

Comparative chronotropic activity of β -adrenoceptive antagonists

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Summary

1. Chronotropic dose-response curves (non-cumulative) for β -adrenoceptive antagonists were constructed from results in rats anaesthetized with pentobarbitone and depleted of catecholamines by pre-treatment with syrosingopine.
2. Depletion of catecholamines lowered resting heart rate and reduced the threshold to the chronotropic action of isoprenaline by about 50%. Eight β -adrenoceptive antagonists produced a dose-dependent chronotropic response but the maximum response was in all cases smaller than that obtained with isoprenaline. The order of activity was dichloroisoprenaline > LB 46 > practolol > INPEA > oxprenolol > pronethalol > alprenolol > I.C.I. 45,763 (Kö 592). Propranolol and sotalol were without significant activity. The duration of the chronotropic response to the antagonists was more prolonged than that to isoprenaline. Propranolol caused a parallel shift to the right of the dose-response curves for the agonist effects of the antagonists.
3. Estimation of β -adrenoceptor blocking activity in anaesthetized cats gave an order of activity dissimilar to that found for maximum agonist responses: LB 46 > oxprenolol > alprenolol > propranolol > I.C.I. 45,763 > practolol > dichloroisoprenaline > sotalol > INPEA > pronethalol.
4. Consideration of chemical structure and physico-chemical properties did not explain the differences between the agonist activities of the adrenoceptive antagonists.

Introduction

The first specific antagonist at β -adrenoceptors was dichloroisoprenaline (Powell & Slater, 1958). Pharmacological studies showed it to possess a partial agonist action (Moran & Perkins, 1958), and it is a widely held view that this property militated against its practical use in man.

A naphthyl analogue of isoprenaline, pronethalol, was described by Black & Stephenson (1962) which was said to be devoid of partial agonist properties. Subsequently it was found that this substance produced a marked increase in the level of circulating free fatty acids and that it was indistinguishable from dichloroisoprenaline in this respect (Barrett, unpublished results). Two years later, the more potent propranolol was introduced (Black, Crowther, Smith, Shanks & Dornhorst, 1964), and the original claim that this compound is devoid of agonist activity has

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not been seriously challenged. In recent years a number of other β -adrenoceptor antagonists have been introduced for laboratory and clinical use, the majority of which possess some degree of agonist activity. No attempt appears to have been made to determine the stimulant properties of these agents quantitatively, and the present study was undertaken to provide such a comparison.

Methods

The animals used were male albino rats (190–210 g) of the specific pathogen-free strain (Wistar) bred at Alderley Park. Endogenous catecholamines were depleted by pretreatment, 24 h before use, with syrosingopine (5 mg/kg intraperitoneally). Syrosingopine was selected because Spriggs (1964) found that cardiac stores of noradrenaline were depleted by 90% 24 h after injection of 4 mg/kg subcutaneously. Noradrenaline was determined as described by Iversen (1963).

The heart rates were counted in rats anaesthetized with pentobarbitone sodium 55 mg/kg intraperitoneally by means of a cardiometer triggered by the QRS complex of the electrocardiogram (Horsfall, 1965). Drugs were injected intravenously in volumes of 0.1 ml/100 g, dissolved in 0.9% sodium chloride solution. Each β -adrenoceptive antagonist was injected in four separate rats at each dose level to avoid the possible errors involved in cumulative dose-response curves. The maximum change in heart rate was recorded and the mean value calculated. Some of the experiments were repeated in rats which had received propranolol (0.5 mg/kg subcutaneously) 30 min earlier.

The β -adrenoceptor blocking activity was determined in cats anaesthetized with chloralose 80 mg/kg intravenously. A reproducible submaximal chronotropic response was obtained by intravenous injection of isoprenaline (0.2 μ g/kg). The mean response of sixty cats was 50 ± 4 (S.E.) beats. Each adrenoceptive antagonist was then infused intravenously for a period of 30 min, the isoprenaline challenge being repeated every 10 min. The reduction in the control response after 30 min infusion was calculated and the rate of infusion increased in geometric progression for further periods of 30 min until antagonism approximating to 25, 50 and 75% was attained. The total dose infused over each 30 min period was plotted against the % inhibition and the dose producing 50% antagonism was estimated. Each drug was tested in at least three different cats and the mean dose causing 50% antagonism was calculated with the standard error of the mean.

The drugs used were isoprenaline sulphate, the hydrochlorides of dichloroisoprenaline, pronethalol, propranolol, oxprenolol, alprenolol, practolol, sotalol, INPEA, I.C.I. 45,763 and LB 46 synthesized at I.C.I. and syrosingopine (Ciba). The doses of compounds used all refer to the base and their chemical structures are displayed in Table 1.

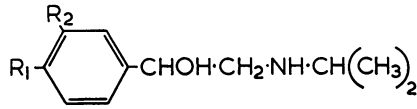
Results

The resting heart rate was considerably lower in rats which had been depleted of catecholamines by 5 mg/kg of syrosingopine than in control rats (Table 2). Syrosingopine reduced the noradrenaline content by at least 95%.

The tachycardia produced by isoprenaline in control rats was dose-dependent (Fig. 1); the threshold dose was 6 ng/kg and the maximum response was produced

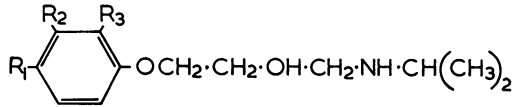
TABLE 1. Chemical constitutions of the drugs used in the experiments

(a) Arylethanolamines



	R ₁	R ₂
Isoprenaline	OH	OH
Dichloroisoprenaline	Cl	Cl
Pronethalol	-CH=CH-	-CH=CH-
Sotalol (MJ 1999)	CH ₃ SO ₂ NH-	H
INPEA	NO ₂	H

(b) Aryloxypropanolamines



	R ₁	R ₂	R ₃
Propranolol	H	-CH=CH-	-CH=CH-
Oxprenolol	H	H	CH ₂ =CH-CH ₂ -O-
Alprenolol	H	H	CH ₂ =CH-CH ₂ -
Practolol	CH ₃ CONH-	H	H
(I.C.I. 50,172)	H	CH ₃	H
I.C.I. 45,763	H	-NH	-CH=CH-
LB 46	H	-NH	-CH=CH-

TABLE 2. Effect of syrosingopine (5 mg/kg intraperitoneally 24 h previously) on resting rate and noradrenaline content of rat hearts (mean ± S.E.)

Treatment	No. of rats	Heart rate (beats/min)	Noradrenaline content (μg/g)
0.9% NaCl solution	12	422 ± 6	0.681 ± 0.059
Syrosingopine	12	300 ± 5	0.031 ± 0.009

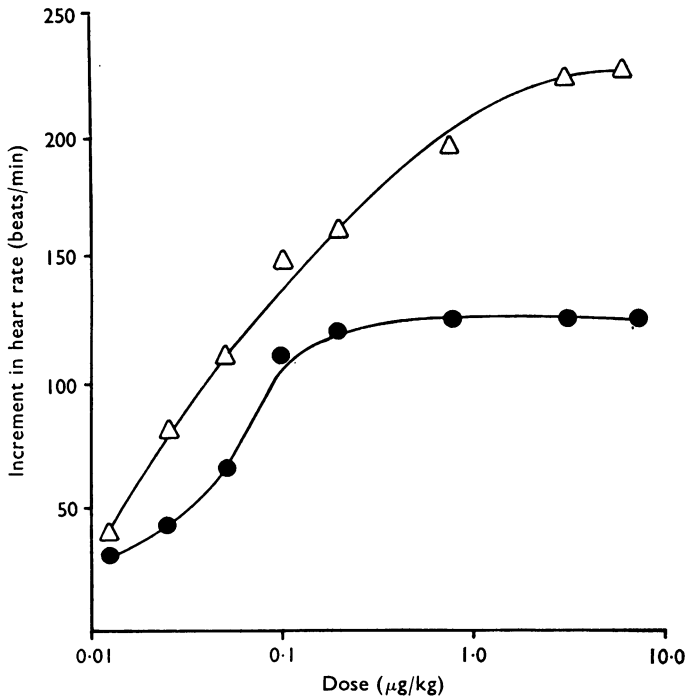


FIG. 1. Increase in heart rate following various doses of isoprenaline in control rats (●) and animals depleted of catecholamines (Δ). Each point represents the mean obtained from four rats.

by 100 ng/kg. The average maximum response was 125 beats/min, corresponding to a final heart rate of 543 beats/min. For rats pretreated with syrosingopine the threshold dose was only 3 ng/kg, and the maximum response was obtained with 3.2 $\mu\text{g}/\text{kg}$, the final heart rate being 521 beats/min. There was no significant difference between the final heart rates in control or depleted rats, although the maximum increment in the syrosingopine-treated rats was almost twice that of the controls because of the low resting rate in the treated animals. Intravenous injection of 0.9% NaCl solution to either control or depleted rats altered the heart rate by less than 5 beats/min.

The relative potencies of isoprenaline and dichloroisoprenaline are illustrated in Fig. 2. The slopes of the two curves were essentially similar but the maximum response to the dichloro analogue was less than that of the parent catechol. The potency difference was approximately 500. The response to isoprenaline, however, was virtually over in 5 min but there was still a substantial increase in heart rate 20 min after injection of dichloroisoprenaline and practolol (Fig. 3).

Dose-response curves were constructed for practolol, oxprenolol, alprenolol, propranolol, sotalol, pronethalol, INPEA, LB 46 and I.C.I. 45,763 (Kö 592) (Fig. 4).

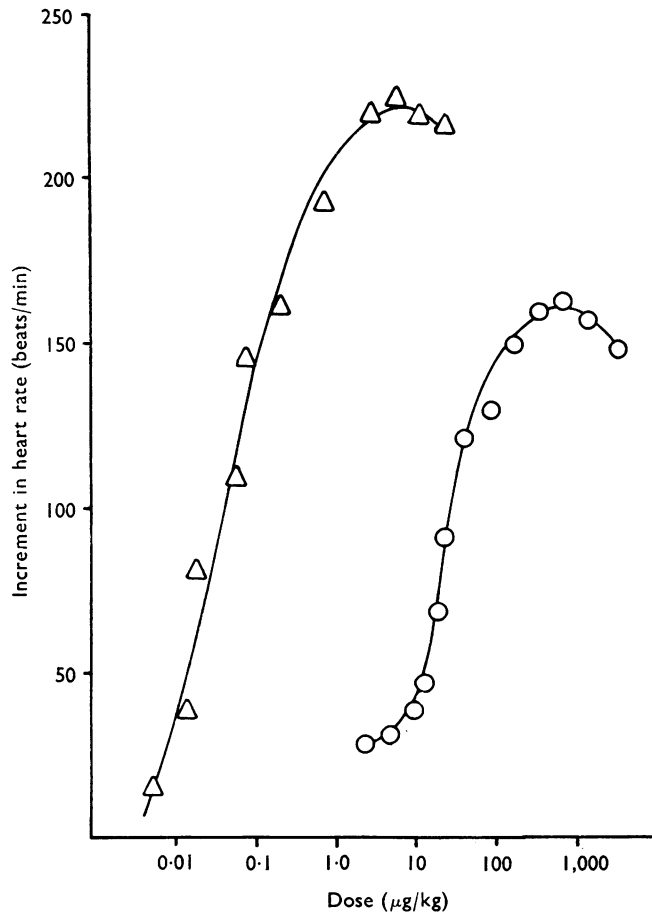


FIG. 2. Increase in heart rate in catecholamine-depleted rats following various doses of isoprenaline (Δ) and dichloroisoprenaline (\circ). Each point represents the mean obtained from four rats.

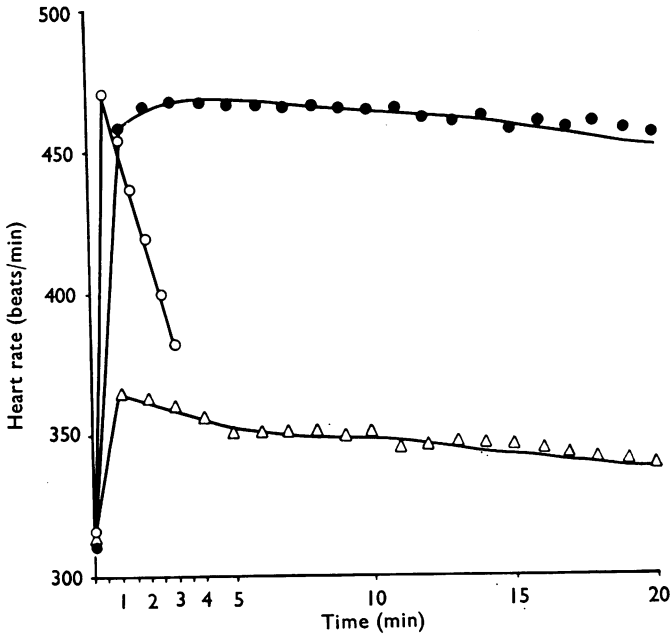


FIG. 3. Duration of tachycardia following submaximal doses of isoprenaline 0.1 $\mu\text{g}/\text{kg}$ (\circ), dichloroisoprenaline 200 $\mu\text{g}/\text{kg}$ (\bullet) and practolol 20 $\mu\text{g}/\text{kg}$ (\triangle) in catecholamine-depleted rats. Each point represents the mean obtained from four rats.

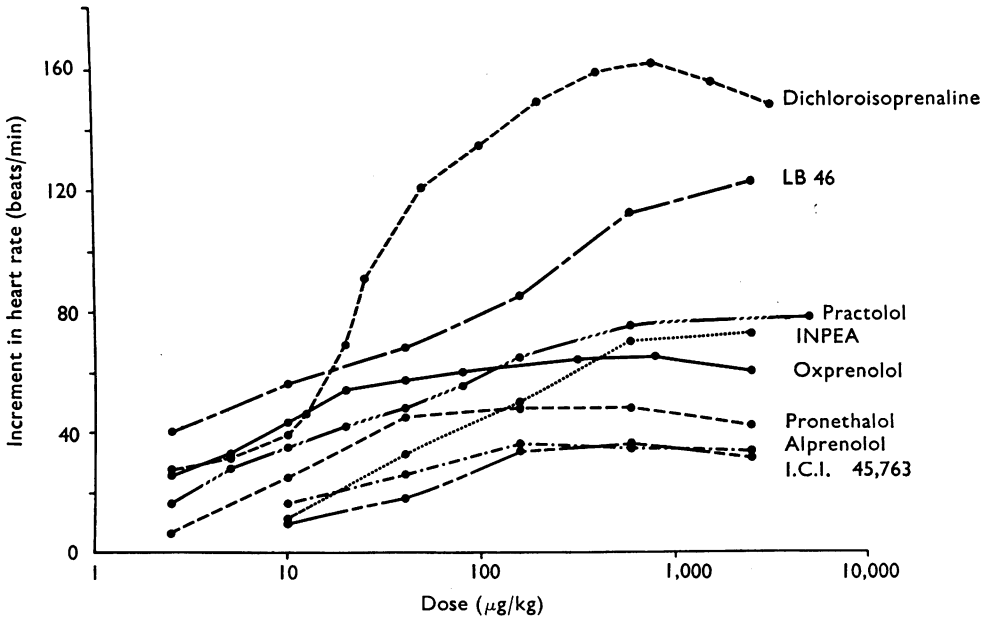


FIG. 4. Increase in heart rate following the intravenous injection of various doses of β -adrenoceptor antagonists in catecholamine-depleted rats. Each point represents the mean value from four animals.

Propranolol and sotalol had no significant effect on heart rate at doses up to 2.5 mg/kg but at higher doses both drugs depressed the heart rate. The maximum response produced by the other drugs varied widely. If the maximum response for each drug is taken as a measure of its agonistic activity, then a figure for each can be calculated taking that for isoprenaline as 1 (Table 3). Estimates of β -adrenoceptive antagonist activity in anaesthetized cats are also included in Table 3 for purposes of comparison. In these experiments, partial agonist activity was not observed directly but it was noted that the degree of bradycardia induced was inversely proportional to agonist potency. There was no obvious correlation between the maximum response for any one drug and its potency as a β -adrenoceptor antagonist.

Dose-response curves for the eight β -adrenoceptor antagonists with agonist activity were also constructed in rats which had been pre-treated with propranolol (0.5 mg/kg subcutaneously). For all compounds, except I.C.I. 45,673, there was a shift in the position of the curve to the right, parallel to the control curve (Fig. 5). I.C.I. 45,673 produced no tachycardia and as the dose was raised heart rate was depressed.

Discussion

It has been suggested that a β -adrenoceptor blocking drug possessing partial agonist properties might be less likely to aggravate cardiac failure than an agent with no agonist activity (Ablad, 1967). Yet it is frequently written in the literature that the clinical utility of dichloroisoprenaline is limited because of its sympathomimetic properties (for example, Lucchesi and Whitsitt, 1969). From such statements one may be tempted to conclude that a moderate partial agonist activity is acceptable whereas an excess is not. Until now there has been no information available with respect to the relative agonist activities of the β -adrenoceptor antagonists currently undergoing clinical evaluation.

It would have been advantageous to make a comparison of partial agonist potencies in a preparation measuring inotropic rather than chronotropic responses. Preliminary experiments gave qualitative information on positive inotropic actions but it was not possible to obtain reproducible dose-response curves. Techniques em-

TABLE 3. *Relative maximal agonist effects of ten β -adrenoceptor antagonists in catecholamine-depleted rats and their antagonist potencies in cats*

Compound	Agonist activity (maximal response to isoprenaline=1)	Antagonist potency (dose required (μ g/kg) to reduce the chronotropic response to isoprenaline (0.2 μ g/kg) by 50%) (mean \pm s.e.)
Isoprenaline	1.00	—
Dichloroisoprenaline	0.73	730 \pm 80
LB 46	0.56	2.5 \pm 2.0
Practolol	0.35	155 \pm 11.0
INPEA	0.34	984 \pm 112
Oxprenolol	0.29	33 \pm 7.4
Pronethalol	0.28	1,260 \pm 270
Alprenolol	0.16	60 \pm 11
I.C.I. 45,763	0.16	81 \pm 10
Sotalol	0	773 \pm 104
Propranolol	0	62 \pm 10

ployed included isolated cardiac muscle from normal and catecholamine-depleted animals and the estimation of contractility in anaesthetized cats and dogs depleted of catecholamines, using either strain gauge arches or dP/dT derived from the left ventricular pressure changes. Other workers have experienced similar difficulties. For example, Ablad, Brogard & Ek (1967) reported that alprenolol produced an increase in cardiac contractility over the dose range of 0.05 to 1 mg/kg but that the effect of 0.05 mg/kg was quantitatively similar to that of higher doses. Similarly, Blinks (1967) found that pronethalol produced definite but variable positive inotropic and chronotropic responses on isolated cardiac tissues. Levy & Richards (1966) noted that I.C.I. 45,763 (Kö 592) showed less direct negative inotropic activity than propranolol or pronethalol whereas both I.C.I. 45,763 and pronethalol raised the cardiac rate in cats depleted of catecholamines while propranolol did not (Shanks, Wood, Dornhorst & Clark, 1966). Oxprenolol has been shown to possess positive

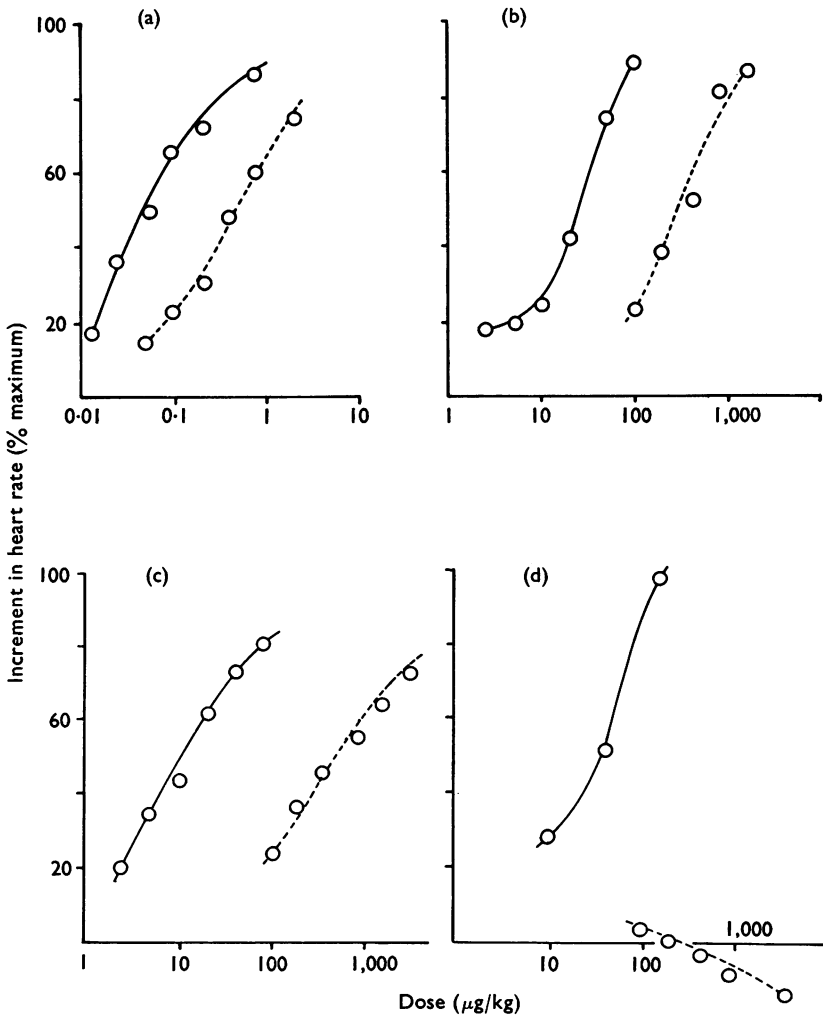


FIG. 5. Increase in heart rate in catecholamine-depleted rats with and without propranolol (0.5 mg/kg) pretreatment: (a) isoprenaline; (b) dichloroisoprenaline; (c) practolol; (d) I.C.I. 45,763. Solid lines indicate control values, broken lines indicate values after propranolol. Each point is the mean obtained from four rats.

inotropic and chronotropic actions in reserpine-treated guinea-pigs and the effects were partly blocked by propranolol (Brunner, Hedwall & Meier, 1968). The stimulant effects of alprenolol were also blocked by propranolol (Ablad *et al.*, 1967). Precise determination of partial agonist activity may be complicated by the fact that some antagonists also depress the myocardium by an action other than on β -adrenoceptors.

The choice of a preparation which would demonstrate positive chronotropic effects *in vivo* rather than *in vitro* was partly due to force of circumstance and partly deliberate inasmuch as potencies *in vitro* do not always parallel those found *in vivo*. For example, practolol possesses only 1/70 the potency of propranolol *in vitro* (Foo, Jowett & Stafford, 1968; Jackson, 1968; Barrett & Shakespear, unpublished results) yet *in vivo* the value is approximately 1:3 (Barrett, Crowther, Dunlop, Shanks & Smith, 1968; Papp & Vaughan Williams, 1969). Depletion of catecholamines before the assessment of partial agonist activity is necessary to obviate the complications of blockade of endogenous noradrenaline and the use of pentobarbitone as an anaesthetic further decreases the contribution of any residual autonomic activity (Barrett, unpublished).

The results of the present investigation clearly demonstrate the relative partial agonist properties of a number of β -adrenoceptor blocking agents. Determination of the maximal responses is independent of variations in uptake, distribution and metabolism of the compounds tested. This will not be true for the dose necessary to produce 50% of the maximum response and therefore such estimates cannot be equated to affinity with confidence. It is of interest, however, that the ED₅₀ value for isoprenaline (50 ng/kg) is very similar to that quoted for isoprenaline *in vitro* (Wale, 1970), if the volume of distribution in blood is 10% of body weight.

The results confirmed the expectation that dichloroisoprenaline had the greatest agonist activity and that propranolol and sotalol were without significant action. All the other compounds tested exhibited intermediate agonist activity. It was not possible to discern a clear structure-activity relationship. Agonist activity was associated with the presence or absence of naphthyl or phenyl nuclei, of a methoxy bridge between the aromatic moiety and the ethanolamine side-chain and of hydrophilic or lipophilic characteristics. Similarly, the absence of agonist activity in propranolol and sotalol could not be related to these characteristics. The fact that propranolol caused a shift of all dose-response curves to the right, in parallel fashion, argues in favour of a common point of action of these drugs for both agonist and antagonist activity. It is obvious, however, that there are unexplained problems emerging from this work which will require further investigation.

In conclusion, it may be held that β -adrenoceptor antagonists with partial agonist activity will not lower heart rate to the same extent as compounds devoid of this property, except in those situations where sympathetic drive is high (for example post-infarction states), when blockade of endogenous sympathetic activity will be more apparent than the stimulant effects of the drug (compare Forsberg & Johnsson, 1967; Lund-Larsen & Sivertssen, 1969). In addition, statements such as "the negative inotropic effect of Ciba 39,089 Ba (oxprenolol) . . . is significantly less than that of propranolol" (Nayler, Chipperfield & Lowe, 1969) must be construed in terms of net effects rather than absolute properties in view of differences in agonist activities of these drugs.

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