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# BLOCKADE OF PERIPHERAL VASCULAR RESPONSES TO ISOPRENALINE BY THREE β-ADRENOCEPTOR ANTAGONISTS IN THE ANAESTHETIZED DOG

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1 A dog's hind limb was vascularly isolated by strong nylon snares and its sympathetic nerve supply interrupted. Blood was perfused at constant flow into the femoral artery and drained from the femoral vein. In some dogs the cardiac nerves were cut.

2 Isoprenaline infused intravenously caused an increase in heart rate and a decrease in arterial resistance.

3 Practolol (2 mg/kg) effectively blocked heart rate responses to isoprenaline infused at up to 10 μg/min but was relatively ineffective in blocking arterial responses. ICI 66082 (2 mg/kg) reduced vasomotor responses and propranolol (0.5 mg/kg) abolished vasomotor responses.

4 Small cumulative doses of β-adrenoceptor antagonists were given to some dogs. Practolol blocked heart rate responses in lower doses than were required to block vasomotor responses. Propranolol preferentially blocked vasomotor responses and ICI 66082 was intermediate between the other two in its effects.

## Introduction

Recently it has become apparent that some β-adrenoceptor antagonists differ in their relative abilities to block the responses to isoprenaline at different sites. Dunlop & Shanks (1968) were the first to describe a drug (practolol) which had the effect of antagonizing cardiac chronotropic responses to injected catecholamines but still allowed systemic hypotensive responses to occur following intravenous injections of isoprenaline. Levy (1973) described another drug (H 64/52) which also blocked cardiac responses to isoprenaline at lower doses than were required to block the hypotensive responses.

In previous pharmacological studies vascular responses were assessed by recording changes in arterial blood pressure or, in some of the experiments described by Levy (1973), by recording changes in perfusion pressure to a limb perfused at constant flow. These methods were not ideal because the responses could be greatly modified by reflex changes in sympathetic vasomotor tone and, in the perfused limb studies, by a change in blood flow through the extensive collateral circulation. In the present study we investigated arterial responses in a limb which was vascularly isolated from the rest of the circulation and in which the sympathetic nerve supply was interrupted, thus eliminating the possible effects of vascular anastomoses and changes in sympathetic tone. Using this preparation we determined the relative abilities of propranolol, practolol and ICI 66082 (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973) to block the arterial response to infused isoprenaline. In some dogs we also compared the relative blocking effects of these drugs on the cardiac and vasomotor responses to infused isoprenaline.

## Methods

Dogs of weight 10-32 kg were anaesthetized by chloralose (0.1 g/kg; Etablissement Kuhlmann, Paris) infused through a catheter which had been passed, under local anaesthesia (decicain 2%), through a saphenous vein so that its tip lay in the inferior vena cava. The chloralose was dissolved to make a solution, 1 g/100 ml in sodium chloride solution (0.9 g/100 ml). Further infusions of chloralose were given during the experiment to maintain a state of light surgical anaesthesia. Following induction of anaesthesia the neck was opened in the mid line, the trachea cannulated and positive pressure respiration started with a Starling

'Ideal' pump. The rate of the pump was 18 strokes/min and the stroke volume was initially 50 ml/3 kg body weight.

The left lumbar sympathetic trunk was exposed retroperitoneally. It was crushed between the fourth and fifth lumbar ganglia and a pair of stimulating electrodes was placed round the nerve immediately caudal to the crushed area. This procedure denervates the sympathetic nerves to the limb and stimulation should activate most of these nerves (Donegan, 1921; Webb-Peploe & Shepherd, 1968). The completeness of denervation of the vessels in the limb was confirmed by the absence of reflex vasomotor responses to carotid clipping. The stimulating electrodes were connected to a Grass stimulator (Model S-4).

To isolate the vasculature of the left hind limb, three strong nylon cords were placed round the main muscle groups at the upper end of the limb. The cords were positioned to include all the tissue except the sciatic nerve and the femoral nerve and vessels. The dog was then given heparin (500 i.u./kg, i.v., followed by 50 i.u./kg every half hour) and the perfusion circuit, which had been filled with dextran solution (Dextraven 150, Fisons Pharmaceuticals Ltd.), was connected to the dog. A cannula was tied into the proximal end of a common carotid artery. Blood was pumped at constant flow into the distal end of the femoral artery with a roller pump (Watson Marlow, Type MHRE). A fine nylon catheter (Portex No. 3) was passed through a metatarsal vein so that its tip lay in the femoral vein. A 5 mm bore polyethylene cannula, with several side holes cut near its tip was tied in the femoral vein. This cannula drained blood into a reservoir, from which it was pumped back into the external jugular vein. When the three nylon snares were tightened by means of cranking devices the cannula in the femoral vein was the only outlet for blood from the limb. The effectiveness of the vascular isolation was shown by observing zero outflow when the inflow pump was stopped. The femoral venous drainage system enabled the pressure in the femoral vein to be held constant throughout each experiment. Changes in limb vascular resistance were calculated as the percentage changes in femoral artery perfusion pressure (flow constant).

In ten dogs, responses were determined in preparations in which the heart had been denervated by cutting the vago-sympathetic trunks in the neck and crushing the stellate ganglia and ansae subclaviae on both sides through small incisions made in the right and left second intercostal spaces. The completeness of denervation was shown by the absence of a change in heart rate for 10-15 s after bilateral carotid clipping.

Pressures were recorded with Statham P23Gb strain gauges attached to cannulae in the right femoral artery (systemic), the left femoral artery (perfusion) and the femoral vein. After amplification by carrier amplifiers (S.E. Laboratories, Feltham, Middlesex) the pressure signals were recorded on photographic paper by a direct-writing ultra-violet light recorder (S.E. Laboratories). Mean pressures were obtained by passing the signals from the strain gauge amplifiers through R-C networks with time constants of 2 s which were incorporated in the amplifiers. Zero pressures were recorded at the ends of the experiments as the pressures with the cannula tips free in air. Records were also obtained of heart rate with a digital cardiotachometer (Gilford Instrument Inc.) triggered by the arterial pressure pulse, and the ECG recorded from limb leads.

To prevent changes in vascular resistance due to muscular activity the dogs were given 0.5 mg/kg suxamethonium chloride (Scoline, Allen and Hanbury), repeated every 15 minutes. Oesophageal temperature was recorded with a thermistor probe (Yellow Springs Instruments Inc.) and was adjusted to 37-39°C by heating elements under the operating table. Arterial blood gases and pH were determined frequently during the experiments with standard glass electrode systems (Norman, Ledsome & Linden, 1965). Before undertaking any experimental procedures $P_{CO_2}$ and pH were adjusted to 35-40 mmHg and 7.35-7.40 units respectively by infusion of molar sodium bicarbonate solution and adjusting the stroke of the respiration pump. Molar sodium bicarbonate was infused at a rate of about 1 ml/min throughout the experiment. $Pa_{O_2}$ was kept greater than 80 mmHg. If it fell below this level breathing room air, oxygen was added to bring the inspired oxygen concentration to 40%. Blood volume was maintained by the constant infusion of dextran at a rate of about 3 ml/minute.

*Experimental procedure*

At the start of each experiment the responsiveness of the preparation was tested by stimulating the sympathetic trunk at supramaximal intensity (10-15 V and 2-4 ms) and different frequencies. Isoprenaline made up to a strength of 10 μg/ml in sodium metabisulphite (0.1 g/100 ml) was infused into a cannula in an external jugular vein at rates starting at 1 μg/min with a Braun constant infusion pump. Infusions were continued at each step until all the measured variables had reached steady states (usually about 4 minutes). One of the β-adrenoceptor blocking drugs was then given intravenously and, when all measured variables had reached steady states (about 15 min), isoprenaline

infusions were repeated, usually continuing to higher rates.

In a further group of dogs, we determined the effects of progressively increasing doses of the β-adrenoceptor antagonists on the vascular and the heart rate responses to infused isoprenaline.

## Results

*Responses to lumbar sympathetic nerve stimulation*

In all experiments stimulation of the left lumbar sympathetic trunk at all frequencies above 1 Hz resulted in an immediate increase in arterial perfusion pressure. The average response to stimulation at 5 Hz was an increase in perfusion pressure of +79% (range, +55 to +125; s.e., 10.1). Responses in one dog to stimulation at 2 Hz and 5 Hz are shown in Figure 1.

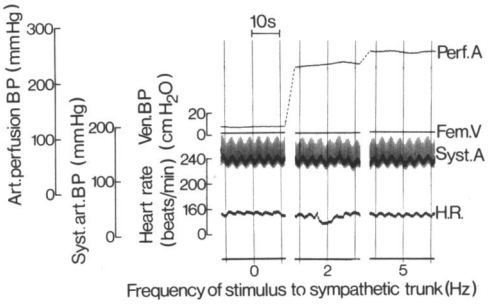


**Fig. 1** Responses of perfused artery of the dog to stimulation of lumbar sympathetic trunk between 4th and 5th lumbar ganglia with 10 V, 2 ms and frequencies of 2 and 5 Hz. Large increases in arterial resistance occurred. Perf. A., pressure in perfused artery (femoral); Fem. V., pressure in femoral vein; Syst. A., systemic arterial pressure; H.R., heart rate.

*Responses to isoprenaline infusion before and after administration of a β-adrenoceptor antagonist:*

*Practolol.* Isoprenaline infused in doses up to 50 μg/min resulted in an increase in heart rate and a decrease in arterial vascular resistance (Figure 2a). After practolol, 2 mg/kg, the heart rate responses to infused isoprenaline were greatly reduced but the decrease in arterial resistance was only slightly reduced (Figure 2b). The difference in the blocking effects of practolol on the cardiac and vascular responses is seen clearly from plots of the responses against log dose of isoprenaline before and after practolol. In Fig. 3 the data obtained from the same experiment as shown in Fig. 2 have been plotted and the small effect of practolol on the vascular responses to isoprenaline is apparent.

In seven dogs, plots of responses of arterial perfusion pressure against log-dose of isoprenaline were drawn for responses obtained before and after administration of practolol. In all dogs the plots were shifted to the right after practolol; i.e. larger doses of isoprenaline were required to produce the same degree of vasodilatation. For each experiment a vasomotor response was selected on the linear part of the log dose-response curve and the rates of isoprenaline infusion which

**Table 1** Effects of practolol on vascular responses to infused isoprenaline in the dog

| | *Before practolol* | | *After practolol* | | | |
|---|---|---|---|---|---|---|
| *Dog* | *Vasc. response (% decrease)* | *Rate isopren. (μg/min)* | *Dose practolol (mg/kg)* | | *Relative increase in isoprenaline* | |
| 1 | 27 | 19.0 | 0.5 | | 1.8 | |
| | | | | 2.0 | | 2.6 |
| 2 | 25 | 9.0 | 0.5 | | 1.3 | |
| | | | | 2.0 | | 3.2 |
| 3 | 33 | 10.0 | | 2.0 | | 2.6 |
| 4 | 10 | 10.0 | | 2.0 | | 1.6 |
| 5 | 20 | 3.7 | | 2.0 | | 2.7 |
| 6 | 30 | 8.0 | | 2.0 | | 1.2 |
| 7 | 25 | 3.2 | | 2.0 | | 3.1 |
| Averages | 24.3 | 9.0 | 0.5 | 2.0 | 1.6 | 2.4 |

Relative increase in isoprenaline is the factor by which the rate of isoprenaline infusion must be increased after practolol to cause the same vascular response. Values obtained from linear ranges of log dose-response curves.

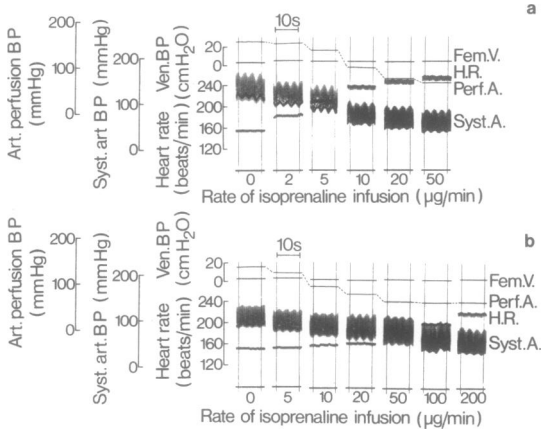


**Fig. 2** Records showing effects in dog of infusion of isoprenaline at different rates (a) before and (b) after administration of practolol, 2 mg/kg. Both vago-sympathetic trunks and ansae subclaviae were cut. Records taken during steady states about 4 min after changing isoprenaline rate. After practolol the heart rate response was competitively blocked but the arterial response and the blood pressure response were relatively unaffected. Perf. A., pressure in perfused (femoral) artery; Fem. V., pressure in femoral vein; Syst. A., systemic arterial pressure; H.R., heart rate.

were required to produce this response before and after practolol were read from the curves. The factor by which the dose of isoprenaline had to be increased to produce the same response was calculated from each experiment. The results, listed in Table 1, show that after 2 mg/kg practolol, isoprenaline had to be increased by an average factor of 2.4 (range 1.2 to 3.2) to cause the same vasomotor response as before practolol.

*ICI 66082.* The effects of ICI 66082 were studied in eight dogs. This drug in the same dose was usually more effective than practolol in blocking the arterial responses to infused isoprenaline. The plots of heart rate and arterial responses against log-dose of isoprenaline were displaced to the right (Figure 4). In all the experiments after 2 mg/kg of ICI 66082 the dose of isoprenaline had to be increased by an average factor of 3.5 (range 2.1 to 4.1) to produce the same arterial responses (Table 2). ICI 66082 in a dose of 2 mg/kg caused a significantly greater block of the vasomotor responses to infused isoprenaline than was obtained after the same dose of practolol ($P < 0.01$).

*Propranolol.* The effects of propranolol were studied in four dogs. In all these animals propranolol, 0.5 mg/kg, caused a high degree of blockade of both the arterial and the heart rate responses to infused isoprenaline. Following 0.5 mg/kg of propranolol doses of isoprenaline in excess of 50 μg/min had to be infused to cause significant vasomotor responses (Figure 5). Even after doses of propranolol as low as 0.05 mg/kg, in two dogs tested, the rate of isoprenaline infusion had to be increased by factors of 8.5 and 12.4 to produce the same decreases in vascular resistance.

**Table 2** Effects of ICI 66082 on vascular responses to infused isoprenaline in the dog

| | *Before 66082* | | | | | *After 66082* | | |
|---|---|---|---|---|---|---|---|---|
| *Dog* | *Vasc. response (% decrease)* | *Rate isopren. (μg/min)* | *Dose 66082 (mg/kg)* | | | *Relative increase in isoprenaline* | | |
| 8 | 21 | 10.5 | 0.5 | | | 1.7 | | |
| 9 | 29 | 5.6 | 0.5 | | | 2.0 | | |
| 10 | 38 | 5.4 | 0.5 | | | 1.7 | | |
| | | | | 2.0 | | | 3.8 | |
| 11 | 38 | 16.0 | | 2.0 | | | 3.1 | |
| 12 | 20 | 15.0 | | 2.0 | | | 3.2 | |
| 13 | 30 | 12.5 | | 2.0 | | | 3.8 | |
| 14 | 14 | 2.8 | | 2.0 | | | 4.1 | |
| | | | | | 7.0 | | | 18 |
| 15 | 13 | 1.7 | | 2.0 | | | 3.1 | |
| | | | | | 7.0 | | | 29 |
| Averages | 25.4 | 8.7 | 0.5 | 2.0 | 7.0 | 1.8 | 3.5 | 23 |

Relative increase in isoprenaline is the factor by which the rate of isoprenaline infusion must be increased after ICI 66082 to cause the same vascular response. Values obtained from linear ranges of log dose-response curves.

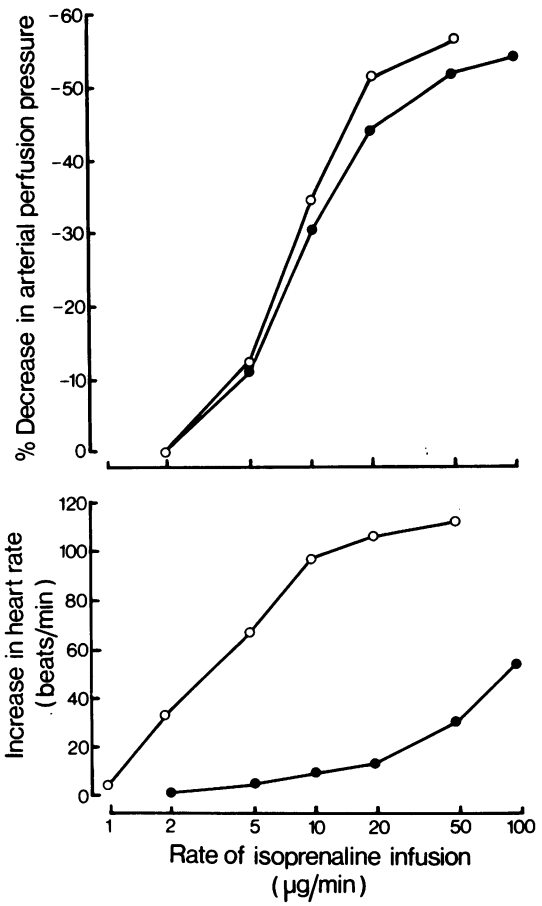


**Fig. 3** Responses of perfused artery and heart rate of dog to isoprenaline infusion. Results from same dog as in Figure 2. (⊖), before and (•), after practolol, 2 mg/kg. Results taken from dog No. 6 in which practolol had the least effect of the series on the vascular responses to isoprenaline.

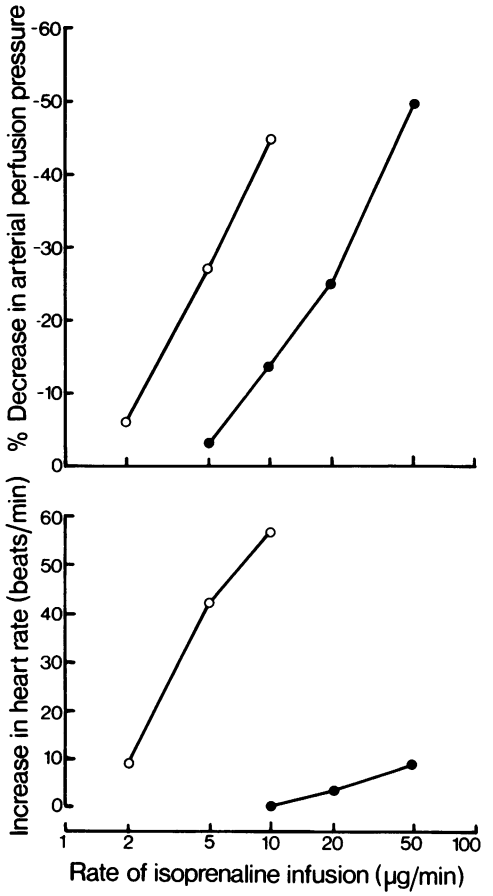


**Fig. 4** Responses of perfused artery and heart rate of dog to isoprenaline infusion (○) before and (•) after ICI 66082, 2 mg/kg. Heart denervated. Results show that dose-response curves for both arterial and heart rate responses are shifted to right after ICI 66082.

*Comparison of blocking effects of the three drugs on heart rate and arterial responses to infused isoprenaline*

Studies were made on ten dogs: four with practolol, four with ICI 66082 and two with propranolol. In all these preparations the heart was denervated. We first recorded the increase in heart rate and decrease in arterial resistance produced by several rates of infusion of isoprenaline. We then recorded the responses to infusion of isoprenaline at the same rates following a small dose of one of the β-adrenoceptor blocking drugs.

A series of log dose-response curves were drawn for heart rate and vasomotor responses to different rates of infusion of isoprenaline showing progressive displacement to the right following cumulative doses of β-adrenoceptor blocking drugs (Figure 6). For each drug, the percentage reduction in the arterial response to each rate of isoprenaline infusion at each stage of blockade, was plotted against the corresponding percentage reduction in the heart rate response (Figure 7). These plots indicate that at low doses of propranolol the vasomotor responses are blocked to a greater extent than the heart rate responses; the vasomotor response to a given rate of infusion of isoprenaline was reduced by about 60% for a dose of propranolol which reduced the heart rate response to isoprenaline by only about 15%. Practolol, however, reduced the heart rate response by about 60% when the vasomotor

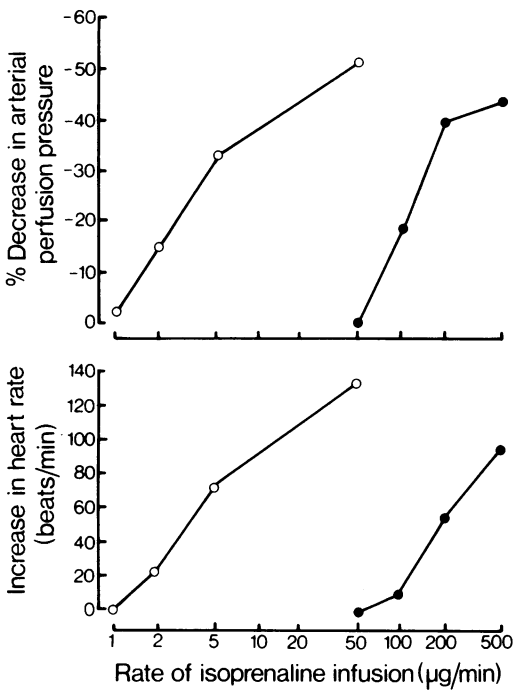


**Fig. 5** Responses of perfused artery and heart rate of dog to isoprenaline infusion (○) before and (●) after propranolol, 0.5 mg/kg. Heart denervated. Both curves show massive shift to right after propranolol.

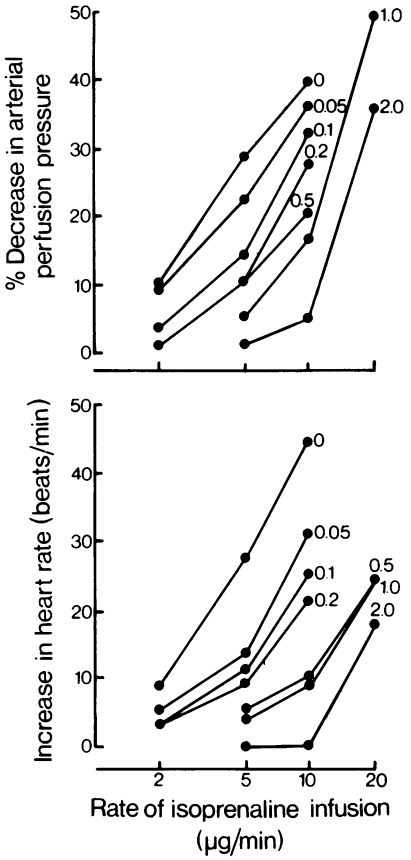


**Fig. 6** Series of log dose-response curves for arterial resistance and heart rate responses of dog to isoprenaline infusion. Heart denervated. Curves obtained before ICI 66082 and following cumulative doses of ICI 66082. The total doses in mg/kg are written alongside each curve.

response was reduced by about 15%. ICI 66082 was slightly more effective against the heart rate responses than against the vasomotor responses and produced, on average, a reduction in the vasomotor response of 40% when heart rate response was reduced by 60%.

## Discussion

Our study of the blocking effects of practolol, propranolol and ICI 66082 on the peripheral vasodilation induced by isoprenaline has been approached in two ways. Firstly, we selected doses of the drugs which have been regarded by other workers as producing a considerable degree of competitive blockade of the response of the heart rate to infused isoprenaline, and tested the effects of these doses on the vasomotor responses to isoprenaline. The second approach was to compare the degree of blockade of the vasomotor responses with the degree of blockade of the heart rate responses to infused isoprenaline with progressively increasing doses of the antagonists. This method permits a comparison to be made of the relative abilities of each of these antagonists to block preferentially either the heart rate or the vasomotor responses.

In the first part of the study, the doses of the antagonists selected were found to be effective in abolishing or greatly reducing the increase in heart rate from infusion of isoprenaline at rates of up to 10 μg/min and in the case of propranolol (0.5 mg/kg) responses were abolished up to even higher rates of infusion of isoprenaline. The effects of these three drugs on the vasomotor responses to infused isoprenaline were different. Propranolol was the most effective and practolol the least effective in blocking the vasomotor changes.

Determination of the vascular effects of β-adrenoceptor antagonists at a particular dose provides only limited information. It only permits

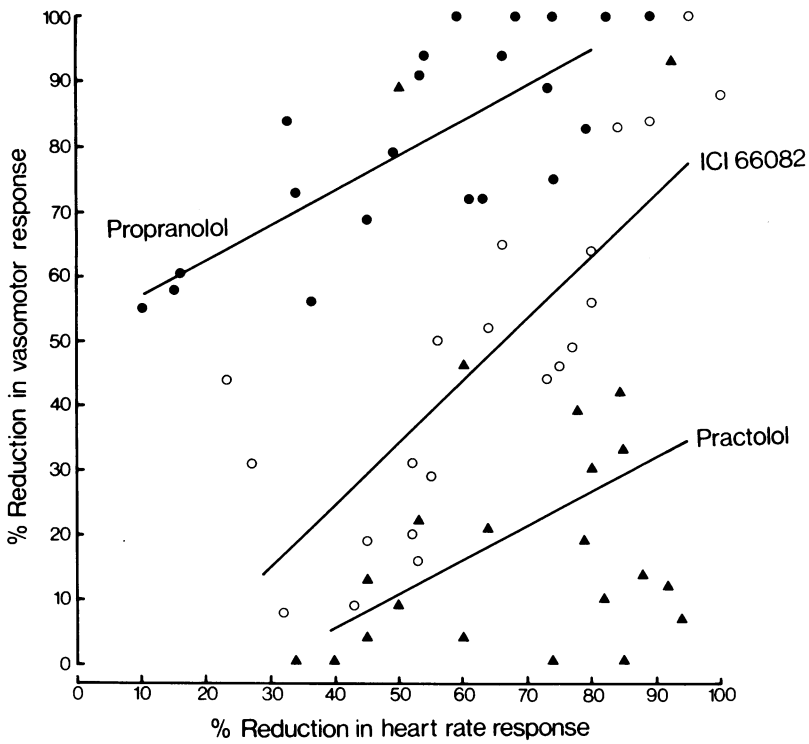


**Fig. 7** Comparison of reduction in vasomotor response to isoprenaline with corresponding reduction in heart rate response in the dog following several doses of propranolol, ICI 66082 and practolol. Points obtained by infusion of isoprenaline at different rates before and after cumulative doses of β-receptor antagonists and by comparison of the reduction in the vasomotor responses with the coincident percentage reductions of the heart rate responses.

a prediction of the vascular effects likely to occur when the drug is used at that dose. More information regarding differential blocking action can be obtained from the comparative studies of the heart rate and vascular effects with several doses of the antagonists. This enables the vascular blocking effects to be 'titrated' against the heart rate blocking effects. The results from these studies, summarized in Fig. 7, show clearly the difference between the three drugs. Since, with no blockade, all lines must pass through the origin and with complete blockade of both responses the lines must reach the (100, 100) point, these plots show only the relative effects of partial blockade by the three drugs.

Our results showing the high potency of propranolol and the low potency of practolol in blocking the vascular response to isoprenaline agree with other published results (Dunlop & Shanks, 1968; Boissier, Advenier, Guidicelli & Viars, 1971). However, from our results ICI 66082 does appear to be more effective in blocking the vascular response or less effective in blocking the heart rate response than the results of Barrett *et al.* (1973) would suggest. According to Barrett *et al.*, ICI 66082 is as 'cardioselective' as practolol. There are two possible explanations for this discrepancy. First, there may be a species variation. Barrett *et al.*, determined the heart rate effects in the cat and blood pressure changes in the dog. Secondly, their method for assessing vasomotor responses is different from ours. Instead of merely observing blood pressure changes or even flow changes, we isolated the vasculature of a limb and interrupted its sympathetic nerve supply. We showed that the blood vessels of this limb responded normally to nerve stimulation and then we determined the responses under conditions where changes in systemic blood pressure or changes in cardiovascular reflex activity could not have affected the responses.

β-Adrenoceptor blocking drugs have been classified into different groups according to whether their effect is predominantly on the heart or on the peripheral blood vessels or whether there is no apparent selectivity (Lands, Luduena &

Buzzo, 1967; Levy & Wilkenfeld, 1970). Such a classification is unlikely to be very rigid and all gradations are likely to occur. However, from our results it would appear that practolol is much more effective against heart rate responses than against vasomotor responses, propranolol is more effective against vascular responses and ICI 66082 appears to be intermediate with perhaps a slightly greater effect against the heart rate responses.

There is a clinical requirement for 'selective' β-adrenoceptor blocking drugs. In particular a drug is required which effectively antagonizes sympathetic stimulation of the heart without causing myocardial depression and which does not antagonize vasodilator or bronchodilator effects. Propranolol antagonizes all effects of β-sympathetic stimulation and in large doses depresses the myocardium (Harry, Kappagoda, Linden & Snow, 1973). Practolol is relatively 'cardioselective' in its blocking action, but it is a partial agonist (Dunlop & Shanks, 1968). The effect of this was seen in the present experiments as an increase in the control heart rates following practolol. ICI 66082 does not have this stimulating action and can be used in doses of up to 40 mg/kg without causing myocardial depression (Harry, Knapp & Linden, 1974), but it appears to be less 'cardioselective' than practolol.

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