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Study on some selective β-adrenoreceptor blocking effects of 1-(4-nitrophenyl)-1-hydroxy-2-methyl isopropylaminoethane (α-methyl INPEA)

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1. The effect of methyl substitution in the α-carbon position of the ethanolamine side chain of INPEA was investigated on its β-adrenoreceptor blocking activity in the isolated turtle heart and anaesthetized cats.

2. In the turtle heart preparation, the pA_2 value for erythro-α-methyl INPEA was 5·3, as compared with 6·9 for INPEA, while threo-α-methyl INPEA was extremely weak and its pA_2 value could not be determined.

3. In the intact cat experiments, α-methyl INPEA produced a competitive blockade of the peripheral vasodilator effect of isoprenaline in doses ranging from 5 to 20 mg/kg. However, this agent had a minimal effect on the cardiac stimulant effect of the catecholamine.

4. These results are consistent with the hypothesis that the β-adrenoreceptors comprise a family of nonhomogeneous receptors and a selective blockade of only some, but not all, responses mediated through their activation can be achieved by specific molecular modification of the β-adrenoreceptor blocking agents.

Studies on the structure-activity relationship of β-adrenoreceptor blocking agents have clearly demonstrated that most of these agents selectively antagonize various pharmacological responses mediated through activation of β-receptors. In recent years, however, several reports have appeared to suggest that a selective blockade of only a few pharmacological effects of β-receptor activation can be achieved by some structurally related compounds. Thus, Van Deripe & Moran (1965) reported that substitution with a methyl group at the α-carbon atom in the ethanolamine side chain of dichloroisoprenaline (α-methyl DCI) altered the pharmacological activities in such a manner that the agent selectively abolished the vasodilator effects of isoprenaline, but inhibited the positive inotropic effects of both calcium chloride and the catecholamines. Methoxamine and *n*-isopropylmethoxamine have been shown to inhibit the metabolic effects of catecholamines in doses which had little or no effect on haemodynamic actions of the sympathomimetic amines (Burns, Colville, Lindsay & Salvador, 1964 ; Levy, 1964). More recently, ICI 50172 has been found to antagonize selectively the cardiostimulant effects of sympathomimetic amines with little or no effect on other smooth muscle responses to β-receptor activation

(Dunlop & Shanks, 1968). In the present study, the effect of α-methyl substitution in the side chain of *n*-isopropyl-*p*-nitrophenylethanolamine (INPEA) was investigated on the β-adrenoreceptor blocking activity. The structure of α-methyl INPEA is shown in Fig. 1.

Methods

Isolated turtle heart preparation

A modified Straub heart preparation was used in all experiments and the technique has been described in detail in a previous publication (Laddu & Somani, 1969). Briefly, the ventricles of the turtle *Pseudomys elegans* were rapidly removed and suspended from a cannula-reservoir containing the borate-acetate buffer solution at pH 7·5. The composition of the buffer solution (g/l.) was: boric acid 0·309 ; NaCl 4·74 ; KCl 0·222 ; sodium acetate ($3{\cdot}H_2O$) 0·68 ; $CaCl_2$ ($2{\cdot}H_2O$) 0·294. The ventricles were paced electrically by square wave stimuli of threshold voltage (3–6 V) and 10 msec duration at the rate of 24 beats/min. The ventricular contractile force was recorded by connecting the frenulum of the ventricles to a Grass FT-03 force-displacement transducer over a pulley.

The positive inotropic effect of cumulative doses of isoprenaline, ranging from 1×10^{-13} to 1×10^{-7}M, was obtained by adding the drug directly to the reservoir in the absence and presence of four different concentrations (1×10^{-6} to 1×10^{-4}M) of the antagonist. At least six preparations were used for each concentration of the blocking agent. The increase in ventricular contractile force in response to isoprenaline, expressed as per cent of maximum effect obtained with the highest concentration of the agonist, was plotted on a semilogarithmic paper to determine the nature of the shift in the dose-response curves by the antagonist. The pA_2 values were calculated according to the method of Arunlakshana & Schild (1959).

Experiments on cats

Adult cats of either sex, weighing 2–5 kg, were anaesthetized with Dial-urethane (0·6 ml./kg intraperitoneally). The trachea, a femoral vein and a carotid artery were cannulated. The systolic, diastolic and mean arterial blood pressure were recorded via a Statham pressure transducer from the carotid artery. The heart rate (HR) was counted from the lead II electrocardiogram (e.c.g.). Positive pressure artificial respiration with room air was maintained by a Harvard pump. The thorax was opened by a midline incision and a small Brodie–Walton strain gauge arch for cats was sutured to the right ventricle in such a manner that a 50% stretch was produced between the two legs of the arch. All the cats were vagotomized bilaterally.

Experimental design

These investigations were carried out in sixteen cats. During the control period, the effect of four different doses of isoproterenol (0·125–1 μg/kg intravenously) on

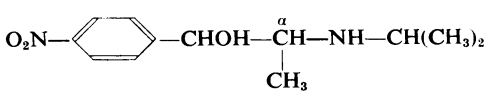

FIG. 1. Structure of 1-(4-nitrophenyl)-1-hydroxy-2-methyl isopropylaminoethane (α-methyl INPEA).

HR, myocardial contractile force (CF) and arterial blood pressure was obtained. In a series of nine animals, the effect of isoprenaline (0·125–2 μg intravenously) was determined after the administration of 5 mg/kg of α-methyl INPEA. In the second series of seven animals, the blocking agent was administered in 10 and 20 mg/kg dose and the response to various doses of isoprenaline was elicited before and after administration of the antagonist. This protocol was necessitated by a very small supply of α-methyl INPEA at our disposal in the early phase of the investigation. The following drugs were used: The *threo* and *erythro* forms of 1-(4-nitrophenyl)-1-hydroxy-2-methylisopropyl aminoethane (α-methyl INPEA) were synthesized by Drs. L. Almirante and W. Murmann, of Selvi & Co., Milan, Italy. The hydrochloride salts, which are freely water soluble, were used in this study. Fresh solutions were prepared daily in 0·9% NaCl solution or in the borate-acetate buffer solution. Since the *threo*-α-methyl INPEA was found to be extremely weak (see **Results**), further experiments were carried out with only *erythro*-α-methyl INPEA, which will be referred to as α-methyl INPEA in the present paper.

Results

Turtle heart preparation

A cumulative dose-effect relationship to various concentrations (1×10^{-13}M to to 1×10^{-7}M) of isoprenaline was obtained by addition of the agonist directly to the buffer solution in the reservoir. In a control series of ten experiments, repeated estimates of the dose-response relationship to the agonist did not show any appreciable change from the initial response over a period of 2 hr. Addition of *threo*-α-methyl INPEA in 1×10^{-6}M concentration to the buffer solution produced an average of $36{\cdot}5 \pm 7{\cdot}9\%$ (mean ± S.E. ; $n=6$) increase in the myocardial contractile force. Addition of higher concentrations of this agent to the perfusion medium, however, produced a fairly large depression of the myocardial contractility, the latter being reduced by an average of $18{\cdot}8 \pm 1{\cdot}1\%$ (mean ± S.E. ; $n=6$) in 5×10^{-5}M concentration, and $31 \pm 2{\cdot}1\%$ (mean ± S.E. ; $n=6$) in 1×10^{-4}M concentration. *Threo*-α-methyl INPEA did not produce any β-adrenoreceptor blockade in concentrations as large as 1×10^{-4}M, higher concentrations could not be tested properly because of an overt depression of the myocardial contractile force.

Exposure of the turtle hearts to α-methyl INPEA did not produce any initial sympathomimetic effect; an average of $13{\cdot}0 \pm 1{\cdot}8\%$ (mean ± S.E. ; $n=6$) depression in the myocardial contractile force was observed in preparations exposed to 5×10^{-5}M concentrations of the drug. Pretreatment with α-methyl INPEA produced a competitive blockade of the positive inotropic effect of isoprenaline, as indicated by a parallel shift to the right of the dose-effect curve of the agonist in presence of the antagonist. However, α-methyl INPEA was found to be approximately 40 times less active than INPEA in this respect, for the calculated pA_2 value for the former agent was 5·3, as compared with 6·9 for INPEA (Laddu & Somani, 1969).

Experiments in cats

Direct effects of α-methyl INPEA

Slow intravenous infusion of α-methyl INPEA at the rate of 1 mg/kg per min produced a substantial decrease in heart rate, myocardial contractile force and arterial blood pressure (Table 1). Both the negative inotropic and negative chrono-

TABLE 1. *Direct cardiovascular effects of slow infusion of α-methyl INPEA in the cat*

	5 mg/kg (nine animals)	10 mg/kg (seven animals)	20 mg/kg (six animals)
Heart rate (beats/min)			
Before	157±14	144±7	119±9
After	128±14	106±5	96±6
Δ	29±6	35±5	23±4
	$P<0.01$	$P<0.01$	$P<0.01$
Contractile force (% decrease from control*)	50±2	51±4	40±8
	$P<0.01$	$P<0.01$	$P<0.01$
Systolic blood pressure (mm Hg)			
Before	111±10	102±8	89±7
After	104±7	75±8	64±6
Δ	7·5±6	27±9	26±9
		$P<0.05$	$P<0.05$
Diastolic pressure (mm Hg)			
Before	75±11	65±8	56±8
After	72±4	40±7	30±5
Δ	3±2	26±8	25±8
		$P<0.01$	$P<0.01$
Mean blood pressure (mm Hg)			
Before	94±10	83±9	72±7
After	89±8	58±9	46±6
Δ	5±4	25±9	26±8
		$P<0.05$	$P<0.01$

* The contractile force was measured in mm deflection of the pen and the % decrease from control indicates the change in contractility from immediately before to immediately after the drug infusion. All values are mean±S.E.M. *P* values were calculated by paired analysis. Δ=Difference.

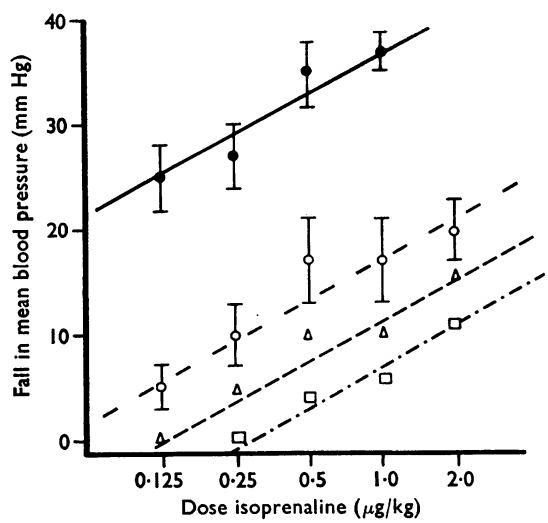

FIG. 2. Effect of pretreatment with various doses of α-methyl INPEA on the lowering of mean blood pressure by different doses of isoprenaline: ●——●, control; ○--○, 5 mg/kg; △-·-·-△, 10 mg/kg; □-·-·-□, 20 mg/kg. Each point represents a mean of at least six experiments. Brackets indicate S.E.M.

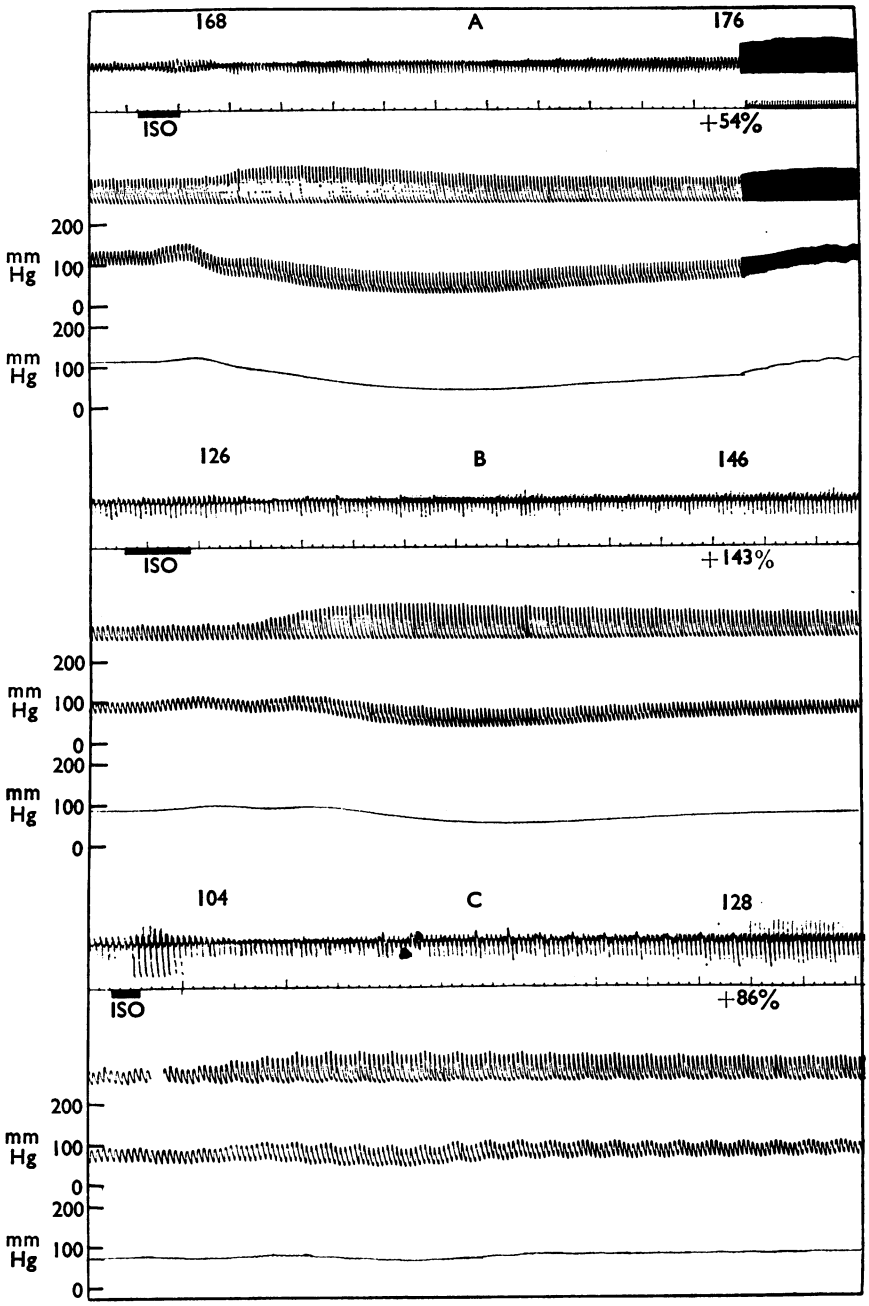

FIG. 3. Effect of isoprenaline (0·5 μg/kg intravenously) on heart rate, myocardial contractile force and arterial blood pressure in an anaesthetized cat before and after treatment with α-methyl INPEA. Panel A, Control response; Panel B, response after 10 mg/kg α-methyl INPEA; Panel C, response after 20 mg/kg α-methyl INPEA. In each panel, tracings from top downwards include: lead II e.c.g.; time marks at 1 and 5 sec intervals; myocardial contractile force (CF) recorded through a strain-gauge arch; systolic-diastolic, and mean arterial blood pressure; numbers above e.c.g. indicate heart rate (beats/min) before and after the administration of isoprenaline and numbers above CF refer to % increase in CF following isoprenaline. Isoprenaline was injected at the signal (ISO).

tropic effects were obtained in a cumulative dose of 5 mg/kg, but in most experiments this dose of α-methyl INPEA produced a slight increase in the arterial blood pressure. A significant decrease in all these haemodynamic measurements was observed with 10 and 20 mg/kg doses of the antagonist. Such a marked reduction in the cardiovascular function of the cat was transient, although some depression of these parameters persisted for a period of more than 15–30 min after the end of infusion of the antagonist in spite of repeated administration of isoprenaline.

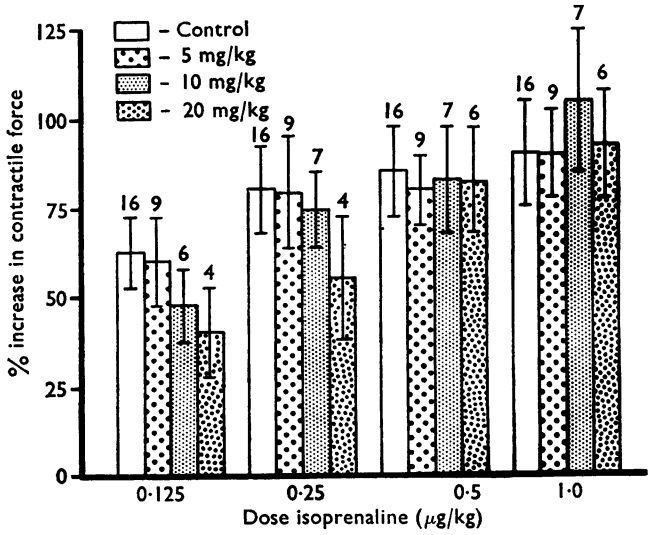

FIG. 4. Effect of pretreatment with various doses of α-methyl INPEA on the increase in heart rate produced by different doses of isoprenaline in cats. In each bar, brackets indicate S.E.M. and numbers above represent the number of experiments.

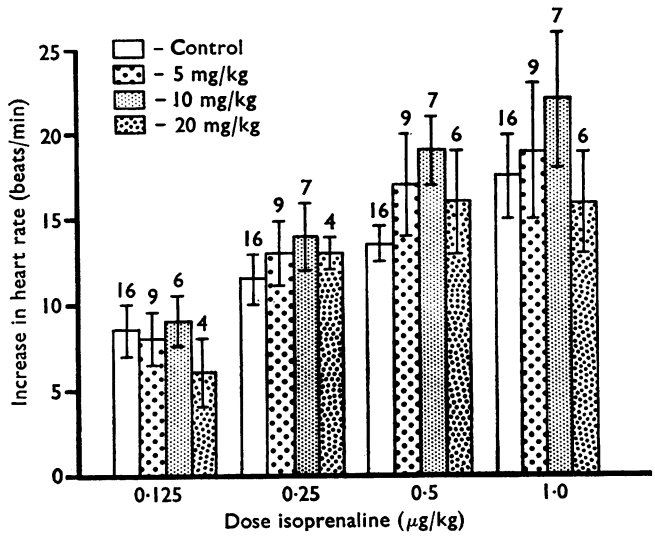

FIG. 5. Effect of pretreatment with various doses of α-methyl INPEA on the increase in myocardial contractile force produced by different doses of isoprenaline in cats. Explanations as in Fig. 4.

Blockade of the vasodilator effect of isoprenaline

Intravenous injection of isoprenaline produced a dose-dependent fall in diastolic and in mean (Fig. 2) blood pressure. Pretreatment with 5, 10 and 20 mg/kg doses of α-methyl INPEA caused a reduction in the vasodilator response to isoprenaline (Figs. 2 and 3). Thus, it may be observed that α-methyl INPEA reduced not only the intensity but also the duration of the fall in blood pressure elicited by isoprenaline (Fig. 3). A parallel shift to the right in the dose-effect curve of isoprenaline clearly demonstrates a competitive blockade of the vasodepressor action of the agonist.

Cardiac effects of isoprenaline

Intravenous injection of various doses of isoprenaline produce an increase in myocardial contractile force and heart rate. Pretreatment with a 5, 10 or 20 mg/kg dose of α-methyl INPEA had little or no blocking effect on either the positive inotropic or the positive chronotropic effect of isoprenaline, except a tendency towards a slight reduction in the effects of the smallest dose of the agonist after a 20 mg/kg dose of α-methyl INPEA (Figs. 4 and 5). It may be observed that in many experiments there was even a slight potentiation of the positive inotropic and positive chronotropic effect of isoprenaline following the administration of α-methyl INPEA (compare Figs. 3, 4 and 5). Larger doses of the antagonist could not be used in these experiments because of a marked depression in cardiac contractility.

Discussion

Specific blockade of the cardiac stimulant and smooth muscle relaxant effects of sympathomimetic amines had led to the classification of propranolol-like agents as specific β-adrenoreceptor blocking agents (Moran, 1967). Synthesis and pharmacological investigations of drugs such as dichloroisoprenaline, pronethalol, INPEA, MJ 1999 [4-(2-isopropyl-amino-1-hydroxyethyl)-methanesulfonanilide], propranolol, H 56/28 [1-O-allylphenoxy)-3-isopropylamino-2-propane], ICI 45763 [1-isopropyl-amino-3-(3-tolyloxy)-2-propanol] and other similar agents has provided support for the classification of adrenergic receptors into α and β categories (Ahlquist, 1948). In recent years, however, evidence has accumulated that a simple classification of the adrenoreceptors into α and β categories is not sufficient to explain the results obtained with newer synthetic agents which antagonize some, but not all, of the effects believed to be mediated through the activation of β receptors. Thus, isopropylmethoxamine was found to selectively block the metabolic effects of sympathomimetic amines with little or no blockade of the haemodynamic effects (Burns *et al.*, 1964 ; Meester, Hardman & Barboriak, 1965). Similarly, butoxamine and dimethyl isopropylmethoxamine were reported to be more potent in inhibiting the metabolic and vasodepressor rather than the cardiac effects of isoprenaline (Burns & Lemberger, 1965 ; Salvador & April, 1965 ; Levy, 1966a, 1966b, 1967). More recently, Dunlop & Shanks (1968) have reported that ICI 50172 selectively blocked the cardiostimulant action of catecholamines in doses which failed to affect the vasodilator action of isoprenaline.

Van Deripe & Moran (1965) have shown that α-methyl substitution in DCI results in a modification of the β-adrenoreceptor blocking activity in such a manner that α-methyl DCI selectively antagonized the vasodilator effect of isoprenaline but

inhibited equally the positive inotropic action of both isoprenaline and calcium chloride. In the present study, it was found that in the intact animal, α-methyl INPEA produced a selective and competitive blockade of the vasodilator effect of isoprenaline with little or no effect on the myocardial stimulant action of the agonist. Thus, α-methyl INPEA appears to be more selective than α-methyl DCI. It must be pointed out, however, that in the isolated turtle heart preparation, α-methyl INPEA did inhibit the positive inotropic effect of isoprenaline, although it was found to be approximately 40 times less potent than INPEA in this respect. On the isolated epididymal fat cells of the rat also, α-methyl INPEA has been observed to be more than 40 times less potent than INPEA in antagonizing the release of free fatty acids by noradrenaline or isoprenaline (Lech & Somani, unpublished observations).

Differences in relative potencies of several sympathomimetic amines on various isolated tissues (Lands & Brown, 1964 ; Lands, Arnold, McAuliff, Luduena & Brown, 1967 ; Furchgott, 1967), and a differential blockade of some but not all the effects of isoprenaline has led some investigators to propose a subclassification of the β-adrenoreceptors into β^1 and β^2 categories (Lands *et al.*, 1967 ; Levy & Wilkenfeld, 1969). Thus, β-receptors in the heart and intestinal smooth muscle are termed β^1 and those in the blood vessels and rat uterine smooth muscle are labelled as β^2. Data obtained in the present study suggest that α-methyl INPEA is more potent in antagonizing the β^2-receptors than the β^1-receptors. Such a selectivity of action of α-methyl INPEA as compared with the effect of INPEA (Somani & Lum, 1965) appears to be acquired when a methyl group is present on the α-carbon atom in the ethanolamine side chain. It is interesting to note that the presence of an α-methyl group in IMA, butoxamine, α-methyl DCI, H 35/25 [1-(4-methylphenyl)-2-isopropylamino propanol] and dimethyl isopropylmethoxamine is a common structural feature of all these drugs which seem to block the effects mediated through the activation of the β^2-adrenoreceptors.

Results obtained in the present study also demonstrate that the *erythro* rather than the *threo* configuration is more important in this respect, because the *threo* α-methyl INPEA was extremely weak in inhibiting the myocardial effects of isoprenaline. It should be pointed out, however, that the *threo* isomer produced a 36% increase in the myocardial contractile force in 1×10^{-6}M concentration, suggesting that it is capable of activating the cardiac β^1-adrenoreceptors. Furthermore, a depression of myocardial contractile force, either *in vitro* or *in vivo*, by both the *erythro* and the *threo* isomers of α-methyl INPEA could not be explained on the basis of blockade of the β^1-adrenoreceptors in the myocardium. It is interesting to note that isopropylmethoxamine has also been reported to produce a marked depression of myocardial contractility in doses which fail to antagonize the cardiostimulant action of the sympathomimetic amines (Burns *et al.*, 1964).

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