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SELECTIVE BLOCKADE OF ADRENOCEPTIVE BETA RECEPTORS IN THE HEART

BY

D. DUNLOP AND R. G. SHANKS*

From Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

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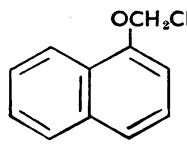
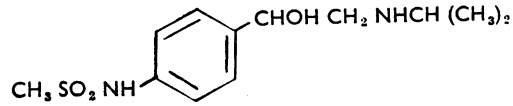
On the basis of a study of the activity of five sympathomimetic amines, Ahlquist in 1948 classified adrenoceptive receptors into two main types, which he designated alpha and beta. This classification has been vindicated by the development of drugs which specifically block the effects of stimulation of one type of receptor but not the other. Classical adrenergic blocking drugs such as phenoxybenzamine, dibenamine, phentolamine, tolazoline and dihydroergotamine block the effects of stimulation of alpha receptors but not beta receptors (Nickerson, 1949; Levy & Ahlquist, 1961; Moran & Perkins, 1961). These drugs are now described as adrenergic alpha receptor blocking agents. Recently several compounds have been described which block beta receptors but not alpha receptors. These, adrenergic beta receptor blocking agents, include dichloroisoprenaline (Powell & Slater, 1958), pronethalol (Black & Stevenson, 1962), INPEA (Teotino, Friz, Steis & Della Bella, 1963), H 13/57 (Corrodi, Persson, Carlsson & Roberts, 1963) propranolol (Black, Duncan & Shanks, 1965), MJ 1999 (Lish, Weikel & Dungan, 1965), I.C.I. 45763 (Kö 592) (Shanks, Wood, Dornhorst & Clark, 1966) and H 56/28 (Johnsson, Norrby, Sölvell & Åblad, 1966). Structurally these compounds are closely related to each other and may be considered as derivatives of isoprenaline; in each case the side chain is identical with that of isoprenaline, or as in the last three compounds differs by the addition of an $-OCH_2$ group. The blocking activity of these compounds is similar qualitatively, in that they block all beta receptors, but differs quantitatively. Another group of compounds, which block some but not all beta receptors, has recently emerged. These compounds include *N*-isopropylmethoxamine (Levy, 1964) which blocks beta receptors in the rat uterus; *N* tertiary butylmethoxamine (Levy, 1966a), and dimethyl isopropylmethoxamine (Levy, 1966b) which block beta receptors in the rat uterus, canine intestine and peripheral blood vessels. None of these compounds blocks the cardiac inotropic or chronotropic actions of catecholamines. Structurally these compounds are characterized by having a methyl group attached to the alpha carbon atom of the side chain. These observations suggest that beta receptors are not a homogenous group and may be capable of division into sub-groups. This hypothesis is further substantiated by the present paper in which

* Present address: Department of Therapeutics and Pharmacology, The Queen's University, Belfast, Northern Ireland.

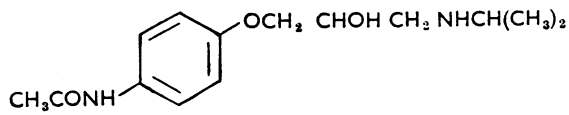
a number of the properties of a new compound, I.C.I. 50172, 4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide are described and compared with the properties of propranolol. The structure of these compounds and MJ 1999 are as follows:



Propranolol



MJ 1999



I.C.I. 50172

METHODS

Measurement of heart rate in anaesthetized cats

Observations were made in cats weighing between 2 and 3.5 kg and anaesthetized by the intravenous injection of chloralose 80 mg/kg. Spontaneous respiration was assisted by the insertion of a tracheal cannula. The femoral veins were cannulated for the injection of drugs. Heart rate was measured by means of a cardiotachometer triggered by the QRS of the electrocardiogram (Horsfall, 1965). Increases in heart rate were produced by the intravenous infusion of isoprenaline and by stimulation of the right stellate ganglion. The right stellate ganglion was approached from the back by removal of part of the second rib: the cardioaccelerator nerves were isolated, their central connexions severed and miniature shielded bipolar electrodes applied to them. The effect of I.C.I. 50172 on the resting heart rate was studied in two cats anaesthetized 16 hr after the subcutaneous administration of syrosingopine (5 mg/kg) to deplete cardiac noradrenaline stores (Orlans, Finger & Brodie, 1960); depletion was confirmed by the absence of an increase in heart rate on maximum stimulation of the cardioaccelerator nerves and on the injection of tyramine (0.5 mg/kg).

Measurement of cardiac function and peripheral blood flow in anaesthetized dogs

Dogs were anaesthetized by the intravenous injection of pentobarbitone, 30 mg/kg, or by the intravenous injection of thialbarbitone, 30 mg/kg, followed by chloralose, 60 mg/kg. A cuffed endotracheal tube was inserted; some animals breathed spontaneously and others were artificially respired with room air. Drugs were injected through a catheter in a foreleg vein. Arterial pressure was recorded from a cannula in the left carotid artery or in a femoral artery with an inductive-type differential pressure transducer (New Electronic Products). Heart rate was measured by means of a cardiotachometer. Cardiac contractile force was measured by suturing a strain gauge arch to the epicardial surface of the right ventricle near to its apex (Shanks, 1966a).

In separate experiments blood flow to the hind-limb of dogs was measured by placing a probe for an electro-magnetic flowmeter (Medicon, K2000) around the external iliac artery as previously described (Shanks, 1967). The probe was calibrated at the end of each experiment. Drugs were injected into the external iliac artery through a fine polyethylene catheter inserted into the artery (Shanks, 1967).

In the appropriate experiments, heart rate, arterial pressure, cardiac contractile force and mean flow through the external iliac artery were continuously recorded on a multi-channel tape recorder (Ampex SP300 or Precision Instruments PI 6108). At the completion of each experiment the tape-recorder was re-run at eight or ten times the recording speed and a permanent record of the responses obtained on an ink-writing recorder (Mingograph, Elema-Schonander).

Measurement of heart rate and blood pressure in conscious dogs

Observations were made on beagle dogs trained to lie or stand quietly. Heart rate was measured by means of a cardiotachometer using fine needle electrodes inserted under the skin. Arterial pressure was recorded from a polythene catheter which had been implanted at least 1 week previously in the right brachial artery. One end of the catheter lay in the aorta and the other end was taken out through the skin at the back of the neck and was connected to a light plastic stopcock. Between observations the catheter was filled with heparinized saline. Pressure was recorded on a Mingograph by connecting the stopcock to an inductive type differential pressure transducer. Drugs were injected through fine indwelling catheters in veins in the ear or administered orally in gelatine capsules.

Studies of cardiac arrhythmias

Arrhythmias were produced with a cardiac glycoside in dogs and cats anaesthetized by the intravenous injection of pentobarbitone (30 mg/kg) and artificially respired with room air. Ouabain, 60 μg/kg, was injected intravenously and was followed at 20 min intervals by 30 μg/kg and then 10 μg/kg which was repeated until a ventricular tachycardia or frequent ventricular ectopic beats developed. The test compounds were infused through a catheter in a femoral vein from a motor-driven syringe. Leads 1, 2 and 3 of the electrocardiogram were obtained from fine needle electrodes inserted into the skin.

Ventricular fibrillation was produced in dogs anaesthetized by the intravenous injection of pentobarbitone by the intra-tracheal administration of methyl chloroform, (1,1,1,trichloroethane) 0.1 ml./kg, followed in 10 sec by the injection, through a catheter in a femoral vein, of adrenaline 10 μg/kg. When dogs, which were pre-treated with an adrenergic blocking compound, survived this first methyl chloroform and adrenaline challenge, it was repeated at hourly intervals for up to 3 hr.

In three dogs the anterior descending branch of the left coronary artery was ligated and the animals allowed to recover (Shanks & Dunlop, 1967). Observations were made on each dog, when conscious, before and on the second, third and fourth days after coronary artery ligation. Electrocardiograms and the responses to the intravenous injection of adrenaline were recorded on these days as described previously (Shanks & Dunlop, 1967).

Measurement of local anaesthetic activity

The local anaesthetic activity of several compounds was compared using the isolated sciatic nerve of a frog. The nerve was immersed in a bath containing 0.8 ml. of Ringer solution at room temperature. One end of the nerve was placed on a pair of stimulating electrodes and the other end on a pick-up electrode. The two sets of electrodes were insulated from each other and from the Ringer solution in the bath, which was earthed. A square wave pulse of 2.0 msec duration and 5 V amplitude was applied through a RF coupler to the nerve at the frequency of 1 pulse/sec. The conducted action potential was displayed on an oscilloscope (Tektronix 502A) and the amplitude was measured. When the height of the action potential had been constant for 30 min, the solution in the bath was replaced by a solution of I.C.I. 50172 or procaine. If the action potential was not reduced by 50% at the end of 30 min, the bath was washed and a more concentrated solution added.

Relaxation of tracheal and bronchial smooth muscle

Pairs of isolated tracheal chains were prepared from guinea-pigs as described by Foster (1966). Control responses were obtained by the selection of a dose of adrenaline which produced approximately 40% of the maximum relaxation of the tissue; a second and similar dose of adrenaline was then added to the organ bath and the cumulative effect recorded. Recovery of the tissue to its normal tone was facilitated by changing the Krebs solution in the organ bath every 5 min for 30 min. I.C.I. 50172 was added to one organ bath and propranolol to the other and after 10 min the effects of the cumulative doses of adrenaline recorded. This procedure was continued using doubling doses of I.C.I. 50172 and propranolol until the effect of adrenaline was reduced to less than that of the first control dose. Groups of four guinea-pigs were put in a cylindrical

Perspex chamber and a 1:80 solution of histamine (base) sprayed into the chamber for 45 sec. After 10 min air was drawn through the chamber for 5 min and the number of surviving guinea-pigs was counted after 15 min. I.C.I. 50172 and propranolol were administered by subcutaneous injection 30 min before exposure to histamine, and isoprenaline 0.1 mg/kg was injected subcutaneously 15 min before exposure.

Drugs

The following drugs were used: (−)-adrenaline bitartrate (Burroughs Wellcome); (−)-noradrenaline bitartrate (Winthrop); (±)-isoprenaline sulphate (Burroughs Wellcome); atropine sulphate (May & Baker); pempidine tartrate (I.C.I.); (±)-propranolol hydrochloride (Inderal, I.C.I.); MJ 1999 (Mead Johnson); I.C.I. 50172, 4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide, as the hydrochloride. Drugs were dissolved in 0.9% saline at the required concentration; doses are expressed in terms of the salt.

RESULTS

Changes in heart rate in anaesthetized cats

The increases in heart rate produced in anaesthetized cats by the intravenous infusion of isoprenaline were recorded before and after the intravenous infusion of I.C.I. 50172. The mean results are given in Fig. 1, which for comparison contains previously published results for pronethalol and propranolol (Black *et al.*, 1965). The infusion of I.C.I. 50172 produced little change in resting heart rate and reduced the isoprenaline-induced tachycardia. These effects were similar to those of pronethalol.

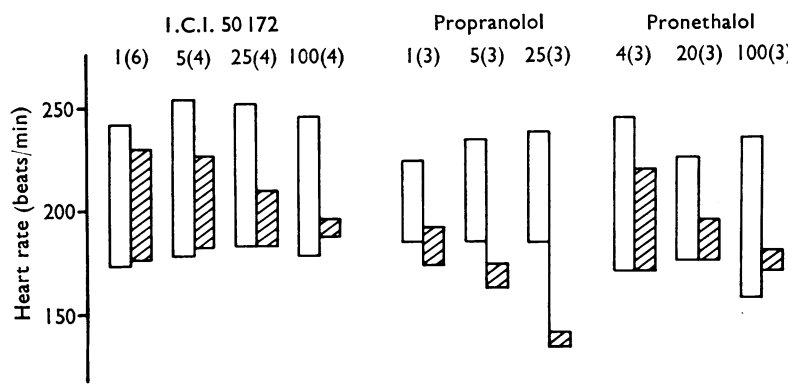


Fig. 1. Cats anaesthetized with chloralose. Open rectangles, increase in heart rate produced by the intravenous infusion for 10 min of isoprenaline (0.2 μg/kg/min); hatched rectangles, response to isoprenaline after the intravenous infusion of I.C.I. 50172, propranolol and pronethalol (doses in μg/kg/min) for 30 min. Each set of results is the mean of observations in the number of cats shown in brackets; the bottom of each rectangle is the resting value and the top of each rectangle the maximum value during the infusion of isoprenaline.

In two cats pretreated with syrosingopine, the intravenous infusion of I.C.I. 50172 increased heart rate to the same extent as pronethalol but less than dichloroisoprenaline (Table 1).

The intravenous infusion of I.C.I. 50172 reduced the increases in heart rate produced in cats by stimulation of the right stellate ganglion (Fig. 2).

TABLE 1

CHANGES IN HEART RATE PRODUCED BY THE INTRAVENOUS INFUSION FOR 30 MIN OF DICHLOROISOPRENALINE (DCI), PRONETHALOL, PROPRANOLOL AND I.C.I. 50172 IN CATS ANAESTHETIZED WITH CHLORALOSE 16 hr AFTER TREATMENT WITH SYROSINGOPINE

Drug and No. of cats	Rate of infusion (μg/kg/min)	Total dose (mg/kg)	Resting heart rate (beats/min)		
			Control	Treated	Change
DCI (1)	100	3·0	157	254	+97
Pronethalol (2)	100	3·0	180	206	+26
	100	3·0	148	170	+22
Propranolol (2)	25	0·75	160	162	+ 2
	25	0·75	150	144	− 6
I.C.I. 50172 (2)	25	0·75	157	184	+27
	25	0·75	122	155	+33

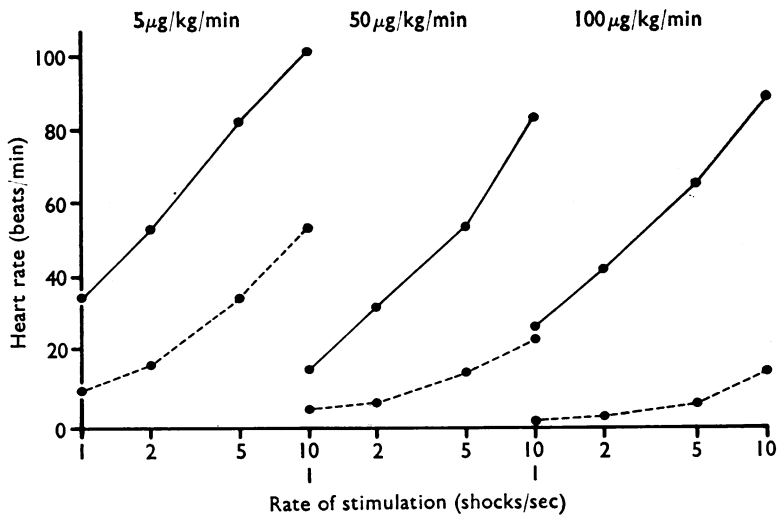


Fig. 2. Cats anaesthetized with chloralose. Increases in heart rate produced by stimulation of the right stellate ganglion at four rates for periods of 20 sec before and after the intravenous infusion for 30 min of I.C.I. 50172 at 5, 50 and 100 μg/kg/min. Continuous lines, responses before drug; interrupted lines, responses after drug. Mean of observations in three cats for each rate of infusion.

Changes in cardiac function in anaesthetized dogs

The intravenous injection of isoprenaline increased heart rate and cardiac contractile force and reduced mean and diastolic arterial pressure (Fig. 3). After the intravenous injection of I.C.I. 50172 (0.5 mg/kg) the increases in heart rate and cardiac force produced by isoprenaline were less and the fall in arterial pressure prolonged. Increasing the dose of I.C.I. 50172 to 5.0 mg/kg completely prevented the cardiac responses to isoprenaline and diminished the fall in blood pressure. The tracings from a typical experiment are shown in Fig. 3. The mean results from three anaesthetized dogs in which the effects of isoprenaline were studied before and after three doses of I.C.I. 50172 are given in Fig. 4. The results from similar experiments with propranolol and MJ 1999 are also included in Fig. 4. I.C.I. 50172 reduced resting heart rate and diastolic pressure

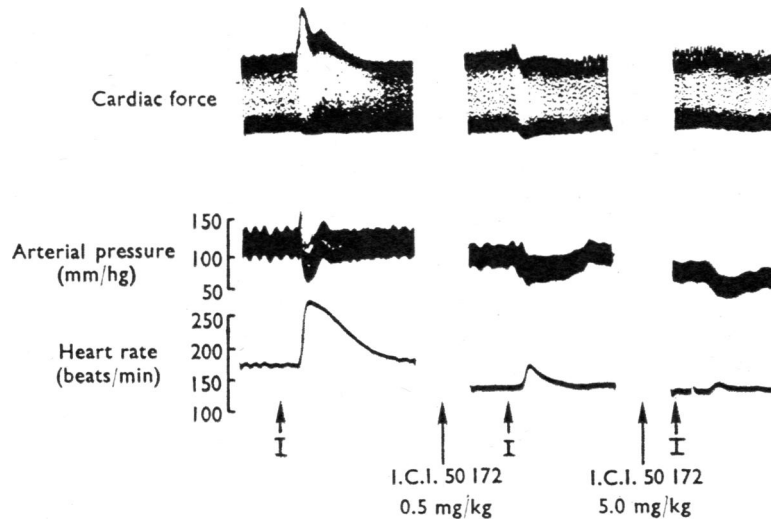


Fig. 3. Dog anaesthetized with pentobarbitone. Records of cardiac contractile force (right ventricle), femoral arterial pressure and heart rate. Responses to the intravenous injection of isoprenaline (I) 0.4 μg/kg before and after the intravenous injection of I.C.I. 50172 (0.5 and 5.0 mg/kg).

slightly but produced no change in cardiac force. It reduced the chronotropic and inotropic effects of isoprenaline but had no effect on the fall in diastolic pressure. This contrasts with propranolol and MJ 1999 which reduced all the effects of isoprenaline.

Further comparisons of the effects of I.C.I. 50172, propranolol and MJ 1999 on the responses to isoprenaline are given in Fig. 5. It was not possible to assess the potency ratio of I.C.I. 50172 accurately, because the slope of the dose response curves for heart rate and cardiac contractile force were different from those for propranolol and MJ 1999. In a low dose producing 20% reduction of the cardiac effects of isoprenaline, I.C.I. 50172 was as active as propranolol but in doses that produced 80% reduction in the responses it was one-eighth to one-tenth as potent than propranolol and equi-potent with MJ 1999.

The effects of adrenaline and noradrenaline on heart rate, cardiac contractile force and mean arterial pressure are shown in Fig. 6. The administration of I.C.I. 50172 reduced the cardiac responses to these amines. The increase in arterial pressure produced by adrenaline was only slightly reduced while there was a greater reduction in the pressor response produced by noradrenaline.

Changes in peripheral blood flow

The injection of isoprenaline into the external iliac artery increased blood flow through the artery; the response increased with the dose (Fig. 7). These responses were not affected by the prior administration of I.C.I. 50172, 0.05, 0.25 or 1.0 mg/kg; the effects of two of these doses are shown in Fig. 7. The intra-arterial injection of I.C.I. 50172, 0.05 and 0.25 mg/kg, did not change flow while 1.0 mg/kg produced a small transient vasodilatation which averaged 4.7 ml./kg/min in the four experiments.

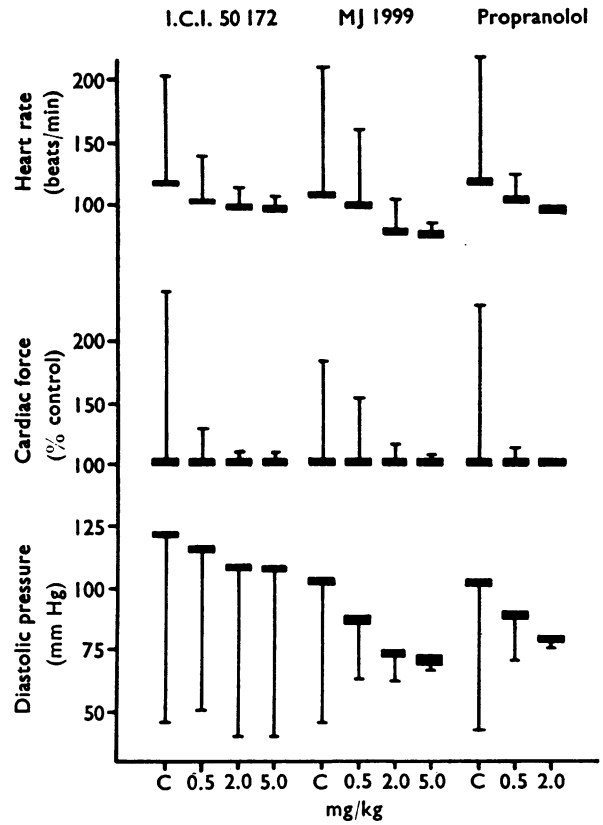


Fig. 4. Observations in dogs anaesthetized with thialbarbitone and chloralose. Changes in heart rate, cardiac contractile force (expressed as % of initial resting value) and arterial diastolic pressure produced by the intravenous injection of isoprenaline (2.0 μg/kg) before and after the intravenous injection of increasing doses of I.C.I. 50172, MJ 1999 and propranolol. Mean of observations in three dogs for each drug. The resting level of each parameter is represented by a thick horizontal line and the maximum response to isoprenaline by a thin horizontal line.

Comparison of activity of I.C.I. 50172 *in conscious and anaesthetized dogs*

The geometrically progressive reduction by I.C.I. 50172 of an isoprenaline-induced tachycardia which was found in anaesthetized dogs was not found in conscious dogs (Fig. 8). In the conscious dog, 0.1 mg/kg produced a much greater reduction of the isoprenaline tachycardia than did the succeeding doses. After I.C.I. 50172, 6.4 mg/kg, the isoprenaline tachycardia was reduced by 97% in the anaesthetized dogs but in the conscious dogs by 60%. I.C.I. 50172 in doses which reduced an isoprenaline tachycardia in conscious dogs did not affect the decrease in diastolic pressure (Fig. 9).

The administration of pempidine potentiated the blocking effect of I.C.I. 50172 on an isoprenaline tachycardia but not on the depressor response (Fig. 9 and Table 2). Pempidine did not influence the effect of propranolol on an isoprenaline tachycardia (Table 2). In conscious dogs pre-treated with pempidine, increasing doses of I.C.I. 50172 progressively reduced an isoprenaline tachycardia in the same manner as in the anaesthetized dogs (Fig. 8).

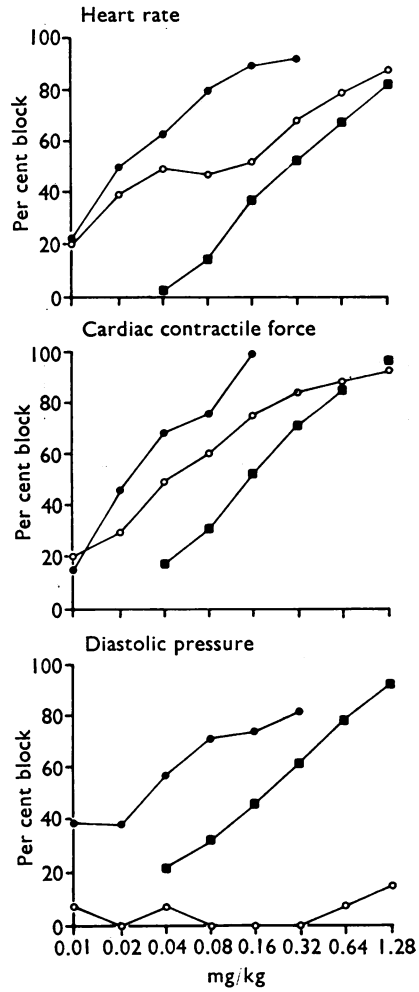


Fig. 5. Dogs anaesthetized with thialbarbitone and chloralose. The changes in heart rate, cardiac contractile force and arterial diastolic pressure produced by the intravenous injection of isoprenaline 0.4 μg/kg were recorded before and after each of a series of doses of I.C.I. 50172 (○——○), propranolol (●——●) and MJ 1999 (■——■), and are expressed as the percentage reduction of the control responses. Results are the mean of observations in three dogs for each drug.

Duration of action of I.C.I. 50172 *in conscious dogs*

A comparison of the duration of the effect of I.C.I. 50172 and propranolol, on oral and intravenous administration, on an isoprenaline tachycardia was made in normal dogs and in dogs pre-treated with pempidine. The effect of I.C.I. 50172 was more prolonged than that of propranolol for both routes of administration and in the two groups of dogs (Figs. 10 and 11). Pre-treatment with pempidine potentiated the effect of I.C.I. 50172 on the isoprenaline tachycardia but not that of propranolol.

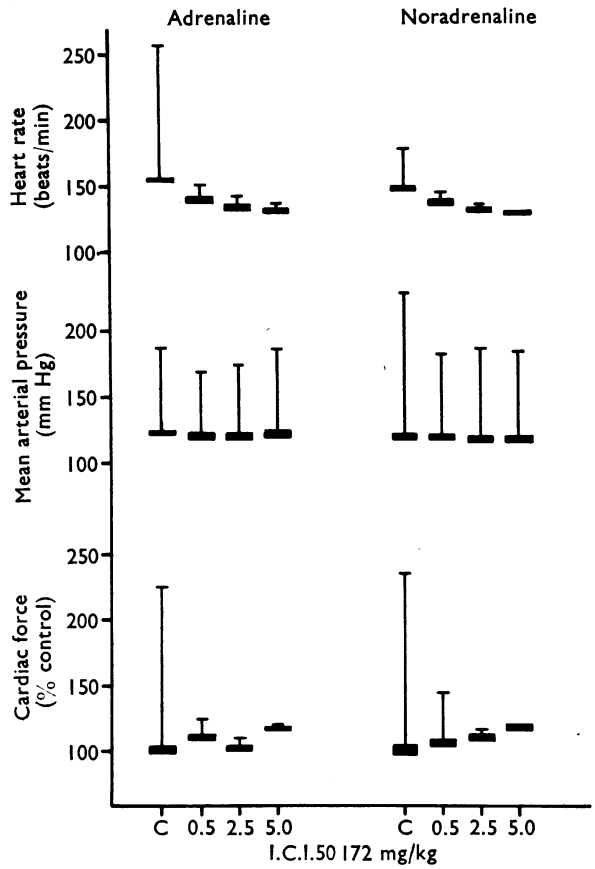


Fig. 6. Dogs anaesthetized with pentobarbitone. Effect of the intravenous injection of a series of doses of I.C.I. 50172 on the increases in heart rate, mean arterial pressure and cardiac contractile force (expressed as % of initial resting level) produced by the intravenous injection of adrenaline 1.0 μg/kg and noradrenaline 1.0 μg/kg. Mean of observations in three dogs. Symbols as in Fig. 4.

Experimental cardiac arrhythmias

A ventricular tachycardia was produced in three dogs anaesthetized with pentobarbitone by the intravenous injection of ouabain in divided doses. Ten minutes after establishment of the arrhythmia, I.C.I. 50172 was infused intravenously at 2 mg/kg/min for 20 min without affecting the arrhythmia. In three dogs the intravenous infusion of propranolol, 2 mg/kg/min for 2–3 min abolished the arrhythmia with a return to sinus rhythm. In similar experiments in four anaesthetized cats, the intravenous infusion of I.C.I. 50172 did not abolish an established cardiac arrhythmia produced by ouabain.

Ventricular fibrillation occurred in four cats after the intra-tracheal administration of methyl chloroform followed by the intravenous injection of adrenaline. In three cats challenged with methyl chloroform and adrenaline 10 min after the intravenous injection of I.C.I. 50172, 1.0 mg/kg, ventricular fibrillation did not develop. On repetition of the

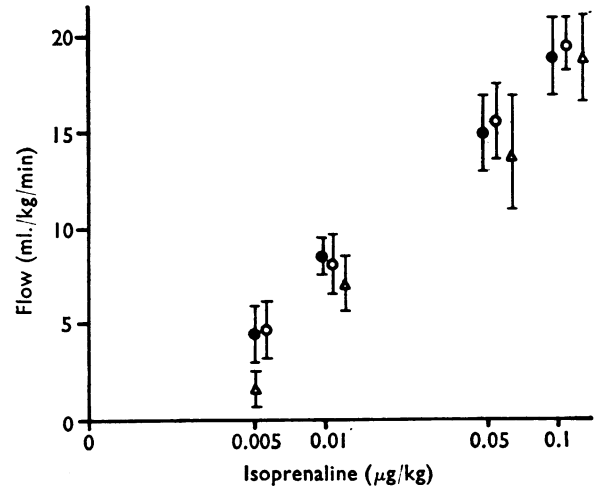


Fig. 7. Mean increases in blood flow to the hind limb of four dogs produced by the intra-arterial injection of isoprenaline before (●) and after the intra-arterial injection of I.C.I. 50172, 0.05 (○) and 1.0 (▲) mg/kg. Standard errors of the mean are included.

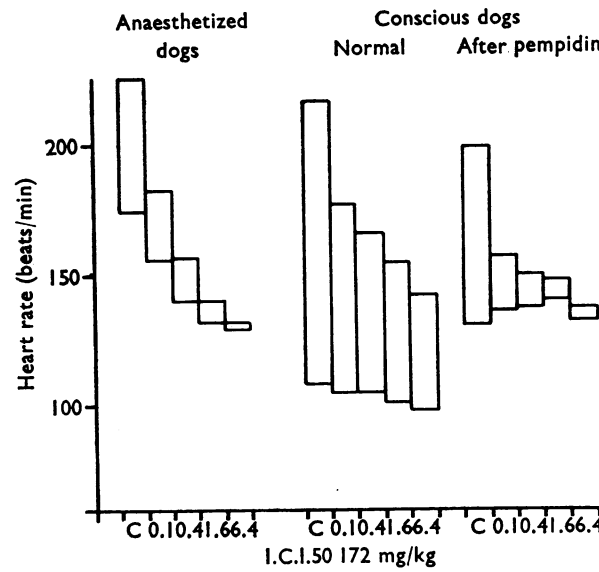


Fig. 8. Mean increases in heart rate produced by the intravenous injection of isoprenaline (0.4 μg/kg) before and 5 min after the intravenous injection of each of a series of doses of I.C.I. 50172 in three dogs anaesthetized with pentobarbitone, three normal conscious dogs and three conscious dogs 30 min after the intravenous injection of pempidine 0.5 mg/kg. The bottom of each rectangle represents resting heart rate and the top the maximum rate produced by isoprenaline.

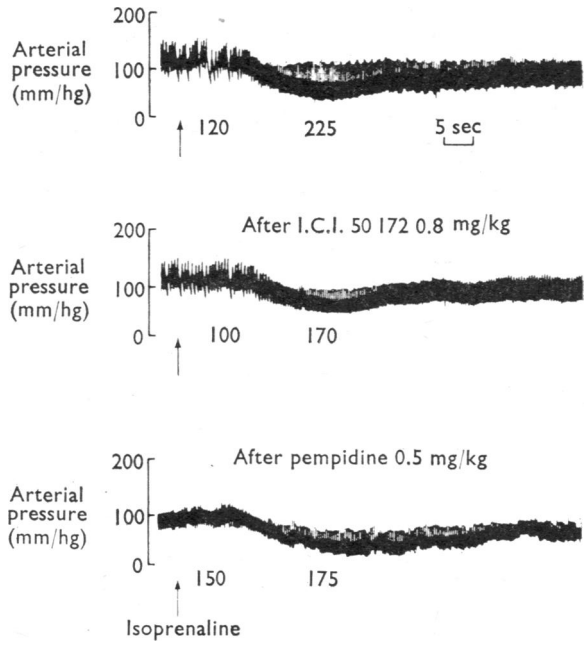


Fig. 9. Arterial pressure recorded from a catheter in the right brachial artery of a conscious dog. Heart rate obtained from a cardiotachometer is given in beats/min below each trace. Isoprenaline 0.4 μg/kg was injected intravenously before and 5 min after the intravenous injection of I.C.I. 50172 0.8 mg/kg and 20 min after the intravenous injection of pempidine 0.5 mg/kg. Pempidine was given 10 min after I.C.I. 50172.

TABLE 2

INCREASE IN HEART RATE PRODUCED BY THE INTRAVENOUS INJECTION OF ISOPRENALINE 0·4 μg/kg ADMINISTERED BEFORE AND AFTER THE INTRAVENOUS INJECTION OF I.C.I. 50172 OR PROPRANOLOL AND AGAIN 20 MIN AFTER THE INTRAVENOUS INJECTION OF PEMPIDINE 0·5 mg/kg

Pempidine was given 10 min after the blocking drug. Observations in six conscious dogs

Heart rate (beats/min)								
Control			After I.C.I. 50172 (0·8 mg/kg)			After pempidine (0·5 mg/kg)		
Rest	Isop.	Increase	Rest	Isop.	Increase	Rest	Isop.	Increase
120	210	90	95	145	50	145	172	29
120	225	105	100	170	70	150	175	25
110	225	115	100	170	70	140	165	25
120	220	100	110	180	70	160	190	30
Control			After propranolol (0·2 mg/kg)			After pempidine		
100	220	120	80	140	60	120	190	70
100	195	95	75	100	25	110	130	20

challenge with methyl chloroform and adrenaline, ventricular fibrillation developed in one cat after 1 hr but had not occurred in the remaining two at the end of 3 hr. In further experiments I.C.I. 50172, 5 mg/kg, and propranolol 1 mg/kg, prevented the development of ventricular fibrillation for at least 3 hr (Table 3).

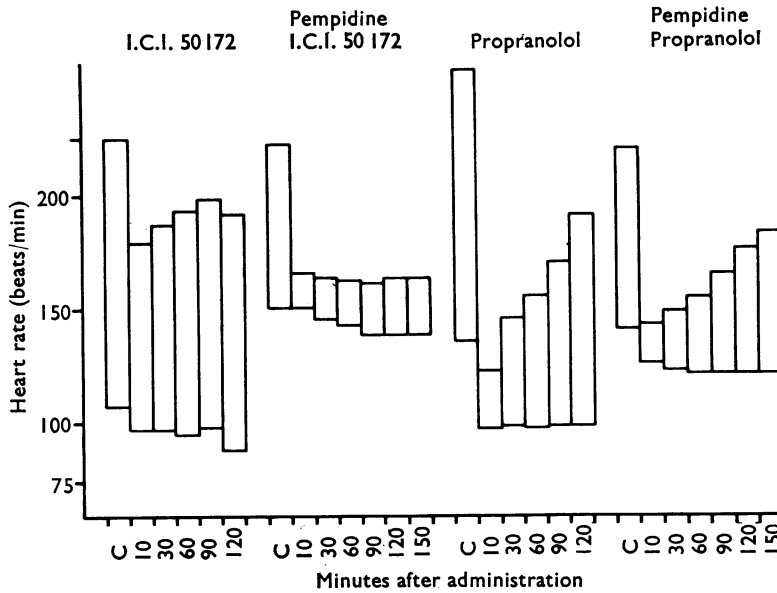


Fig. 10. Observations on four groups of conscious dogs with three in each group. Mean increases in heart rate produced by the intravenous injection of isoprenaline (0.4 μg/kg) were recorded before and at intervals after the intravenous administration of I.C.I. 50172 0.8 mg/kg and propranolol 0.2 mg/kg in normal dogs and in dogs pre-treated with pempidine 0.5 mg/kg intravenously. The bottom of each rectangle represents resting heart rate and the top the maximum rate produced by isoprenaline.

In conscious dogs 2–4 days after acute ligation of the anterior descending branch of the left coronary artery, the intravenous injection of adrenaline, 10 μg/kg, produced a ventricular tachycardia or multiple ventricular ectopic beats (Maling & Moran, 1957). The effect of I.C.I. 50172 on these arrhythmias was studied in three dogs and the results are given in Fig. 12. I.C.I. 50172 reduced the ectopic response to adrenaline on the second day after ligation and on the third and fourth days reduced or prevented it. On all 3 days 1 mg/kg was as effective as 3 mg/kg.

Tracheal smooth muscle relaxation

Observations were made on four pairs of isolated guinea-pig tracheal chains. The concentrations of I.C.I. 50172 and propranolol required to reduce the tracheal relaxation produced by a double dose of adrenaline to that produced by the control single dose, were determined and the ratio of these for each pair of chains calculated. I.C.I. 50172 was much less effective than propranolol in inhibiting the tracheal relaxation produced by adrenaline. From the four pairs of chains the ratio of activity propranolol to I.C.I. 50172 was 1:184 (range 100 to 288).

These observations were confirmed by the experiments on guinea-pigs exposed to histamine. The results are summarized in Table 4. The subcutaneous injection of isoprenaline, 0.1 mg/kg, protected the majority of guinea-pigs from the bronchoconstrictor action of histamine. The prior administration of propranolol, 0.1 mg/kg, abolished this protection whereas I.C.I. 50172, 1.0–4.0 mg/kg did not influence the effect of isoprenaline.

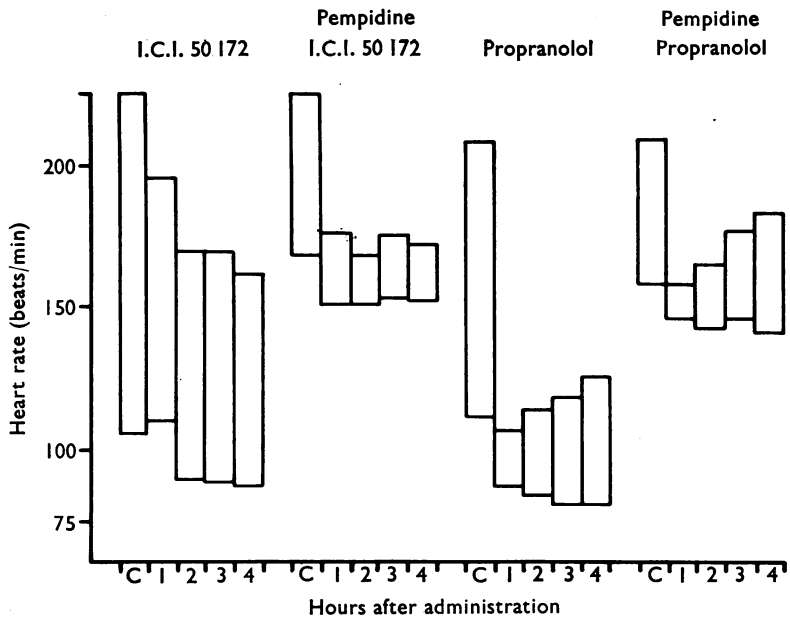


Fig. 11. Mean increases in heart rate produced by the intravenous injection of isoprenaline before and after the oral administration of I.C.I. 50172 5 mg/kg and propranolol 2 mg/kg. Observations in normal dogs and in dogs pre-treated with pempidine; four dogs in each group. Symbols as in Fig. 10.

TABLE 3

PROTECTION AGAINST METHYL CHLOROFORM ADRENALINE ARRHYTHMIAS IN ANAESTHETIZED DOGS BY THE INTRAVENOUS INJECTION OF I.C.I. 50172 AND PROPRANOLOL

The time in minutes indicates the interval between the administration of the drug and the methyl chloroform adrenaline challenge

Drug	Dose (mg/kg)	Number of dogs, fibrillated/tested			
		10 min	70 min	130 min	190 min
Control	—	4/4	—	—	—
I.C.I. 50172	1·0	0/3	1/3	0/2	0/2
	5·0	0/3	0/3	0/3	0/3
Propranolol	1·0	0/4	0/4	0/4	0/4

Local anaesthetic activity

The effect of I.C.I. 50172 on the height of an action potential conducted along an isolated frog sciatic nerve was compared with that of procaine. In five experiments the average concentration of procaine required to reduce the height of the action potential by 50% was 85 μg/ml. In six preparations I.C.I. 50172 200 μg/ml. did not affect the height of the action potential. In three cases increasing the concentration of I.C.I. 50172 in the bath to 400 μg/ml. produced only a slight reduction in the action potential. In three preparations the administration of procaine after I.C.I. 50172 reduced the action potential. These observations indicate that I.C.I. 50172 is devoid of local anaesthetic activity.

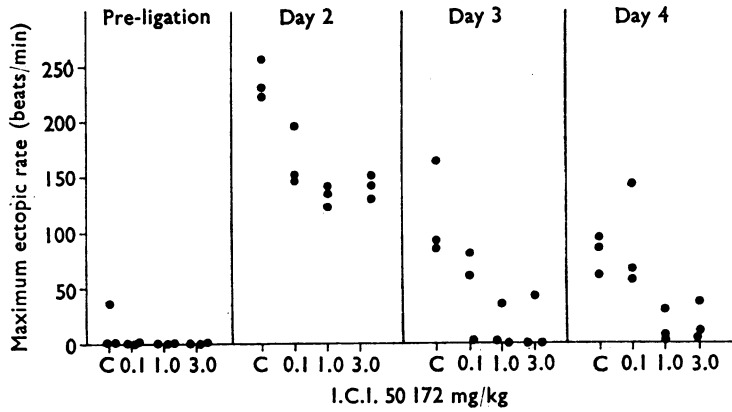


Fig. 12. Observations from three conscious dogs before and on second, third and fourth days after coronary artery ligation. Maximum ectopic responses, expressed as ventricular beats/min, to adrenaline (10 μg/kg) before and after the intravenous injection of I.C.I. 50172, 0.1, 1.0 and 3.0 mg/kg.

TABLE 4

EFFECT OF THE SUBCUTANEOUS INJECTION OF ISOPRENALINE 0·1 mg/kg ON THE MORTALITY RATE OF GUINEA-PIGS EXPOSED TO A HISTAMINE AEROSOL AND OF THE INFLUENCE ON THIS ACTION OF THE SUBCUTANEOUS INJECTION OF PROPRANOLOL AND I.C.I. 50172

Isoprenaline (0·1 mg/kg)	Antagonist	Dose (mg/kg)	Number of animals (dead/tested)
+	None	—	2/7
+	Propranolol	0·1	4/4
+	Propranolol	0·2	4/4
+	Propranolol	0·4	4/4
+	I.C.I. 50172	1·0	0/4
+	I.C.I. 50172	2·0	0/4
+	I.C.I. 50172	4·0	0/4
—	None	—	3/4

DISCUSSION

I.C.I. 50172 seems to be a new type of adrenergic beta receptor blocking agent, because its properties differ from those of previously described drugs. In anaesthetized and conscious dogs, I.C.I. 50172 in doses which reduced or abolished the inotropic and chronotropic effects of isoprenaline, adrenaline and noradrenaline did not affect the fall in arterial diastolic pressure produced by isoprenaline. The increase in blood flow to the hind limb of the dog produced by the injection of isoprenaline into the external iliac artery was not reduced by the intra-arterial injection of I.C.I. 50172. I.C.I. 50172 was much less effective than propranolol in blocking the relaxation of tracheal and bronchial smooth muscle produced by catecholamines. These results indicate that I.C.I. 50172 blocked beta receptors in the heart but not in the smooth muscle of blood vessels or respiratory tract.

In Ahlquist's classification of adrenotropic receptors, most excitatory responses elicited by sympathomimetic amines arose from activation of alpha receptors and most inhibitory responses from activation of beta receptors (Ahlquist, 1948). The one impor-

tant exception was cardiac stimulation as both the inotropic and chronotropic responses, although excitatory, were classified as beta receptor actions. The classical adrenergic beta receptor blocking agents, dichloroisoprenaline, pronethalol, propranolol, MJ 1999 and I.C.I. 45763 block both the inhibitory and excitatory actions arising from beta receptor stimulation (Shanks, 1966b). It would seem that I.C.I. 50172 blocks only the excitatory responses and not the inhibitory responses associated with beta receptors although all the inhibitory responses have not yet been studied. These observations suggest that adrenergic beta receptors may be divided into two groups—excitatory and inhibitory. This division is supported by the findings of Levy, who has shown that *N* tertiary butylmethoxamine (Levy, 1966a) and dimethylisopropylmethoxamine (Levy, 1966b) block some of the inhibitory responses associated with adrenergic beta receptors but not the cardiac stimulation.

In the experiments in which hind limb blood flow was measured, the intra-arterial injection of I.C.I. 50172 (1 mg/kg) did not reduce the isoprenaline vasodilatation whereas propranolol 0.005 mg/kg has previously been shown to produce a significant reduction (Shanks, 1967). Thus I.C.I. 50172 has less than a two-hundredth the activity of propranolol in blocking the peripheral vasodilator action of isoprenaline. A more accurate potency ratio was not obtained as the intra-arterial injection of I.C.I. 50172 in amounts greater than 1 mg/kg has an effect on the general circulation and thus may influence the tone of peripheral vessels by reflex action. The observations in Fig. 5 show that a total cumulative dose of I.C.I. 50172 2.55 mg/kg reduced the fall in diastolic pressure produced by isoprenaline to a lesser extent than propranolol 0.01 mg/kg. This small decrease in the depressor response may have resulted from reduction of the coronary vasodilatation as a consequence of the antagonism of the inotropic action of isoprenaline (Winbury, 1964). The observations on isolated tracheal chains indicated that I.C.I. 50172 antagonized the relaxation produced by adrenaline but its action was less than one-hundredth that of propranolol. Thus I.C.I. 50172 may not be entirely without effect on the inhibitory responses to catecholamines but there is a wide margin between the doses required to block the cardiac responses and those which affect the inhibitory responses.

It was difficult to calculate an accurate potency ratio for I.C.I. 50172 with respect to propranolol for blockade of cardiac adrenergic receptors because the dose response curves for I.C.I. 50172 were much flatter than those for propranolol (Fig. 5). This was also seen when the effects of I.C.I. 50172 and propranolol were compared for antagonism of the increases in heart rate produced by stimulation of the right stellate ganglion in cats (results for propranolol taken from Black *et al.*, 1965). The intravenous infusion of I.C.I. 50172 and propranolol at 5 μg/kg/min reduced the tachycardia to the same extent; increasing the dose of propranolol to 50 μg/kg/min completely abolished the tachycardia whereas this dose of I.C.I. 50172 and even 100 μg/kg/min only produced a further slight reduction but did not abolish it. It was concluded from a consideration of all the results that propranolol was at least three to four times as active as I.C.I. 50172 in blocking cardiac beta receptors.

The present observations suggest that the increase in heart rate in response to isoprenaline in conscious dogs may have two components. One results from a direct action of isoprenaline on the sino-atrial node and the other arises as a reflex response to the fall in arterial pressure produced by isoprenaline. As propranolol and similar adrenergic

beta receptor blocking agents abolish both the direct effects of isoprenaline on the heart and the fall in arterial pressure, they produce complete inhibition of the increase in heart rate in response to isoprenaline. On the other hand, I.C.I. 50172 does not reduce the fall in arterial pressure produced by isoprenaline so that the reflex component to the tachycardia may remain after complete blockade of the direct effects on the heart. After interruption of reflex activity by the administration of pempidine, I.C.I. 50172 antagonized an isoprenaline tachycardia as effectively as it did in anaesthetized dogs and, after allowance for differences in potency, as propranolol in conscious dogs. The reflex effect of isoprenaline on heart rate was not obvious in dogs anaesthetized with pentobarbitone or thialbarbitone and chloralose. These agents may have depressed baroreceptor reflexes sufficiently to abolish reflex effects on the heart although previous studies have shown that a reflex decrease in heart rate occurred in response to an increase in arterial pressure in dogs anaesthetized with thialbarbitone and chloralose (Shanks, 1966a). The reflex increase in heart rate in response to the fall in arterial pressure produced by isoprenaline may result either from an increase in sympathetic activity or from a reduction in vagal activity. If it is assumed that I.C.I. 50172 in doses that greatly reduce an isoprenaline tachycardia after pempidine also blocks the effect of sympathetic nervous activity on the heart, it would seem that the increase in heart rate in response to the fall in arterial pressure produced by isoprenaline is the result of a reduction in vagal activity. This conclusion has recently been confirmed by showing that after the administration of atropine, I.C.I. 50172 abolished an isoprenaline tachycardia in conscious dogs (Barrett, Crowther, Dunlop, Shanks & Smith, 1967).

The increase in heart rate produced in conscious dogs by the intravenous injection of isoprenaline was less after the administration of pempidine; in eleven dogs the mean increases were 109 ± 5 and 58 ± 3 beats/min before and after pempidine, respectively. This decrease in the isoprenaline tachycardia did not result from the increase in resting heart rate from 120 ± 4 to 155 ± 8 beats/min produced by pempidine because analysis of observations in twenty-eight conscious dogs has shown that the increase in heart rate produced by isoprenaline is independent of resting heart rate. These results further support the suggestion that there may be a reflex component to the increase in heart rate in response to isoprenaline in conscious dogs.

The absence of an effect of I.C.I. 50172 on the cardiac arrhythmias produced by ouabain further supports the suggestion that the effect of pronethalol, propranolol and their dextro isomers in abolishing these arrhythmias is not due to blockade of adrenergic beta receptors but to some other action which may be related to their local anaesthetic properties (Lucchesi, 1964, 1965; Howe & Shanks, 1966; Lucchesi, Whitsitt & Stickney, 1967). Like other drugs which block cardiac adrenergic beta receptors, I.C.I. 50172 abolished or prevented arrhythmias which were provoked by the administration of adrenaline (Moran, Moore, Holcomb & Mushet, 1962; Somani & Lum, 1965; Lucchesi *et al.*, 1967; Shanks & Dunlop, 1967).

In anaesthetized dogs, the increase in arterial pressure produced by noradrenaline results from an increase in cardiac output and a peripheral vasoconstriction. As propranolol abolished the increase in cardiac output, it reduced the pressor response to noradrenaline (Shanks, 1966a). In the present experiments I.C.I. 50172 reduced the pressor response to noradrenaline, presumably by a similar mechanism. Adrenaline

dilates some blood vessels and constricts others and increases mean arterial pressure (Clark, 1934). Propranolol blocks the vasodilator action of adrenaline and potentiates the increase in mean pressure (Shanks, 1966a). Because I.C.I. 50172 did not potentiate the pressor response to adrenaline, it is concluded that it did not affect the peripheral vasodilator action of adrenaline. These results suggest that I.C.I. 50172 did not affect the peripheral vasoconstrictor action of noradrenaline or adrenaline which results from adrenergic alpha receptor stimulation.

SUMMARY

1. I.C.I. 50172 reduced or abolished the increases in heart rate and cardiac contractile force produced by adrenaline, isoprenaline and noradrenaline in anaesthetized dogs but did not affect the fall in arterial diastolic pressure in response to isoprenaline.

2. The injection of I.C.I. 50172 into the external iliac artery did not reduce the increase in flow to the hind limb of the dog produced by the intra-arterial injection of isoprenaline.

3. In anaesthetized cats, the increases in heart rate produced by the intravenous infusion of isoprenaline and by stimulation of the right stellate ganglion were reduced by I.C.I. 50172.

4. I.C.I. 50172 possessed some sympathomimetic activity because it increased heart rate in normal and in syrosingopine pre-treated cats.

5. The dose-response curves for I.C.I. 50172 for antagonism of the effects of isoprenaline in increasing the heart rate and cardiac contractile force in anaesthetized dogs were flatter than those for propranolol and MJ 1999. Although an accurate potency ratio was not obtained, the results indicated that I.C.I. 50172 had one-third to one-quarter the activity of propranolol.

6. I.C.I. 50172 was about one one hundred and fiftieth as active as propranolol in antagonizing adrenaline-induced relaxation of isolated tracheal chains prepared from guinea-pigs.

7. The oral and intravenous administration of I.C.I. 50172 produced a much smaller reduction in an isoprenaline tachycardia in conscious dogs than in anaesthetized dogs. In conscious dogs the blocking effect of I.C.I. 50172 was increased after the administration of pempidine. It was concluded that in conscious dogs the increase in heart rate produced by isoprenaline resulted both from a direct action on the sino-atrial node and from a reflex increase in heart rate in response to the fall in mean arterial pressure.

8. Arrhythmias produced by ouabain in anaesthetized cats and anaesthetized dogs were not abolished by I.C.I. 50172 but it prevented the development of ventricular fibrillation in anaesthetized cats on the administration of methyl chloroform and adrenaline and reduced the number of ventricular ectopic beats produced by adrenaline in conscious dogs following coronary artery ligation.

9. I.C.I. 50172 had no local anaesthetic activity.

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