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THE ACTIONS OF A NEW β-ADRENOCEPTOR BLOCKING DRUG, ICI 66082, ON THE RABBIT PAPILLARY MUSCLE AND ON THE DOG HEART

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1 The actions of 4-(2-hydroxy-3-isopropylaminopropoxy) phenyl acetamide (ICI 66082), a new β-adrenoceptor blocking drug, on the twitch response of the isolated papillary muscle of the rabbit and on d*P*/d*t max* and free heart rate of a denervated dog heart preparation, are described.

2 ICI 66082 (up to 1 mg/ml) did not produce any depression of the twitch response of the rabbit papillary muscle. ICI 66082 antagonized the action of isoprenaline on this preparation at a concentration of 0.01 μg/ml.

3 ICI 66082 (0.5-1.0 mg/kg intravenously) reduced the control value of d*P*/d*t max* in four dog preparations by a mean value of 529 mmHg/s (s.e. mean ± 139 mmHg/s), with no significant change in free heart rate. Antagonism of the effect of isoprenaline on d*P*/d*t max* and on free heart rate was demonstrated with ICI 66082 (0.1 mg/kg).

4 ICI 66082 (1.0-1.5 mg/kg) produced no significant changes in d*P*/d*t max* or in free heart rate in four dogs pretreated with reserpine. A significant reduction (16% of the control value) in d*P*/d*t max* was observed with ICI 66082 at a high dose of 40-50 mg/kg.

5 It is concluded that ICI 66082 is a competitive antagonist against the actions of isoprenaline on cardiac muscle, has no negative inotropic action (unless the dose exceeds 40 mg/kg) and lacks intrinsic sympathomimetic activity.

Introduction

Drugs which antagonize the action of sympathomimetic amines at β-adrenoceptor sites on the heart have been shown to possess other actions on the heart; they have an intrinsic sympathomimetic action (e.g. Barrett & Carter, 1970), are local anaesthetic agents (e.g. Davis, 1970), possess a quinidine-like action (e.g. Vaughan Williams & Papp, 1970), are antiarrythmic agents (e.g. Barrett & Cullum, 1968) and are said to depress the myocardium by an action unrelated to blockade of β-adrenoceptors (e.g. Meier, 1970). In addition, some of these drugs have been shown to block preferentially β-adrenoceptors in the heart–the so-called 'cardioselectivity' (e.g. Dunlop & Shanks, 1968). Consequently, the evaluation of any compound said to be an antagonist of the effects of sympathomimetic amines at β-adrenoceptors in the heart must include investigations designed to decide if the compound possesses any or all of these properties.

ICI 66082 has been introduced as a new β-adrenoceptor blocking drug (Barrett, Carter, Fitzgerald, Hall & Le Count, 1973). The purpose of this investigation has been to confirm that ICI 66082 acts as an antagonist to sympathomimetic amines which affect β-adrenoceptors in the heart, to decide whether ICI 66082 possesses a negative inotropic or chronotropic action on the heart and to determine if it has intrinsic sympathomimetic activity. The preparations used were isolated papillary muscles from the rabbit and carefully controlled intact dog heart preparations. Preliminary results of these experiments have been reported (Harry, Knapp & Linden, 1973).

Methods

Rabbit papillary muscles

Papillary muscles from the right ventricle of the rabbit were mounted in organ baths containing 5 ml Krebs solution maintained at a temperature of 32.5°C and aerated by a mixture of 95% oxygen and 5% carbon dioxide. The muscles were mounted between platinum strip electrodes. Supramaximal pulses (0.5 Hz; 5 ms) were used to produce a maximal isometric twitch tension from

a resting tension between 0.5 and 1.0 g, which was kept constant throughout the experiment. Isometric twitch tension was recorded by means of a force displacement transducer (Model FT03C; Grass Instruments Co., Quincy, Mass., U.S.A.); the signal from the transducer was passed to a carrier amplifier (S.E. Laboratories, Feltham, Middlesex) and the amplified signal was recorded by a direct writing u.v. recorder (Model SE2100; S.E. Laboratories).

Experimental procedures

Effect of ICI 66082 on twitch tension. Two papillary muscles obtained from the right ventricle of a rabbit heart were set up in separate baths. One muscle was subjected to increasing concentrations of ICI 66082 made up in saline (0.9% w/v NaCl solution) and the other to saline alone; twitch tension was recorded after 10 min contact time at each concentration. Between each application of drug or saline the tissues were washed twice in Krebs solution at minute intervals.

Effect of ICI 66082 on responses to isoprenaline. The responses of the papillary muscle to cumulative doses of isoprenaline were recorded; each dose was in contact with the tissue for 3 minutes. ICI 66082 was then added to the bath and after 10 min contact time the response of the muscle to isoprenaline was again recorded.

Dog heart

Dogs weighing between 13 and 25 kg were given an intramuscular injection of morphine sulphate (dose 0.5 mg/kg); 0.5-1 h later under local anaesthesia a catheter was inserted through a saphenous vein into the inferior vena cava. The dogs were anaesthetized with an infusion through this cannula of a solution of α-chloralose (Etablissement Kuhlmann, Paris; dose 0.12 g/kg); two thirds of the anaesthetic solution was sodium chloride (0.9 g/100 ml) containing α-chloralose (1 g/100 ml) and $NaHCO_3$ (0.21 g/100 ml); and one third was Dextravan 150 (Fisons Pharmaceuticals Ltd, Loughborough, England) containing α-chloralose (1 g/100 ml), NaCl (0.72 g/100 ml) and $NaHCO_3$ (0.21 g/100 ml). Subsequently a steady state of light anaesthesia was maintained by further intravenous infusions of chloralose (approximately 0.01 g/kg every 15 minutes). The trachea was cannulated and artificial respiration started using a mixture of 40% oxygen in nitrogen, supplied by means of a modified Starling 'Ideal' pump (Ledsome, Linden & Norman, 1967). When the chest was opened a resistance to expiration was produced by placing the expiratory outlet of the pump under 3 cm of water.

The chest was opened in the midline and the right and left ansae subclaviae were dissected free, clamped and crushed for 10 min at their origins from the stellate ganglia. The vagal nerves were sectioned in the neck. A soft string was placed in position around the descending thoracic aorta and used to produce constriction of the aorta.

Pressures in the cardiovascular system were recorded through metal cannulae (Inconel, 1.5 mm bore; Johnson, Matthey & Co., London) treated with a solution of dialkyl dimethyl ammonium chloride (Arquad; Armour Hess Chemicals (Leeds) Ltd). Pressure in the aorta was recorded through a cannula inserted into the aortic arch through the right common carotid artery. Pressure in the left ventricle was recorded through a cannula which was inserted through the apical dimple of the left ventricle. Occlusion of the tip of the cannula in the cavity of the left ventricle at any point in the cardiac cycle was excluded by confirming before and after each recording that blood could be aspirated without interruption through the cannula during several cardiac cycles. To each of the two cannulae was attached a strain gauge manometer (Model SEM 4-82; S.E. Laboratories) and after amplification by means of a carrier amplifier (Model SE 511/S; S.E. Laboratories) the pressure was recorded by a direct writing u.v. recorder (Model SE 2100; S.E. Laboratories). The frequency response obtained by the method of Ardill, Fentem & Wellard (1967), of the ventricular system was flat (±5%) to better than 100 Hz and of the aortic system to better than 40 Hz. The strain gauges were calibrated in a stepwise manner with mercury and saline manometers. The rate of change of pressure in the left ventricle was derived by applying the pressure signal from the carrier amplifier to an analogue differentiating circuit (Furnival, Linden & Snow, 1970). The output of the differentiator was connected to a galvanometer driver amplifier (Model SE 425; S.E. Laboratories) and was recorded on the u.v. recorder. The response of the system was linear to above 100 Hz.

Nylon cannulae were inserted into both femoral arteries and both femoral veins. The left femoral artery cannula was connected to a strain gauge manometer and after amplification of the signal, the pressure was recorded on the u.v. recorder. The signal from the pressure pulse was used to drive a digital cardiotachometer (Model 121; Gilford Instrument Laboratories Inc., Oberlin, Ohio, U.S.A.). Samples of arterial blood were withdrawn anaerobically from the right femoral artery and the pH, P_{O_2} and P_{CO_2} were measured by the methods of Norman, Ledsome & Linden

(1965). End-tidal P_{CO_2} was monitored continuously by aspirating air from the trachea into an infra-red carbon dioxide analyser (URAS 4, Hartmann and Braun, Frankfurt, Main, West Germany). The arterial P_{CO_2} was kept as near as possible to 40 mmHg by adjusting the respiratory pump, and the pH of the arterial blood was maintained at about 7.4 by periodic infusions of a solution of 0.5 M sodium bicarbonate solution.

The ECG was recorded from the right foreleg and the left hindleg. Oesophageal temperature was recorded from a thermistor probe (Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, U.S.A.), and was maintained at 38 ± 1°C (in acutely denervated dogs) and at 36.5 ± 1.5°C (in dogs treated with reserpine) by heating lamps above and heating elements below the dog. The heart was paced by stimulation of the right atrial appendage with monopolar supramaximal pulses from a Model S4 stimulator (Grass Instrument Co., Quincy, Mass., U.S.A.).

Test for cardiac denervation

The response to occlusion of both common carotid arteries below the carotid sinuses was used as a test for cardiac denervation. In animals with the vagal nerves cut and sympathetic nerves crushed the absence of any increase in heart rate in the first 10 s after occlusion was taken to indicate that the reflex release of noradrenaline from the sympathetic nerve endings in the heart had been prevented and hence that the sympathetic nerves to the heart had been effectively crushed.

Test for depletion of catecholamines

The response to occlusion of both common carotid arteries below the carotid sinuses was also used as a test for the absence of sympathetic activity in dogs which had been treated with reserpine. In animals with the vagal nerves sectioned if there were no changes in heart rate, mean systemic blood pressure and pulse pressure after 1 min of occlusion, then it was assumed that there was no release of catecholamines from sympathetic nerves or from the adrenal medulla. Hence it was assumed that there were no catecholamines to be liberated into the circulation of these dogs.

Experimental procedures

Effect of ICI 66082 on control values of free heart rate and dP/dt max. Free heart rate and d*P*/d*t max* were recorded in the following way. Records were obtained of pressures in the aorta and the left ventricle, the rate of change of pressure in the left ventricle and the free heart rate. In six experiments the heart was then paced and further records were obtained; the pacing frequency selected ranged from 11 to 63 beats/min above the free heart rate at the start of the experiment and was kept constant throughout the experiment. By recording d*P*/d*t max* at the same paced heart rate throughout each series of observations the secondary inotropic effects brought about by changes in heart rate were eliminated. In two of the experiments in which the dogs had been treated with reserpine, all measurements of d*P*/d*t max* were obtained at the free heart rate; in these experiments the maximum variation in free heart rate was 6 beats/min and 8 beats/min, respectively. In all the experiments all records of d*P*/d*t max* were obtained at a constant mean aortic pressure.

At the beginning of each experiment a series of records of free heart rate and d*P*/d*t max* were obtained at 5 min intervals to establish that the preparation was in a steady state. The first dose of ICI 66082 was injected through the right femoral vein and free heart rate and d*P*/d*t max* were recorded 5 min later. Further intravenous doses of ICI 66082 were given and free heart rate and d*P*/d*t max* were recorded 5 min after each dose.

Effect of ICI 66082 on responses to isoprenaline. At the beginning of each series of observations on the effect of isoprenaline on the heart, control records were obtained of pressures in the aorta and the left ventricle, the rate of change of pressure in the left ventricle and the free heart rate. The heart was then paced at a rate just below that which produced pulsus alternans and further records were obtained. Isoprenaline was then infused at a constant rate into the left femoral vein with an infusion pump (Braun, Melsungen, West Germany). When a steady state had been reached 3-4 min after the start of infusion, the above procedure of recording was repeated. On each occasion when the heart was paced, the mean aortic pressure was kept constant at the value obtained during the control period by snaring the thoracic aorta; care was taken to correct only for small changes in blood pressure and to maintain the corrected blood pressure constant for at least 2 min before the records were obtained. By recording d*P*/d*t max* at the same paced heart rate and at the same mean aortic pressure throughout any series of infusion rates of isoprenaline, the secondary inotropic effects brought about by changes in heart rate and mean aortic pressure were eliminated and only the direct

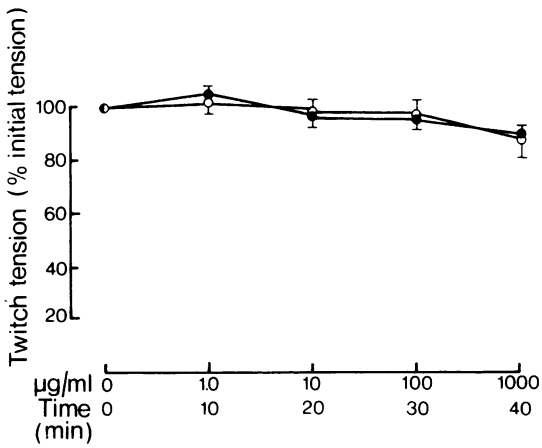

Fig. 1 The effect of ICI 66082 on rabbit papillary muscle twitch response to electrical stimulation. Each point represents the mean twitch tension with s.e. mean from five muscles. The scales on the abscissae are log concentration of ICI 66082 for the muscles to which ICI 66082 was added and time for control muscles to which no ICI 66082 was added. (○) Saline + ICI 66082; (●) saline alone.

inotropic effect of isoprenaline on the left ventricle was recorded (Furnival *et al.*, 1970). In each experiment isoprenaline was infused at three or four different rates. Further control records were obtained when a steady state was reached 10-15 min after the last infusion was stopped. ICI 66082 was then injected into the right femoral vein, and the free heart rate and dP/dt *max* were recorded 3-5 min later. Greater amounts of isoprenaline were then infused and the free heart rate and dP/dt *max* recorded at each infusion rate as described above. The whole procedure was repeated with increasing concentrations of ICI 66082.

Drugs used

(±)-ICI 66082 (4-(2-hydroxy-3-isopropylamino-propoxy) phenyl acetamide), (±)-practolol (I.C.I. Pharmaceuticals Ltd) and (±)-oxprenolol (Ciba Laboratories, Horsham) were dissolved in a solution of NaCl (0.9 g/100 ml). (±)-Isoprenaline sulphate (Evans Medical Ltd, Liverpool) was made up in a solution containing NaCl (0.9 g/100 ml) and $Na_2S_2O_5$ (0.1 g/100 ml). Reserpine (Halewood Chemicals Ltd, Staines, Middlesex) was given by subcutaneous injection; two doses of 0.5 mg/kg were given 24 h apart and the experiment performed 16 h after the second injection, when a further intravenous dose

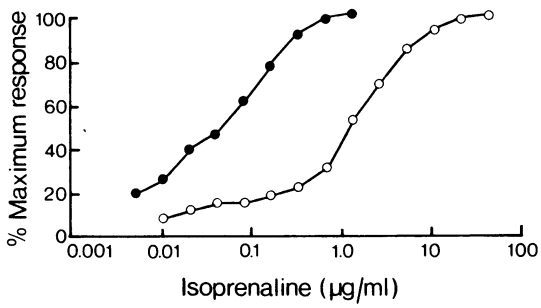

Fig. 2 Log_{10} dose-response curves of twitch tension response to isoprenaline, obtained from one rabbit papillary muscle in the absence (●) and in the presence of (○) of ICI 66082 (1 μg/ml). The twitch response to isoprenaline is expressed as a percentage of the maximum response in the absence of ICI 66082.

(0.5 mg/kg) was given. All drug concentrations are expressed as the salt.

Results

Rabbit papillary muscle

Effect of ICI 66082 on twitch tension. In five experiments the initial tension of the test muscles was 0.80 g (mean; range 0.69-0.91) and of the control muscles was 0.81 g (mean; range 0.54-1.05). The results from five experiments on papillary muscles are summarized in Fig. 1, which shows the twitch tension in the presence of increasing concentrations of ICI 66082 compared with the twitch tension obtained from papillary muscles to which saline had been added as a control. Figure 1 shows that concentrations of ICI 66082 up to 1 mg/ml produced no significant depression of the twitch response.

Effect of ICI 66082 on the response of the twitch tension to isoprenaline. In the papillary muscle from the rabbit isoprenaline produced dose-dependent increases in the twitch response to electrical stimulation, and the effect of ICI 66082 on this response induced by isoprenaline was tested. The results from one experiment are plotted in Fig. 2, which shows the dose-response curves to isoprenaline in the absence and presence of ICI 66082 (1 μg/ml); in the presence of ICI 66082 higher concentrations of isoprenaline were required to produce the same responses of the papillary muscle to isprenaline as were obtained in the absence of ICI 66082. The dose-response curves obtained in the absence and in the presence

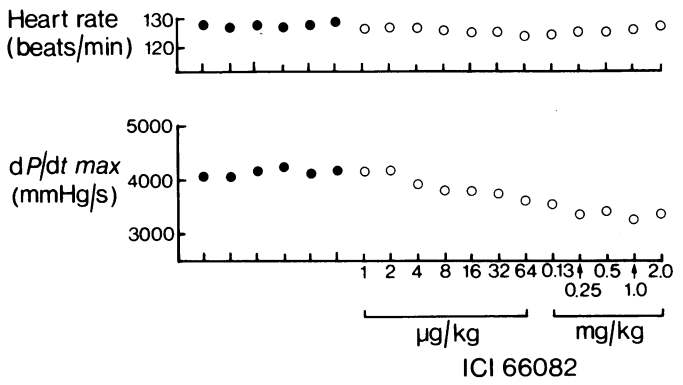

Fig. 3 The results from an experiment on one dog showing the effect of cumulative doses of ICI 66082 on free heart rate and on d*P*/d*t max*. (•) Measurements obtained before ICI 66082 was given; (o) measurements obtained in the presence of ICI 66082.

of ICI 66082 were parallel and blockade of the response to isoprenaline could be completely overcome by increasing the concentration of isoprenaline. The same result was obtained from four such experiments. In the presence of 0.01 µg/ml of ICI 66082 the isoprenaline concentration had to be doubled to obtain the same response as that obtained in the absence of ICI 66082; and in the presence of 1 µg/ml ICI 66082 the isoprenaline concentration had to be increased by 10 to 30 times to obtain the same response as in the absence of ICI 66082.

Acutely denervated heart in the dog

When recording commenced 2-3 h after giving the initial dose of anaesthetic, the mean arterial pressure in the aorta was 154 mmHg (mean; range 122-190), the heart rate was 130 beats/min (mean; range 108-146), and the d*P*/d*t max* was 3286 mmHg/s (mean; range 2313-4031). The pH of the arterial blood was 7.35 (mean; range 7.20-7.42), the P_{CO_2} of the arterial blood was 41 mmHg (mean; range 36-46) and the P_{O_2} of the arterial blood was 196 mmHg (mean; range 160-229).

Effect of ICI 66082 on dP/dt max *and free heart rate.* In four dogs the effect of ICI 66082 on the initial steady state values of d*P*/d*t max* and free heart rate was determined. The values of d*P*/d*t max* and free heart rate obtained in one dog before ICI 66082 was given and in the presence of increasing doses of ICI 66082 are plotted in Figure 3. As the dose of ICI 66082 was increased d*P*/d*t max* decreased gradually until, at a cumulative dose of 0.25 mg/kg a new steady state was attained at a value which was 857 mmHg/s lower than the initial steady state value; free heart rate was unchanged. Results similar to the above have been obtained in four dogs (see Table 1);

Table 1 Effect of ICI 66082 on free heart rate and d*P*/d*t max* in acutely denervated dog heart

	Free heart rate (beats/min)		*dP/dt* max *(mmHg/s)*		
Dog number	*Before ICI 66082*	*Change after ICI 66082*	*Before ICI 66082*	*Change after ICI 66082*	*Cumulative dose (mg/kg)*
32/72	122	0	3192	−315	1.0
34/72	127	−2	4124	−857	1.0
37/72	120	−4	3000	−661	0.5
20/73	145	−6	2351	−281	1.0
Mean change		−3		−529	
s.e. mean		±1.3		±139	
P		>0.1		<0.05	

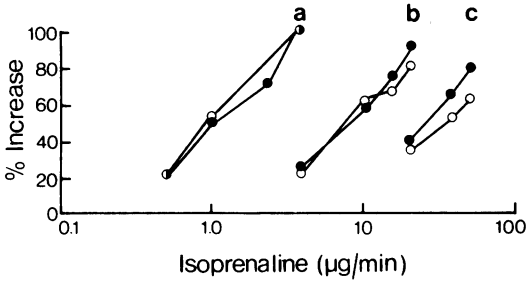

Fig. 4 Dose-response curves obtained from one dog, relating free heart rate (•) and d*P*/d*t max* (○) to the intravenous infusion rate of isoprenaline (a) in the absence of ICI 66082 and in the presence of (b) 0.1 and (c) 0.5 mg/kg ICI 66082. The increases in free heart rate and d*P*/d*t max* in response to isoprenaline are expressed as a percentage of the largest increase obtained in each of these measurements before ICI 66082 was given.

after 0.5-1.0 mg/kg ICI 66082 given cumulatively, there was a significant reduction in d*P*/d*t max* of 529 ± 139 mmHg/s (mean ± s.e.), with no significant change in free heart rate. In these four dogs, further increases in dose of ICI 66082 (to 2-4 mg/kg) produced no further change in d*P*/d*t max* and no change in free heart rate.

Effect of ICI 66082 on responses to isoprenaline. Intravenous infusions of isoprenaline produced dose-dependent increases in d*P*/d*t max* and free heart rate (Furnival *et al.*, 1970). In five dogs d*P*/d*t max* and free heart rate responses to increasing rates of infusion with isoprenaline were recorded before ICI 66082 was given and in the presence of two or three different doses of ICI 66082. The results of such an experiment in one dog are plotted in Fig. 4, which shows the dose-response curves for d*P*/d*t max* and free heart rate responses to isoprenaline in the absence and in the presence of ICI 66082. In the presence of ICI 66082, a higher rate of infusion of isoprenaline was required to obtain the same responses in d*P*/d*t max* and free heart rate as were obtained in its absence; the shift to the right of the dose-response curves was greater the higher the dose of ICI 66082. The results of the above experiment in five dogs are summarized in Table 2, which shows that in the presence of ICI 66082 (0.1 mg/kg) the isoprenaline infusion rate had to be increased by approximately five times to produce increases in d*P*/d*t max* and free heart rate of the same magnitude as those obtained in the absence of ICI 66082.

Acutely denervated heart in dogs treated with reserpine

When recording commenced 2-3 h after giving the initial dose of anaesthetic the mean arterial blood pressure in the aorta was 97 mmHg (mean; range 80-125), the heart rate was 106 beats/min (mean; range 90-115) and d*P*/d*t max* was 2379 mmHg/s (mean; range 1674-3116). The pH of the arterial blood was 7.43 (mean; range 7.35-7.48), the P_{CO_2} of arterial blood was 39 mmHg (mean; range 37-42) and the P_{O_2} of the arterial blood was 195 mmHg (mean; range 180-210).

Effect of ICI 66082 on dP/dt max *and free heart rate.* In dogs pretreated with reserpine the effect of ICI 66082 on initial steady state values of d*P*/d*t max* and free heart rate was determined, following the same experimental procedure as for acutely denervated dog heart preparations. The results of experiments in four dogs are summarized in Table 3, which shows that after a cumulative dose of 1.0-1.5 mg/kg ICI 66082 there was no significant change in d*P*/d*t max* or free heart rate from the initial steady state values. Further increases in the dose of ICI 66082 (up to 20 mg/kg) did not produce any depression of d*P*/d*t max*, but at a dose of 40-50 mg/kg there was a significant decrease in d*P*/d*t max*, of 16% ($P = <0.01$) from the initial steady state value.

Table 2 Results of experiments on five dogs showing the effect of isoprenaline on free heart rate and d*P*/d*t max* before and in the presence of ICI 66082.

	Free heart rate		dP/dt max	
	Isoprenaline infusion rate, μg/min (mean ± s.e.)	*Heart rate increase, beats/min (mean ± s.e.)*	*Isoprenaline infusion rate, μg/min (mean ± s.e.)*	*dP/dt max increase, mmHg/s (mean ± s.e.)*
Before ICI 66082	2.05 ± 0.5	53 ± 3.4	2.3 ± 0.6	2886 ± 429
After 0.1 mg/kg ICI 66082	12.0 ± 2.5	55 ± 3.1	10.0 ± 1.5	2935 ± 375

Effects of oxprenolol and practolol on dP/dt max and free heart rate. In three dogs pretreated with reserpine, oxprenolol (0.064-0.16 mg/kg) produced a mean maximum increase in d*P*/d*t max* of 990 mmHg/s and a mean maximum increase in free heart rate of 13 beats/minute. In four dogs treated with reserpine, practolol (0.064-0.128 mg/kg) produced a mean maximum increase in d*P*/d*t max* of 589 mmHg/s and a mean maximum increase in free heart rate of 18 beats/minute.

Discussion

The results from the experiments with papillary muscles from the rabbit allow the conclusion that ICI 66082 behaves as a competitive antagonist against the action of isoprenaline. Thus, by the criteria of competitive blockade of Ariens & Simonis (1964), ICI 66082 caused a shift to the right of the dose-response curves produced by isoprenaline; the curves remained parallel, and the maximum response of the muscle to isoprenaline could be obtained again in the presence of ICI 66082. Results similar to the above have been obtained in experiments with the intact dog heart in which the effect of ICI 66082 on the changes in d*P*/d*t max* and free heart rate caused by isoprenaline, was determined; the dose-response curves to isoprenaline for each of these parameters was shifted to the right by ICI 66082 and remained parallel. The results from both these investigations agree with the conclusions of Barrett *et al.* (1973) where evidence was presented from experiments on other isolated and intact animal preparations that ICI 66082 is a competitive antagonist to the actions of isoprenaline on the heart.

Another purpose of this investigation was to determine whether ICI 66082 had any direct negative, or any intrinsic sympathomimetic effect on cardiac muscle; this problem was investigated by determining the effect of ICI 66082 on isolated papillary muscles from the rabbit and on the intact dog heart. In experiments on isolated papillary muscles from the rabbit, no significant difference was found between muscles subjected to ICI 66082 (1 mg/ml) and muscles subjected to saline alone. The highest concentration of ICI 66082 used was 10,000 times higher than the concentration at which effective blockade of β-adrenoceptors was demonstrated and 50 times higher than the concentration of propranolol which produced a significant reduction in isometric twitch tension in the same preparation (Harry, Linden & Snow, 1971). Thus no evidence has been found that ICI 66082 has a direct negative inotropic effect on rabbit papillary muscle.

Before considering the direct effect of ICI 66082 on the intact dog heart preparation it is necessary to comment on the use of the maximum rate of rise of pressure in the left ventricle (d*P*/d*t max*) as an index of the changes in inotropic responses of the heart. The maximum rate of rise of pressure in the left ventricle (d*P*/d*t max*) can be used as a reliable and sensitive index of inotropic responses of the heart, provided that factors known to have a secondary effect on this measurement are kept constant (Furnival *et al.*, 1970). They showed that the measurement of d*P*/d*t max* in the dog heart in which the autonomic nerve supply has been sectioned is affected by heart rate and by aortic blood pressure but not by end diastolic pressure within described limits. Thus in any one series of observations obtained in this investigation d*P*/d*t max* was recorded at a constant heart rate and mean aortic pressure. Such precautions have not always been taken in other investigations in which attempts have been made to determine the effect of β-adrenoceptor blocking drugs on the heart (e.g. Shanks, 1966). Further, in the study of the effect

Table 3 Effect of ICI 66082 on free heart rate and d*P*/d*t max* in dogs treated with reserpine

	Free heart rate (beats/min)		*dP/dt max (mmHg/s)*		
Dog number	*Before ICI 66082*	*Change after ICI 66082*	*Before ICI 66082*	*Change after ICI 66082*	*Cumulative dose (mg/kg)*
7/73	107	+1.0	3202	+130	1.0
11/73	91	0	1581	−155	1.5
15/73	112	+2.0	1731	+19	1.0
25/73	114	−2.0	2883	−139	1.0
Mean change		+0.25		−36	
s.e mean		±0.85		±68	
P		>0.5		>0.5	

of β-adrenoceptor blocking drugs on cardiac muscle, it is important to distinguish between a reduction in a positive inotropic effect resulting from blockade of catecholamines and a direct depressant effect the drug may have on the myocardium. 'Negative inotropic response' should be defined as a depression of cardiac muscle over and above removal of sympathetic activity (Harry, Kappagoda, Linden & Snow, 1973).

In the present investigation the heart was not under the influence of sympathetic nerves, only of endogenous sympathomimetic amines present in the blood stream. It has already been shown that the endogenous sympathomimetic amines in the blood stream in this dog preparation affect the myocardium (d*P*/d*t max*) but do not affect the sino-atrial node and this difference results from the greater uptake of catecholamines by the sympathetic nerves in the sino-atrial node than by those in the muscle (Furnival, Linden & Snow, 1971). Consequently, blockade of β-adrenoceptors in the heart in this preparation produces a reduction in d*P*/d*t max* but no change in heart rate (Harry *et al.*, 1973). ICI 66082 in a dose of up to 4 mg/kg produced a reduction of 529 mmHg/s in d*P*/d*t max* with no significant change in free heart rate from the initial control period. This reduction in d*P*/d*t max* is of the same order as the reduction produced by propranolol and by reserpine in dogs in a previous investigation (Harry *et al.*, 1973); but the doses of ICI 66082 (0.25-1.0 mg/kg) required to produce effective blockade of circulating catecholamines were higher than the dose of propranolol (0.1 mg/kg) needed to obtain equivalent blockade in the same preparation (Harry *et al.*, 1973). These results suggest that ICI 66082 up to a dose of 4 mg/kg is a β-adrenoceptor blocking drug with no negative inotropic action on the heart.

Agreement with this conclusion comes from experiments in dogs which had been depleted of all endogenous catecholamines by prior treatment with reserpine; ICI 66082 in doses up to 20 mg/kg produced no significant change in d*P*/d*t max* or in free heart rate. At higher doses however there is evidence of a direct negative inotropic effect on the myocardium by ICI 66082; in dogs treated with reserpine, ICI 66082 in doses in excess of 40-50 mg/kg caused depression of d*P*/d*t max* and free heart rate; this effect could be regarded as a negative inotropic action of this drug. This dose is far in excess of that (0.1 mg/kg) required to produce blockade of stimulation of β-adrenoceptors by isoprenaline.

Oxprenolol (e.g. Meier, 1970) and practolol (e.g. Dunlop & Shanks, 1968) are two β-adrenoceptor blocking drugs which possess intrinsic sympathomimetic activity and both of these compounds stimulate free heart rate and d*P*/d*t max* in this dog preparation which has been pretreated with reserpine. ICI 66082 (up to 50 mg/kg) produced no increase in either free heart rate or d*P*/d*t max* when given to dogs pretreated with reserpine. We conclude that ICI 66082 does not possess intrinsic sympathomimetic activity.

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