenergic receptor blocking drugs were used: Dichloroisoproterenol (DCI) (Aldrich Chemical Co.); pronethalol and propranolol (supplied by Ayerst Laboratories, N. Y. and Imperial Chemical Industries, England); MJ-1998 and MJ-1999 (4-(2-methylamino-1-hydroxypropyl) methane sulfonanilide HCl and 4-(2-isopropyl amino-1-hydroxyethyl) methane-sulfonanilide HCl) (supplied by Mead Johnson Research Center, Indiana); Kö-592 (1-(3-methylphenoxy)-3-isopropylaminopropanol HCl) (supplied by C. H. Boehringer Sohn, Ingelheim, Germany). The racemic and *l*-isomers used were supplied as the HCl salt. The *d*-isomers were supplied as the free base, but rendered water soluble by addition of HCl. All drug concentrations are expressed in terms of the salt.

TABLE 1. *Beta*-Adrenergic Blocking Action of Some Phenethanolamine and Phenoxyisopropanolamine Compounds on Electrically Driven Rabbit Left Atria.

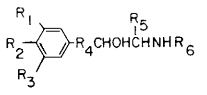
Compound	No. of exp	Mean blocking conc* (μM $\pm$ S.E.M.)	Relative potency (*dl*-pronethalol = 1.0)
dl-Kö-592	5	.97 $\pm$ .01	7.98
dl-propranolol	9	1.15 $\pm$ .35	6.73
DCI	5	1.22 $\pm$ .14	6.34
l-pronethalol	5	3.72 $\pm$ .68	2.08
l-MJ-1999	4	4.18 $\pm$ .58	1.85
dl-pronethalol	7	7.74 $\pm$ 2.10	1.00
d-propranolol	4	10.19 $\pm$ 2.86	.76
dl-MJ-1999	8	12.40 $\pm$ 1.50	.62
d-pronethalol	3	54.61 $\pm$11.30	.14
dl-MJ-1998	8	65.87 $\pm$ 9.16	.12
d-MJ-1999	4	135.49 $\pm$12.22	.06

* Amount of compound needed to produce a 50% attenuation of the maximal positive inotropic response to a test dose of isoproterenol HCl (9.4×10^{-8} M). Atria pretreated for 3-5 min with antagonist before agonist given.

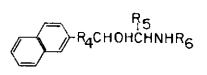
Results. Fig. 1 shows the chemical structures of the compounds studied. Particular attention should be given to the differences in structure of propranolol and Kö-592. Both compounds are phenoxyisopropylaminopropanol derivatives. However, propranolol is characterized by a naphthyl nucleus while Kö-592 has a methyl substitution at the 3 position of the benzene ring. As will be shown below, these two drugs show marked differences in their actions on cardiac force, rate and electrical functions.

Beta-*blocking potency.* Table I indicates the absolute (μM) and relative potency (compared to *dl*-pronethalol) of the *beta*-blockers. Several features are apparent: First, *dl* Kö-592 was the most potent of the drugs studied, although statistically there was no difference in activity from *dl*-propranolol or DCI. Second, the *d*-isomers studied were approximately 15-30 times weaker than the *l*-isomers, or approximately 10 times less potent than the corresponding *dl*-isomers. Third, MJ-1998 (erythro racemate), which differs from *dl*-MJ-1999 only by the presence of a methyl group on the ethylamine side-chain, is 5.3 times weaker than the latter drug. Fourth, although DCI was almost equipotent with *dl*-

BETA-ADRENERGIC BLOCKING DRUGS



DRUG	R_1	R_2	R_3	R_4	R_5	R_6
DCI	H	Cl	Cl	-	H	$CH(CH_3)_2$
MJ-1999	H	CH_3SO_2NH	H	-	H	$CH(CH_3)_2$
MJ-1998	H	CH_3SO_2NH	H	-	CH_3	CH_3
Kö-592	CH_3	H	H	OCH_2	H	$CH(CH_3)_2$



DRUG	R_4	R_5	R_6
Pronethalol	-	H	$CH(CH_3)_2$
Propranolol	OCH_2	H	$CH(CH_3)_2$

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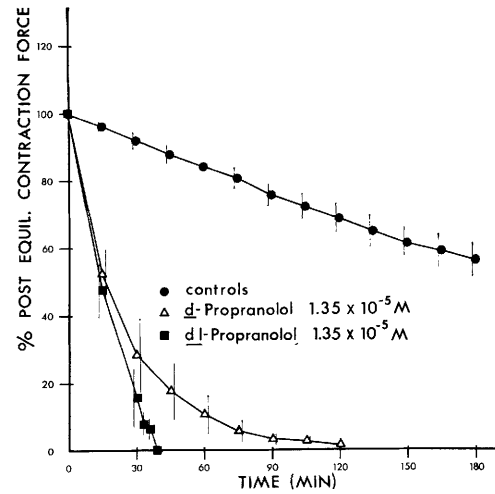

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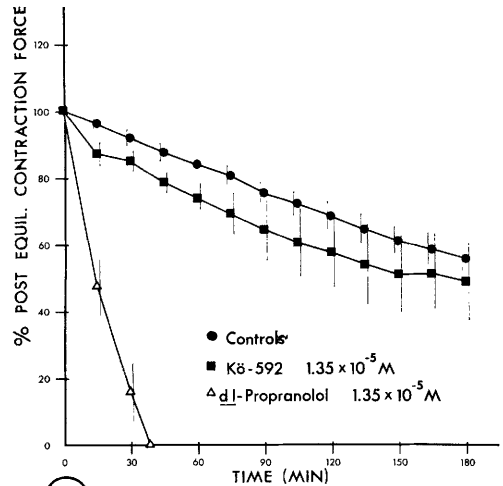

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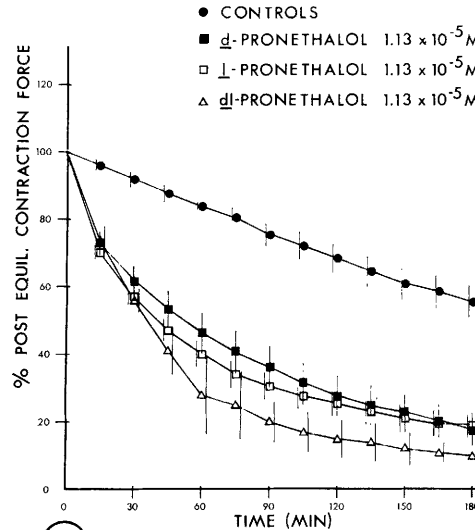

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FIG. 1. Chemical structures of 6 *beta*-adrenergic receptor blocking drugs.

FIG. 2. Time course of effects produced by equimolar concentration (1.35 x 10^{-5}M) of Kö-592 and *dl*-propranolol on isometric force of contraction of electrically driven (120 beats/min) rabbit left atria. Each point is the mean of 4-10 experiments. Vertical lines = ± S.E.M. In this and subsequent Figures, effects are expressed as per cent of the contraction force recorded after 100 min equilibration. Drug effects begin at time 0 in Figures.

FIG. 3. Time course of effects produced by equimolar concentration (1.35 x 10^{-5}M) of *d*- and *dl*-propranolol on force of contraction of electrically driven rabbit left atria. Each point is the mean of 4-10 experiments.

FIG. 4. Time course of effects produced by *d*-, *l*-, and *dl*-pronethalol (1.13 x 10^{-5}M) on force of contraction of electrically driven rabbit left atria. Each point is the mean of 4-10 experiments.

TABLE II. Chronotropic Effects of Several *Beta*-Adrenergic Blocking Drugs on Spontaneously Beating Rabbit Right Atria.

Compound (1.35×10^{-5}M)	No. of exp	Heart rate: Pre-drug 0′ (=100%) BPM*	Heart rate: Post-drug 60′ %Δ†	Heart rate: Post-drug 120′ %Δ†
Untreated	8	156.1 ± 3.5	— 6.3 ± 3.0	— 6.6 ± 2.7
dl-Kö-592	7	155.0 ± 7.9	— 3.9 ± 1.6	— 4.2 ± 1.4
dl-propranolol	8	152.0 ± 5.0	—19.1 ± 2.6	—30.6 ± 4.4
DCI	4	152.8 ± 5.4	+29.5 ± 4.2	+10.3 ± 3.1
l-pronethalol	4	157.0 ± 2.8	— 8.0 ± 1.8	— 8.8 ± 1.9
l-MJ-1999	8	152.4 ± 7.0	— 9.6 ± 4.2	—16.5 ± 5.3
dl-pronethalol	8	133.3 ± 5.5	+ 1.12± 2.7	— 8.5 ± 3.1
d-propranolol	4	159.8 ± 8.1	—19.2 ± 1.7	—25.0 ± 3.3
dl-MJ-1999	4	146.3 ±12.9	— 1.3 ±12.4	— 2.4 ±12.7
d-pronethalol	4	130.3 ± 6.3	— 9.3 ± 1.7	—12.4 ± 1.7
dl-MJ-1998	4	134.0 ± 6.1	— 5.7 ± 2.1	— 6.6 ± 3.4
d-MJ-1999	3	160.3 ±11.3	— 2.1 ± 2.9	— 7.7 ± 2.3

* Beats/min, mean ± S.E.M.
† % Change from pre-drug value, mean ± S.E.M.
Underlined values are significantly different from untreated controls ($P < 0.05$) (Student's "t" test).

propranolol, DCI had significant positive inotropic and chronotropic effects on the heart while propranolol had the opposite effects (see below).

Inotropic effects. Fig. 2 shows the effects of equimolar concentrations of *dl*-Kö-592 and *dl*-propranolol on the force of contraction of electrically driven left atria. Kö-592 was found to have no significant effect on the force of contraction, while propranolol caused a marked negative inotropic effect culminating in asystole at approximately 36 minutes after addition of the drug to the bath.

This observation is of particular interest since these two drugs are almost equipotent in terms of their *beta*-adrenolytic action on the atria. Kö-592 was found to have no *beta*-stimulant actions in the concentrations studied. However, in concentrations of 1.35×10^{-4} M and greater, Kö-592 produced significant negative inotropic effects.

Fig. 3 compares the effects of the *d*- and *dl*- isomers of propranolol on force of contraction. Although the *d*- isomer is approximately 9 times less potent as a *beta*-blocker than the *dl*- form, it nonetheless produces a marked negative inotropic effect. Unlike the more potent *dl*- form, however, it did not produce asystole during a 2-hr observation period.

The *dl*, *d*- and *l*- isomers of MJ-1999 were all devoid of any significant action on the force of contraction in a concentration of 1.3×10^{-5} M. MJ-1998 was also without significant effects on contraction in concentrations of 6.75×10^{-5} M and 1.69×10^{-4} M. In a concentration of 1.35×10^{-6} M, DCI produced a maximum increase in force of contraction of 21.1 ± 2.3% at 6 minutes, with a significantly maintained increase (compared to untreated controls) lasting the full 3 hours of observation. In a concentration of 1.35×10^{-4} M, DCI produced a maximum increase in force of contraction of 29.8 ± 3.2% at 1-2 minutes, followed by a rapid and marked negative inotropic response culminating in asystole at 24 ± 2.2 minutes. The *d* and *l* isomers of pronethalol produced the same significant decrease in force of contraction previously described(2) for the *dl*- form when tested in equimolar concentrations (1.13×10^{-5} M) (Fig. 4).

Chronotropic effects. Table II summarizes the effects of the 6 drugs and their isomers on the spontaneous rate of beating of isolated right atrial preparations. All drugs were studied in a concentration of 1.35×10^{-5} M. DCI produced a consistent and significant increase in rate which lasted over the 2-hour

period of study. All other drugs tested produced either no significant change in heart rate, or a significant decrease, when compared to untreated controls. Thus, only *dl-* and *d-*propranolol caused a statistically significant decrease in heart rate compared to the untreated controls. Kö-592, the most potent of the drugs studied in terms of its *beta*-adrenolytic action, had no significant effect on heart rate in a concentration of 1.35×10^{-5} M. However, in concentrations of 1.35×10^{-4} M and greater, a significant decrease in heart rate was produced. In a concentration of 2.02×10^{-4} M, asystole occurred in 18 ± 3.4 min.

Electrical effects. It has been previously shown that pronethalol and propranolol have significant local anesthetic-like actions on the heart(2,13), while *dl*-MJ-1999 is devoid of such action(2,9,10). In the present study it was determined that in a concentration of 1.35×10^{-5} M, DCI produced only a 5.6% increase in electrical threshold and a 10% decrease in the functional refractory period after 30 minutes exposure to the drug on electrically driven left atria. Neither change in these functions was statistically significant compared to untreated controls. Similarly, Kö-592 in a concentration of 1.35×10^{-5} M produced only an 8.7% decrease in functional refractory period after 30 minutes drug treatment. In contrast to these 2 drugs, *d*-propranolol (1.35×10^{-5} M) caused a marked increase in threshold (>100%) with a concomitant increase in the functional refractory period (28.1%). These changes which occurred at 25 minutes were associated with a marked negative inotropic effect (Fig. 3).

Discussion. The data presented indicate that (a) there is no simple relationship between *beta*-adrenergic blocking action and direct effects on cardiac contraction force, rate, or electrical functions; (b) compounds having the naphthyl nucleus (pronethalol and propranolol and their isomers) are comparatively the most potent cardiac depressants regardless of differences in degree of *beta*-blocking potency; (c) the *l-* isomers of the compounds tested are approximately 15-30 times more potent than the corresponding *d-* isomers, and 2-3 times more potent than the racemic mixtures.

Under the conditions of our study, Kö-592, propranolol, and DCI were the most potent compounds studied. Propranolol, however, possesses significant local anesthetic-like (*i.e.*, depressant) actions, while Kö-592 shows no depressant actions on the heart in concentrations at least 10 times greater than that needed to produce *beta*-adrenergic blockade. Only DCI showed consistent *beta*-stimulant effects in addition to *beta*-blocking action. Less consistently, *dl*-pronethalol also produced a transient increase in cardiac force or rate.

The compounds studied to date strongly suggest that substitutions on the benzene nucleus are critical in terms of obtaining potent *beta*-adrenolytic properties and avoiding cardiac depressant effects. Thus, the naphthyl nucleus characteristic of pronethalol and propranolol appears to be a major feature contributing to the cardiac depression seen with these drugs, regardless of varying degrees of *beta*-blocking action among the isomers of this group. That the phenoxyisopropanolamine structure is not solely responsible for the cardiac depressant effects of propranolol is evidenced by the fact that Kö-592, a methyl substituted phenoxyisopropanolamine compound, was devoid of any significant cardiac depressant effects (when compared in a concentration of 1.35×10^{-5} M or less). However, it has been recently reported that in a concentration approximately 3 times higher, this drug does have a quinidine-like effect (as measured by prolongation of the refractory period, decrease in the maximum rate of rise of the action potential, and prolongation of repolarization in the guinea pig left auricle). Lower concentrations (1.9×10^{-7} M) produced only a slight decrease in force of contraction and refractory period (14). An important conclusion from these studies was that there was no correlation between the "quinidine-like" action of Kö-592 and its *beta*-adrenergic blocking action.

It is interesting that DCI, which, in a high concentration, produced a transient increase in force of contraction followed by a marked negative inotropic effect, is very similar in

molecular size to pronethalol with regard to their aromatic systems. Thus, the two chlorine atoms in DCI are sterically equivalent to the additional benzene ring characteristic of pronethalol and propranolol(15). On an equimolar basis, however, pronethalol and propranolol failed to produce *beta*-adrenergic stimulation like DCI. On the contrary, they caused a significant depression of myocardial function.

The differences in activity between MJ-1998 and MJ-1999 also offer some clues as to the steric requirements for producing myocardial *beta*-blockade. Neither of these 2 agents had any significant effect on myocardial force of contraction in the concentrations tested. Yet there is 5-fold difference in *beta*-blocking action. Both drugs have a *para*-methanesulfonamido group, the only differences between the two compounds being in the lateral amino-alcohol sidechain. The presence of the alkylsulphonyl radical is thought to confer upon the molecules the same ability to align with the adrenergic reactive sites as the phenyl-OH grouping of the catecholamines (16). However, it should be emphasized that the relative lack of activity *in vitro* of MJ-1999 and MJ-1998, both in regard to *beta*-blocking action and direct effect on the myocardium, may be related to the low lipid solubility of these drugs(9). Pronethalol and propranolol show high lipid solubility. The relatively high doses of Kö-592 needed to produce *beta*-blockade *in vivo* (rat) has been attributed primarily to its rapid metabolism by microsomal enzymes in the liver(17).

It also should be emphasized that in the present studies, the ranking of potency of the compounds is based upon the specified experimental conditions. As has been pointed out previously(2), the relative potency of this class of drugs may or may not vary with the type of *beta*-blocking test employed, the pretreatment time, as well as other possible experimental conditions and techniques. The relatively short pretreatment time needed to achieve *beta*-blockade had been previously employed in studies with MJ-1998, MJ-1999, pronethalol, propranolol and DCI(2,6,10,18). Table I is presented to provide an estimate, under a given set of conditions, of the *beta*-blocking actions of the compounds. Our conclusions and interpretations are hence framed only within the design of the present experiments.

The data presented here as well as data previously published(11,12,15,16,18) allow the following generalizations about the structure-activity relations for *beta*-adrenergic blocking drugs of the phenethanolamine and phenoxyisopropanolamine class: (1) Mono alkyl substitution in the *meta* position of the benzene ring of the phenoxyisopropanolamine structure produces the greatest *beta*-adrenolytic action with minor myocardial depressant effects *in vitro*. (2) Double substitution of alkyl or halogen radicals in the 3, 5 or 2, 3 position on the benzene ring of the phenylethylamino structure produces strong *beta*-blocking action, but also significant direct myocardial effects (*e.g.*, DCI, pronethalol, propranolol). (3) Substitution of the acidic methanesulphonamido group in the *para*-position on the benzene nucleus of the phenethanolamine structure yields compounds having slightly weaker *beta*-blocking action *in vitro*, but devoid of any significant *beta*-stimulant or cardiac depressant actions (*e.g.*, MJ-1998 and MJ-1999).

On the basis of the compounds studied to date, and under the described conditions, there does not appear to be any simple interrelationship between (a) chemical structure, (b) direct inotropic and chronotropic effects, (c) *beta*-blocking potency, and (d) ability to depress the electrical properties of the myocardium.

Summary. The inotropic and chronotropic effects of 6 *beta*-adrenergic blocking drugs and their optical isomers were studied on isolated atrial preparations of the rabbit heart. The phenoxyisopropanolamine compound Kö-592 (1-(3-methylphenoxy)-3-isopropylaminopropanol) was the most potent *beta*-adrenolytic compound. DCI and *dl*-propranolol were only slightly less potent; however, pronethalol and 2 methanesulfonanilide substituted compounds (MJ-1998 and MJ-1999) showed weaker actions. The *d*- isomers of pronethalol, propranolol, and MJ-1999 were approximately 7-11 times weaker as *beta*-blockers than their corresponding race-

mates. The *l-* isomers of pronethalol and MJ-1999 were 2-3 times more potent than the *dl-* isomers. The 2 naphthyl compounds (propranolol and pronethalol and their isomers) produced significant depression of myocardial contraction and electrical properties (refractory period and excitability). In equal or higher concentrations, MJ-1998 and MJ-1999 showed no significant effect on the electrically driven atria. DCI produced a significant and sustained increase in force of contraction in a concentration of 1.35×10^{-6} M. A higher concentration (1.35×10^{-4} M) produced a transient increase in force of contraction followed by a rapid decrease leading to asystole. In a concentration of 1.35×10^{-5} M, only *dl-* and *d-*propranolol produced a significant decrease in heart rate of spontaneously beating right atrial preparations. DCI produced a significant increase in rate. On the basis of the compounds studied, there does not appear to be any simple interrelationship between (a) chemical structure, (b) direct inotropic and chronotropic effects, (c) *beta*-blocking potency, and (d) ability to depress the electrical properties of the myocardium.

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Culture of Leucocytes from Rabbit Blood and Lymph: Effect of Phytohemagglutinin and Growth of Macrophages.* (31139)

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Phytohemagglutinin (PHA)(1) has been shown to be mitogenic for human peripheral blood lymphocytes *in vitro*. The mitogenic effect is preceded by and associated with the

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