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Stimulant and Depressant Effects of β-Adrenoceptor Blocking Agents on Isolated Heart Muscle

A Positive Inotropic Effect not Mediated Through Adrenoceptors

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Summary. The chronotropic and inotropic effects of fourteen β-adrenoceptor blocking agents were studied in vitro on preparations of isolated heart muscle from kittens and guinea pigs. Most of the agents exert negative inotropic and chronotropic effects that increase rapidly with concentration above 10^{-6} M. Exceptions are the closely related compounds practolol and atenolol, which have minimal depressant effects in concentrations as high as 2×10^{-3} M. Dose-response relations for the depressant effects are similar for pacemaker frequency and for tension development in atrial and papillary muscle. The cardiodepressant effects of β-blockers are non-stereospecific and are apparently unrelated to the actions of the drugs at β-adrenoceptors. Many β-blockers exert positive inotropic and chronotropic effects at concentrations lower than those that depress. The stimulant effects are slow in onset and do not fade. In most instances these effects are blocked by propranolol and may therefore be considered to be mediated through β-adrenoceptors; (−)-enantiomers are more potent than (+)-enantiomers as adrenoceptor stimulants. Adrenoceptor-mediated inotropic effects are usually more pronounced in atrial than in ventricular muscle. Certain β-blockers, notably INPEA and sotalol, exert positive inotropic effects that are not blocked by propranolol or phenoxybenzamine. The effects are very slow in onset and offset, and are accompanied by the development of contractions with a late tonic component which coincides with a greatly prolonged action potential. Unlike the adrenoceptor-mediated effect, the non-sympathomimetic inotropic effect is more pronounced in ventricular than in atrial muscle, and is not stereospecific. Pindolol also causes the development of a late tonic component, but the accompanying positive inotropic effect is overcome by the simultaneous development of the depressant action of the drug.

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Introduction

The past twenty years have seen the introduction of a large number of new β-adrenoceptor blocking agents, most of which have been reported to have effects significantly different in some respect from those of their predecessors. During much of this time a continuing interest in cardiac adrenoceptors has led us to examine certain properties of many of these compounds as they became available. It is, of course, widely recognized that many β-adrenoceptor blocking agents have both stimulant and depressant effects on the heart (e.g., Fleming and Hawkins, 1960; Åblad et al., 1967; Barrett and Carter, 1970). Nonetheless, there has been uncertainty and even misinformation about certain aspects of these effects. The antagonism of sympathetic influences has sometimes been confused with the directly depressant actions of the drugs (Koch-Weser, 1965). The transient stimulation observed during the onset of action of high concentrations of drugs with biphasic dose-response curves has at times been mistaken for a truly fading drug response. There have been conflicting reports as to whether the stimulant effects of certain β-blockers are direct or mediated through the release of endogeneous norepinephrine (e.g., Dhalla, 1967; Barrett and Carter, 1970). Divergent impressions about the relative prominence of the stimulant effects of various β-blockers have resulted from the comparison of stimulant effects in different tissues or in different species. The experiments reported in this paper were prompted by our need to have clear information about these matters before we could proceed with a more quantitative analysis of the actions of the compounds in question. An unexpected bonus was the discovery that certain β-blockers increase the strength of myocardial

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contraction by an unusual and previously unsuspected mechanism.

Methods

Preparations. Experiments were carried out on tissues from the hearts of kittens (body weight 300 – 1400 g) and guinea pigs (body weight 180 – 900 g) of both sexes. After the animals had been lightly anesthetized with chloroform (kittens) or killed by a blow on the head (guinea pigs), their chests were opened quickly, and the hearts perfused briefly through the inferior vena cava or ventricular stab wounds to clear them of blood. The hearts were then removed and dissected quickly in a shallow dish of freshly oxygenated physiological salt solution at room temperature (20 – 22 °C). Spontaneously beating right atria and quiescent left atrial strips were prepared from both species; thin papillary muscles were also removed from the right ventricles of the kitten hearts. The preparations were mounted, usually in pairs, in one or more units of an apparatus described previously (Blinks, 1965) in which their isometric contractions could be recorded. The muscles were suspended vertically, clamped at the bottom to an electrode holder and tied at the top to stainless steel wire hooks suspended from Statham type G7 strain gauge tension transducers. Records were made with a Sanborn model 154100B oscillograph equipped with 150 – 1100 AS carrier preamplifiers. The left atrial strips, which were cut from the outer surface of the left atrial appendage, were roughly triangular, approximately 5 mm wide at the base and 1 cm long from base to apex. They were clamped at the base and connected to the transducer hook with a short piece of cotton thread tied at the apex. The thinnest available papillary muscles were selected; in no case did we use muscles more than 1 mm thick. The papillary muscles were also clamped at the base and were attached to the transducer hook with a thread tied to the corda tendinea. Left atrial strips or papillary muscles that became spontaneously active were usually reclamped or trimmed in an attempt to remove the focus of spontaneous discharge; if this failed, the preparations were discarded. The atrial strips and papillary muscles were driven with square-wave pulses of 5 ms duration delivered through a punctate cathode in contact with the tissue at the level of the clamp; the anode was a large platinum wire distant from the tissue. Stimuli only barely over threshold voltage (ca. 250 mV for papillary muscles; ca.1 V for atrial strips) were used so that the release of endogenous norepinephrine by the driving stimuli would be minimized (Blinks, 1966, 1967). Left atrial strips were driven at 2-s intervals; papillary muscles at 5-s intervals. To avoid changes in frequency due to stretch of the pacemaker (see Blinks, 1956), the spontaneously beating right atria were suspended between a clamp on the inferior vena cava and a thread tied to the free margin of the atrial appendage; resting tension was set no higher than was necessary to count the contractions on the oscillograph record (about 100 mg). Rough length-tension curves were determined for the driven preparations as soon as they had become reasonably stable. Papillary muscles were then left at the length associated with maximal tension development (L_{max}). At L_{max} the resting tension of atrial strips was very high and tended to creep downward with time. For this reason the atrial strips were set to a length at which the resting tension was approximately half that at L_{max}. Experiments were carried out at 32.5 °C in a physiological salt solution containing (mM) Na^+ 140, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 1.0, Cl^- 98.5, SO_4^{2-} 1.0, HCO_3^- 29, $H_2PO_4^-$ 2, acetate 20 [1], glucose 10. The solution was made up in glass-distilled water, and equilibrated with 95 % O_2 – 5 % CO_2. The organ bath contained 50 ml of the solution.

1 Many of the earlier experiments were carried out in a solution that contained 10 mM fumarate, 5 mM pyruvate, and 5 mM (L)-glutamate instead of 20 mM acetate. A series of experiments on the effects of catecholamines revealed no differences in the behavior of the tissues in the two solutions

Drugs. Racemic mixtures of fourteen β-adrenoceptor blocking agents were studied. These agents (structural formulae are shown in Fig. 1) and their sources are: propranolol, ICI-Ayerst; bupranolol (KL 255), Sanol; Kö 592, Boehringer Ingelheim; pronethalol (nethalide), ICI-Ayerst; alprenolol (H 56/28), Hässle-Astra; oxprenolol (39089-Ba), CIBA; pindolol (SB 46), Sandoz; practolol, ICI-Ayerst; H 87/07, Hässle-Astra; atenolol (ICI 66082), ICI; sotalol (MJ 1999), Mead-Johnson; N-isopropylmethoxamine, Burroughs Wellcome; INPEA, Selvi; dichloroisoproterenol (DCI), Lilly. All of these substances were used as hydrochloride salts except for pindolol and practolol, which were supplied as the base and dissolved as the tartrate and acetate, respectively. In addition, the separate optical isomers (enantiomers) of bupranolol, alprenolol, oxprenolol, and INPEA, and the (+) isomer of propranolol were used, all as the hydrochloride salts. Other drugs used were (−)-isoproterenol bitartrate (Sterling-Winthrop) ((−)-isoprenaline), reserpine phosphate (CIBA), and phenoxybenzamine hydrochloride (Smith Kline & French).

Except for reserpine and phenoxybenzamine, the drugs were made up in aqueous stock solutions (10^{-1} or 10^{-2} M) which were kept in capped vials in a dark refrigerator. The stock solution of isoproterenol was acidified with HCl to pH approximately 3 at the time it was made up. In order to retard the oxidation of the catecholamine, we added ethylenediaminetetraacetic acid, disodium salt (EDTA), 0.04 mM, to the physiological salt solution whenever isoproterenol was to be used (see e.g., Blinks and Koch-Weser, 1963). Phenoxybenzamine was dissolved in a stock solution (10^{-2} M) of 95 % ethanol. Reserpine was dissolved in water from a lyophilized powder at the time of administration and was injected (5 mg/kg subcutaneously in kittens; 10 mg/kg intraperitoneally in guinea pigs) 20 – 26 h before the experiment. Concentrations of all other drugs are expressed as the molar concentration in contact with the tissue. The effectiveness of reserpine pretreatment was checked routinely by subjecting a left atrial strip or papillary muscle from each reserpine-treated animal to high-frequency field stimulation (Blinks, 1966) at the start of the experiment. No evidence of norepinephrine release was ever detected.

Dose-response curves were determined by the cumulative addition of drugs, usually in increments that increased the successive concentrations in steps of approximately 0.5 log unit. The time that each concentration was allowed to act was governed as follows. If a particular dose of a drug produced no effect within 10 min, the effect was recorded as zero, and the next dose was added. If a particular dose produced stimulation, that effect was allowed to develop maximally no matter how much time was required. If a dose produced depression, the effect was followed until either a steady state had been achieved or 30 min had elapsed. If the frequency or force was still declining 30 min after a dose had been added, the measurements were recorded at that time and the next dose was added. This arbitrary procedure was adopted because in the presence of high concentrations of some drugs the performance of the preparations appeared to decline without ever reaching a steady state.

Results

Dose-Response Curves for Inotropic and Chronotropic Effects of β-Blockers. The stimulant and depressant effects of the various β-blockers were studied primarily on isolated tissues from the kitten heart because the onset of action of the compounds was generally slow and these preparations exhibit greater long-term inotropic stability than those from other common laboratory animals (A. Clark and J. R. Blinks, unpublished observations). Furthermore, the kitten heart is highly sensitive to the stimulant actions of partial agonists

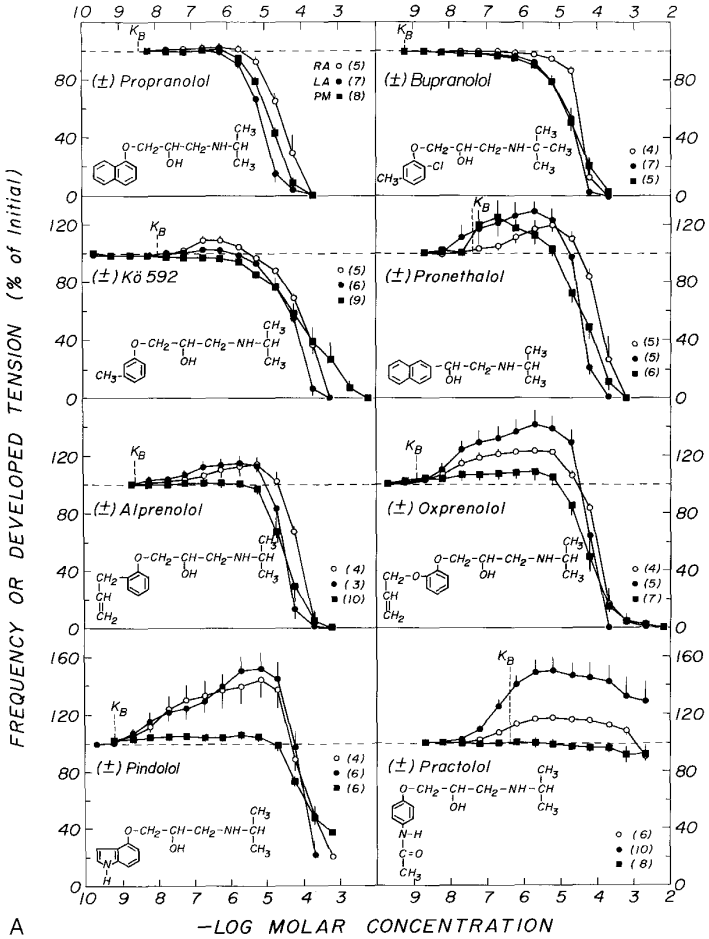
acting on the β-adrenoceptor (see below). Cumulative concentration-effect curves for the racemic mixtures of fourteen β-blockers are shown in Fig. 1. Each of the fourteen panels shows mean dose-response curves for the effects of one drug on the frequency of contraction of right atria and on the tension developed by papillary muscles and left atrial strips. All measurements are expressed as percentages of the initial value for the preparation in question.

Most of the β-blockers studied produced marked depressant effects in concentrations above 10^{-6} or 10^{-5} M. These negative inotropic and chronotropic effects usually increased steeply with concentration, leading in most cases to arrest or inexcitability of the preparation at concentrations between 10^{-4} and 10^{-3} M. Significant exceptions were practolol and the closely related compound atenolol, which had virtually no depressant effect at any of the concentrations tested (up to 2×10^{-3} M). The dose-response curves for the negative inotropic and negative chronotropic effects of most of the agents were strikingly similar, considering the disparate nature of the responses being measured. When marked stimulation occurred (see below) it tended to increase somewhat the concentration at which frequency or force first fell below the control level.

Most of the compounds tested exerted positive inotropic and chronotropic effects in concentrations below those that caused depression. Of the fourteen antagonists studied, only three (bupranolol, atenolol, and N-isopropylmethoxamine) were apparently free of stimulant effects on the kitten heart. The stimulant effects of propranolol were minimal, but definitely present. They were most clearly apparent when concentrations between 10^{-7} and 10^{-6} M were added to previously unexposed preparations that had been allowed to stabilize thoroughly. Under these conditions slight (< 5%) positive chronotropic and inotropic effects could usually be detected in atrial muscle (examples are shown in Blinks, 1967, Figs. 1A and 2). No such stimulation was ever observed when bupranolol was added under similar circumstances. With the notable exception of sotalol the β-blockers tended to have more pronounced inotropic effects on atrial strips than on papillary muscles. The extent of this difference was quite variable, however, as is particularly apparent from a comparison of the effects of practolol (Fig. 1A) and dichloroisoproterenol (Fig. 1B).

Time Course of Stimulation and Depression. The onset of the stimulant effects of β-adrenoceptor blocking agents was characteristically slower than that of catecholamines. All concentrations of catecholamines produced their full effects on our preparations within 3–10 min. Though explicit studies of the time course of onset were not made for all β-blockers, some information on speed of onset was obtained during the determination of the cumulative dose-response curves shown in Fig. 1. In most cases the full stimulant effect was achieved within 10–20 min after each increment in concentration. The onset of action of DCl was particularly slow, especially when low concentrations were used. Thirty minutes or more were sometimes required for the attainment of full effect. A number of experiments were performed in which the onset and persistence of the stimulant effects of various single concentrations of several β-blockers could be followed. When the tissues were exposed to concentrations of the drugs lower than those producing maximum stimulation, there never was any indication of fade – that is, once the maximum effect had been achieved, it persisted as long as the tissue was exposed to the drug. High concentrations (those above the level producing maximum stimulation) produced a biphasic response, but this may be presumed to reflect the onset of the depressant effect of high concentrations of the drugs, and not a fading response in the true sense. Similarly, the development of the stimulant effect of high concentrations of the drugs may appear artificially fast because it is cut short by the onset of depression. Figure 2 illustrates the time course of the stimulant effects of two concentrations of DCl – one submaximal and one supermaximal. The onset of the depressant effects of the β-blockers was characteristically gradual, and sometimes was still progressing slowly at the end of the arbitrary 30-min period between doses adopted for this part of the dose-response curve (see Methods).

Species Differences. The maximum chronotropic (Fig. 3, upper panel) and inotropic (see Kaumann and Blinks, 1980) effects of DCI were only slightly less than those of isoproterenol itself, while the depressant effects of high concentrations of DCI were much more pronounced. These findings are in contrast to those of Fleming and Hawkins (1960), who found that the maximal chronotropic effects of DCI in guinea pig atria were no more than about 30% of those of epinephrine. Because we were interested in finding conditions under which the blocking actions of the various antagonists could be studied with as little interference as possible from stimulant effects, we repeated the comparison between isoproterenol and DCI on guinea-pig atria. The results, shown in the lower panel of Fig. 3, were strikingly different from those in the kitten, and similar to those reported by Fleming and Hawkins. The comparison between the effects of β-blockers in guinea pigs and kittens was then extended to include most of the agents under study. Dose-response curves for racemic mixtures of propranolol, oxprenolol, pindolol, alprenolol, sotalol, INPEA, H 87/07, and N-isopropyl-



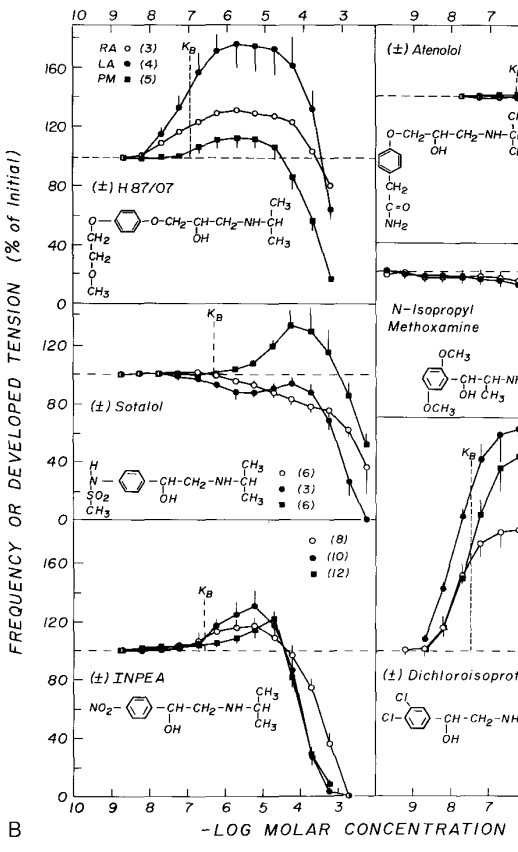


Fig. 1A and B. Stimulant and depressant effects of racemic mixtures of fourteen β-adrenoceptor blocking agents on kitten cardiac muscle. Cumulative concentration-effec 32.5°C for the agents identified in the respective panels. All measurements are expressed as mean percentages of the control frequency or force in the same preparation; SEM.Numbers of experiments are indicated by figures in parentheses in each panel. No preparation was used for more than one curve. Symbols: ○, Frequency of s right atria; ●, tension developed by left atrial strips driven at 2-s intervals; ■, tension developed by papillary muscles driven at 5-s intervals. Stimuli were of threshold stren increased during the phase of depression. K_B indicates apparent equilibrium constant for blockade of chronotropic effects of isoproterenol taken from Kaum Kaumann and Blinks (1980)

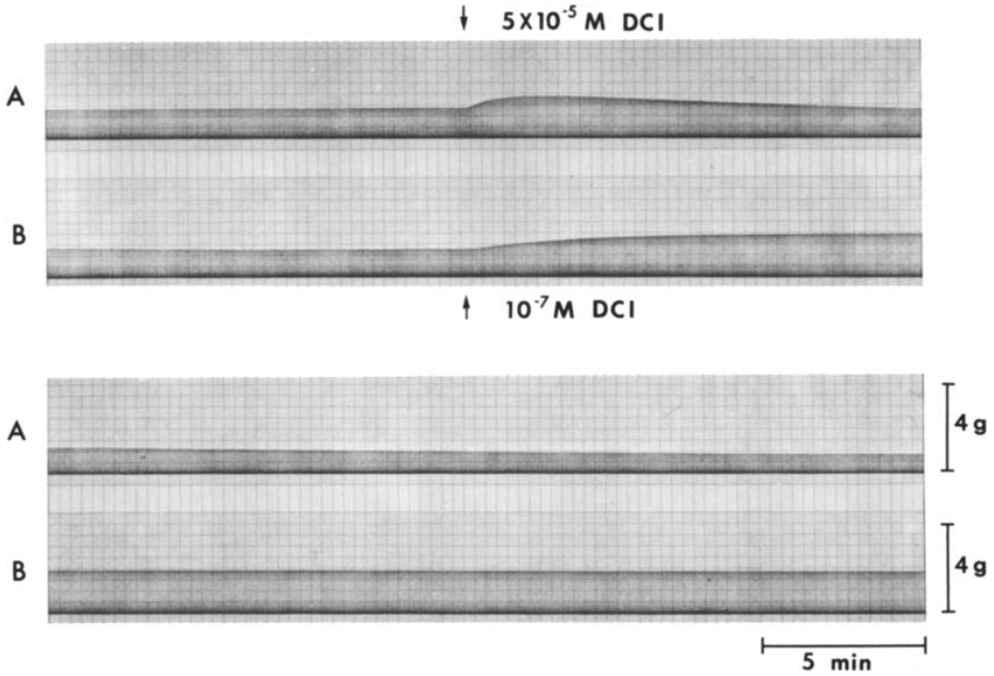


Fig. 2. Responses to sub- and supermaximally effective concentrations of dichloroisoproterenol. Two left atrial strips from the same kitten were set up in separate baths at 32.5°C; they were driven with threshold pulses at 2-s intervals, and isometric contractions were recorded concurrently. At the time indicated by the arrows, (±)-DCI was added to give a concentration of 5×10^{-5} M in strip A, and 10^{-7} M in strip B. Tracings *A* and *B* in the lower panel are continuations of the corresponding ones in the upper panel

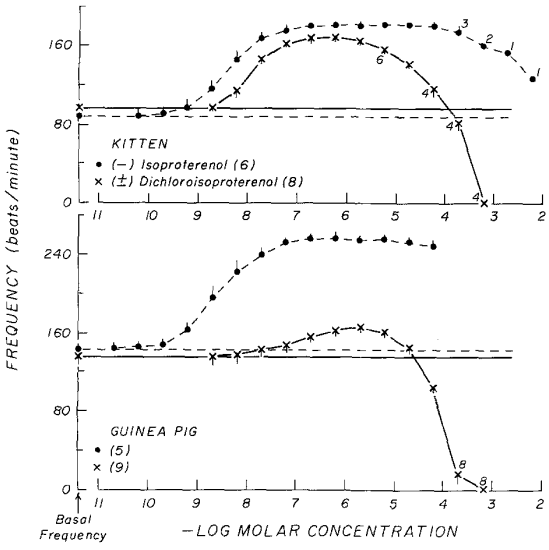


Fig. 3. Species difference in the relative chronotropic effects of isoproterenol and dichloroisoproterenol. Cumulative concentration-effect curves for (−)-isoproterenol (●) and (±)-dichloroisoproterenol (×) determined at 32.5° on spontaneously beating right atria of kittens (upper panel) and guinea pigs (lower panel). Symbols indicate mean ± SEM of absolute frequencies of contraction. Numbers of experiments indicated by figures in parentheses except where indicated by figures beside symbols. Only one curve was determined on a given preparation

methoxamine were determined on spontaneously beating guinea-pig right atria. The negative chronotropic effects of high concentrations of all of these compounds were very similar to those in kitten atria. The positive chronotropic effects were uniformly less pronounced than in kitten atria, however. (Dose-response curves for the stimulant effects are shown in a companion paper – Kaumann and Blinks, 1980.) Only DCI and H 87/07 increased the frequency of contraction of guinea-pig atria by more than 5%. Although full inotropic dose-response curves for only a few of the β-blockers were determined on guinea-pig atria, we had the opportunity to observe the inotropic effects of various individual concentrations of most of the β-blockers during the course of other experiments. The results were generally consistent with the low level of stimulation seen in guinea-pig right atria. Significantly, however, high concentrations of sotalol produced a clear positive inotropic effect in guinea-pig left atrial strips (experiments not shown).

Influence of Propranolol on Stimulation. Whenever any of the β-adrenoceptor blocking agents was found to increase the frequency or force of contraction of a preparation by 10% or more, the dose-response curve was redetermined in the presence of 10^{-6} M (±)-propranolol. (Only a single dose-response curve was

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