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Comparison of the effects of isoprenaline, orciprenaline, salbutamol and isoetharine on the cardiovascular system of anaesthetized dogs

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Summary

1. The intravenous injection of isoprenaline, orciprenaline, salbutamol and isoetharine increased heart rate in anaesthetized dogs. Log dose-response curves obtained with a series of doses of salbutamol and isoetharine were flatter than those for isoprenaline and orciprenaline. The order of activity of the drugs in increasing heart rate was isoprenaline, orciprenaline, and salbutamol = isoetharine.

2. The injection into the external iliac artery of isoprenaline, orciprenaline, salbutamol and isoetharine produced dose dependent increases in femoral blood flow. Log dose-response curves for all drugs were parallel. The order of activity of the drugs was isoprenaline, salbutamol=isoetharine and orciprenaline.

3. Salbutamol and isoetharine were less active than orciprenaline in increasing heart rate but more active in increasing femoral blood flow.

4. These observations indicate that salbutamol and isoetharine have a greater effect on β_2 than on β_1-adrenoceptors in the cardiovascular system.

Introduction

Ahlquist's (1948) classification of adrenoceptors as alpha and beta is now widely accepted. It was based on the effects of six sympathomimetic amines on various effector systems and has been supported during the past 10 years by the development of drugs which specifically block β-adrenoceptors. Recently, it has been suggested that β-adrenoceptors can be subdivided into two groups, designated β_1 and β_2 (Lands, Groblewski & Brown, 1966; Lands, Arnold, McAuliff, Luduena & Brown, 1967). β_1-Adrenoceptors were responsible for lipolysis and cardiac stimulation and β_2 for bronchodilatation and vasodilatation. The subdivision was based on the relative potency of a series of sympathomimetic amines on four different animal preparations (Lands *et al.*, 1967). Two different animal preparations were used to assess the action of these drugs on the β-adrenoceptors in the cardiovascular system. Cardiac stimulation was determined in the isolated perfused rabbit heart and peripheral vasodilatation assessed from the fall in blood pressure produced by the rapid intravenous administration of the test compounds in anaesthetized dogs.

This classification of β-adrenoceptors into β_1 and β_2 suggests that both types of receptor are present in the cardiovascular system. If the concept of two types of

β-adrenoceptors in the cardiovascular system is to be studied, it would appear essential to compare in intact animals the activity on the cardiovascular system of sympathomimetic amines which previous studies have indicated may have a dominant effect on one or other of these subgroups of receptors.

In this paper we have compared the effects of isoprenaline, orciprenaline, salbutamol and isoetharine on the cardiovascular system of anaesthetized dogs. Isoprenaline and orciprenaline stimulate both β_1 and β_2-adrenoceptors (Engelhardt, Hoefke & Wick, 1961; Lands *et al.*, 1967; Shanks, Brick, Hutchison & Roddie, 1967). Although preliminary study has shown that salbutamol has a greater effect on β_2-adrenoceptors than on β_1-adrenoceptors, a detailed comparison of the effects of this drug and isoprenaline on the intact cardiovascular system has not yet been reported (Cullum, Farmer, Jack & Levy, 1969). Isoetharine was first described in 1950 (Land, Luduena, Grant & Ananenko, 1950) and was included by Lands and his colleagues (1967) in the series of sympathomimetic amines used in their observations. Its properties would appear to be similar to those of salbutamol but a comparison of the two drugs has not yet been described.

In our experiments changes in heart rate and femoral blood flow have been used as indices of the effects of the four drugs on heart rate and on the peripheral blood vessels.

Methods

Observations were made on dogs, weighing 20–30 kg, anaesthetized by the subcutaneous injection of morphine sulphate (0·5 mg/kg) followed 1 h later by the intravenous injection of pentobarbitone (20 mg/kg). A cuffed endotracheal tube was inserted and positive pressure respiration with room air maintained by a Palmer 'Ideal' respiration pump at a rate of 20 strokes/min and a stroke of 13 ml/kg body weight. A polythene catheter was inserted into a foreleg vein for the intravenous injection of drugs. The heart rate was measured by a Nielsen Instantaneous Ratemeter (Type 2750). Arterial pressure (1 mmHg$\equiv$1·333 mbar) was measured from the left carotid artery by means of a metal cannula attached to a strain gauge (Consolidated Electrodynamics; Type 4–327–L221). Heart rate, the electrocardiogram (Lead II) and arterial pressure were recorded on a direct writing instrument (Devices M4 or 8) and displayed on an oscilloscope (Airmec).

In six dogs arterial blood flow to each hind limb was measured by means of electromagnetic flowmeters. Both external iliac arteries were exposed through two lower abdominal incisions and probes for the flowmeters were applied to both arteries proximal to the origin of the deep femoral branches. Mean flow was recorded. Zero flow determinations were made by occlusion of the artery by a snare which surrounded the artery distal to the flow probe; the probes were calibrated at the end of each experiment (Shanks, 1967). In these experiments drugs were injected into the external iliac artery through a polythene catheter inserted into the deep femoral artery until its tip lay in the main artery distal to the flow probe. Drugs were injected into the arteries in a volume not exceeding 0·3 ml and washed in with 0·3–0·5 ml saline.

The following drugs were used: $\pm$ isoprenaline sulphate (Burroughs Wellcome); $\pm$ salbutamol sulphate (Allen & Hanburys), $\pm$ orciprenaline sulphate (Boehringer

Ingelheim); ± isoetharine methanesulphonate (Winthrop). All doses are expressed in terms of the base.

An unpaired *t* test was used to assess the significance of differences between the mean responses produced by the different drugs at similar dose levels.

Results

Effects of drugs on heart rate

Observations were made in sixteen dogs. In each dog increasing doses of isoprenaline (0·01, 0·1, 1·0 and 10·0 μg/kg) were injected intravenously. The second and subsequent doses were given when the effects of the previous dose had worn off and baseline values of heart rate and blood pressure were regained. After the administration of isoprenaline, increasing doses of either orciprenaline, salbutamol or isoetharine (0·1, 1·0, 10·0 and 100·0 μg/kg) were given. Orciprenaline and isoetharine were given to five dogs and salbutamol to six dogs. Second and subsequent doses of these drugs were given when the effects of the preceding dose had

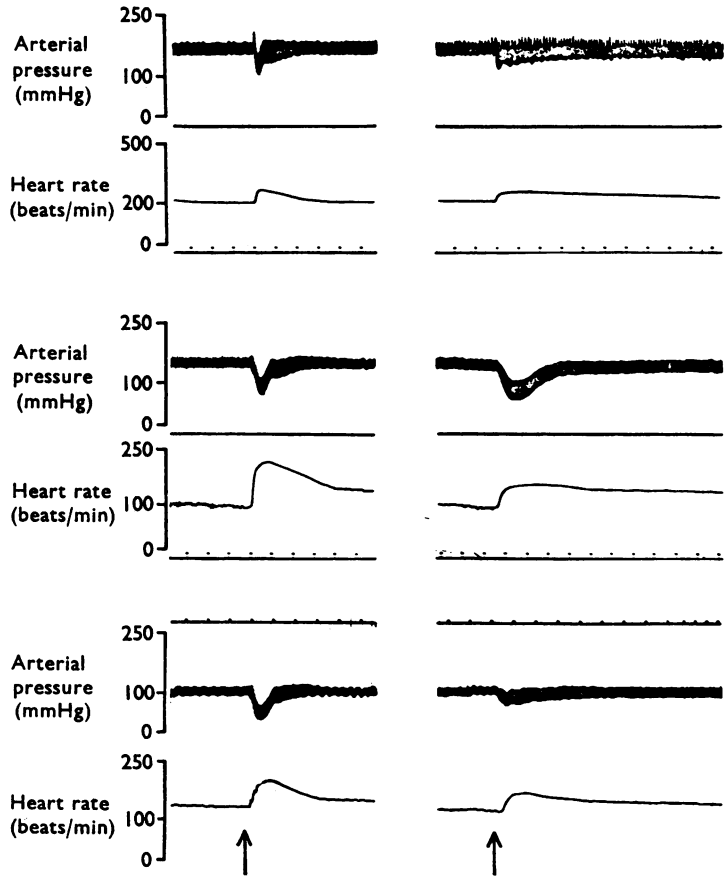

FIG. 1. Records of arterial pressure and heart rate in three anaesthetized dogs showing the responses to the intravenous injection of isoprenaline and orciprenaline in one dog (top trace); isoprenaline and salbutamol in the second dog (middle trace); and to isoprenaline and isoetharine in the third (bottom trace). The dose of isoprenaline in all three dogs was 1·0 μg/kg and of the other three drugs 10·0 μg/kg. The time trace shows intervals of 1 minute.

disappeared. Records taken from three different experiments to demonstrate the responses to isoprenaline (1·0 μg/kg) and orciprenaline, salbutamol and isoetharine (10·0 μg/kg) are shown in Fig. 1. All four drugs increased heart rate and reduced diastolic pressure. There was often an initial transient increase in systolic pressure followed by a fall. The increases in heart rate and falls in diastolic arterial pressure produced by the series of doses of each of the four drugs have been compared.

The effects of different doses of the four drugs on heart rate are given in Fig. 2. Each drug produced a dose dependent increase in heart rate. Significantly greater increases in heart rate were produced by the 0·1, 1·0 and 10·0 μg/kg doses of isoprenaline than by the same doses of the three other drugs ($P<0.001$). The 10 μg/kg dose of isoprenaline produced a significantly greater increase in heart rate than that caused by the 100 μg/kg dose of the other drugs ($P<0.05$). Orciprenaline (10.0 μg/kg) produced a significantly greater increase in heart rate than the same doses of salbutamol or isoetharine ($P<0.001$). There were no statistically significant differences between the tachycardias produced by 0·1, 1·0 and 100·0 μg/kg of salbutamol, orciprenaline and isoetharine.

The mean decreases in diastolic arterial pressure produced by the intravenous injection of the series of doses of the four drugs are given in Fig. 3. Increasing doses of all four drugs caused progressively greater falls in diastolic arterial pressure. The fall produced by isoprenaline (10.0 μg/kg) was not significantly different from the change produced by the largest dose (100.0 μg/kg) of the three other drugs but isoprenaline (0·1, 1·0 and 10·0 μg/kg) produced a significantly greater fall in diastolic arterial pressure than the same doses of salbutamol, orciprenaline and isoetharine ($P<0.001$). The decreases in diastolic pressure produced by orciprenaline, salbutamol and isoetharine (0·1, 10·0 and 100·0 μg/kg) were not significantly different but with 1·0 μg/kg, salbutamol produced a significantly greater fall than orciprenaline ($P<0.001$) or isoetharine ($P<0.02$) and isoetharine a significantly greater fall than orciprenaline ($P<0.05$).

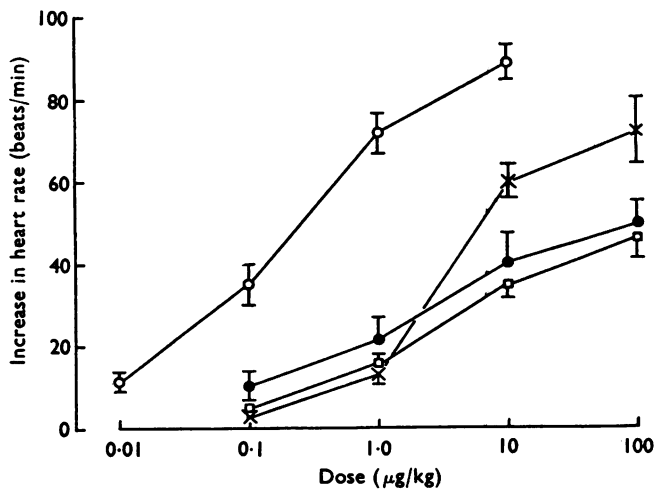

FIG. 2. Increases in heart rate produced in anaesthetized dogs by the intravenous injection of a series of doses of isoprenaline (○), orciprenaline (×), salbutamol (●) and isoetharine (□); mean (± S.E.M.) of observations in sixteen, five, six and five dogs, respectively for each drug.

Effect of drugs on femoral blood flow

Observations were made in six dogs. A separate experiment was carried out on each hind limb giving a total of twelve experiments. The effects of isoprenaline were studied in all twelve experiments and each of the other drugs in four. The

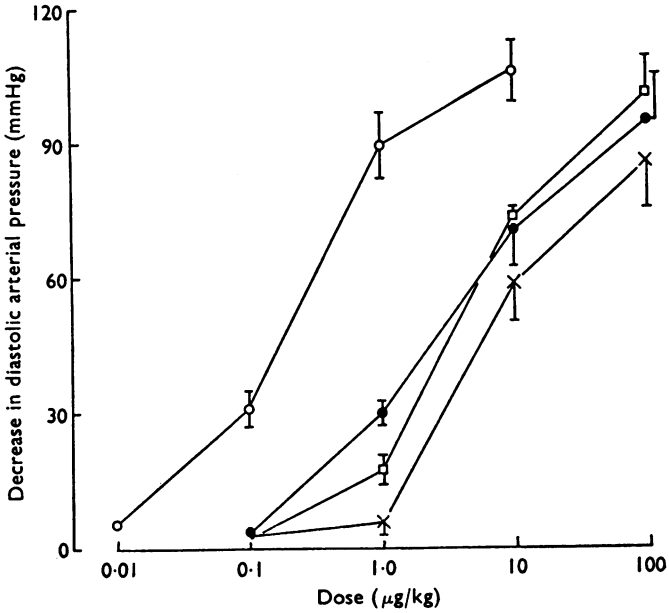

FIG. 3. Decreases in diastolic arterial pressure produced in anaesthetized dogs by the intravenous injection of a series of doses of isoprenaline (○), orciprenaline (×), salbutamol (●) and isoetharine (□); mean (±S.E.M.) of observations in sixteen, five, six and five dogs respectively.

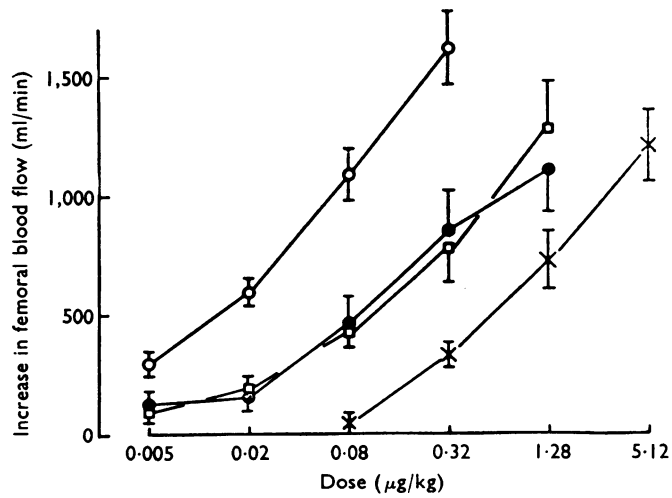

FIG. 4. Increases in femoral blood flow produced in anaethetized dogs by the intra-arterial injection of a series of doses of isoprenaline (○), orciprenaline (×), salbutamol (●) and isoetharine (□); mean (±S.E.M.) of observations in twelve dogs for isoprenaline and four for each of the other drugs.

largest dose of each drug produced transient small increases in heart rate and falls in arterial pressure. The increases in femoral blood flow produced by the injection into the femoral artery of four doses of isoprenaline (0·005, 0·02, 0·08 and 0·32 μg/kg) were determined. The second and subsequent doses were only given when the recovery from the preceding dose was completed. The increases in flow produced by the intra-arterial injection of a series of doses of orciprenaline, salbutamol or isoetharine were then similarly determined in the same limb. One hour later observations were made on the contralateral hind limb. After the responses to the series of doses of isoprenaline had been determined, the responses to one of the two drugs which had not been given to the other hind limb were measured.

Figure 4 shows the increases in femoral arterial blood flow produced by the intra-arterial injection of increasing doses of isoprenaline, salbutamol, orciprenaline and isoetharine. All doses of the four drugs produced increases in femoral blood flow with the exception of orciprenaline (0·005 and 0·02 μg/kg). The maximum increase in flow produced by isoprenaline (0·32 μg/kg) was greater than that produced by any of the other drugs. The increase produced by the maximum dose of each drug was significantly greater than that produced by the penultimate dose: $P<0·001$ for isoprenaline, $P<0·05$ for salbutamol and $P<0·01$ for isoetharine and orciprenaline. All doses of isoprenaline produced significantly greater increases in femoral blood flow than the same doses of salbutamol and isoetharine ($P<0·001$), both of which caused significantly greater increases in femoral flow than the same doses of orciprenaline ($P<0·001$).

Discussion

The results show that isoprenaline, orciprenaline, salbutamol and isoetharine produced dose dependent increases in heart rate. The largest dose of isoprenaline (10·0 μg/kg) probably produced the maximum increase in heart rate that could be obtained as previous observations in dogs using the same anaesthetic procedure have shown that the maximum increase in heart rate produced by isoprenaline occurred with a dose of 10·0 μg/kg even though larger doses (25, 50 and 100 μg/kg) were given (Collins, McDevitt, Shanks & Swanton, 1969). In our experiments, the largest doses of orciprenaline, salbutamol and isoetharine were probably not sufficient to produce their maximum increase in heart rate as the response to the final dose of each drug was always greater than the response to the penultimate dose and less than the maximum response to isoprenaline. Larger doses of these three drugs were not given as the responses to the top dose of each were prolonged and in some experiments resting heart rate was not regained after 90 min: larger doses were shown in preliminary experiments to produce such marked respiratory stimulation with fluctuations in arterial pressure and heart rate that the precise effect of the drug could not be determined.

The dose-response curves for the increases in heart rate produced by isoprenaline and orciprenaline were parallel whereas those for salbutamol and isoetharine were much flatter. The results indicate that isoprenaline is clearly more active than any of the three other drugs in increasing heart rate, and that orciprenaline is probably more active than salbutamol and isoetharine. A quantitative comparison of the activity of the four drugs in increasing heart rate has been obtained from the

results shown in Fig. 2. Fifty per cent of the maximum increase in heart rate produced by isoprenaline is forty-three beats/min and, from the dose response curve in Fig. 2, would have been evoked by isoprenaline (0·19 μg/kg).

The doses of each of the other three drugs that would have increased heart rate by forty-three beats/min are given in Table 1. The activity ratios of orciprenaline, salbutamol and isoetharine with respect to isoprenaline, which has been given a value of 1,000, are given in Table 1.

All four drugs increased femoral blood flow in a dose dependent manner. The maximum dose of each drug produced a greater increase in flow than the penultimate dose. The injection into the femoral artery of larger doses of all drugs produced an increase in heart rate and a fall in arterial pressure. This latter effect alters the perfusion pressure for the hind limb, so that changes in femoral arterial inflow may not be a true index of changes in femoral vascular resistance. As this effect would invalidate this method of measurement of changes in vascular resistance produced by the intra-arterial injection of drugs, the largest dose of each of the four drugs was limited to that which had little effect on the general circulation. It would appear from these experiments that for each of the drugs this dose did not produce maximal dilatation of the vessels in the hind limb. The four dose-response curves were parallel. The relative activities of orciprenaline, salbutamol and isoetharine with regard to isoprenaline, have been calculated in the same way as for the increases in heart rate and the results are given in Table 1.

The reduction in arterial diastolic pressure produced by the intravenous administration of isoprenaline has also been used as an index of the peripheral vasodilator action of isoprenaline (Ahlquist, 1948). Although the intravenous injection of isoprenaline decreases total peripheral resistance, this may not be reflected in changes in diastolic arterial pressure (Fordham & Resnekov, 1970). The fall in arterial pressure produced by the intravenous administration of isoprenaline may elicit reflexes which alter the circulatory effects of isoprenaline (Dunlop & Shanks, 1968). Consequently, changes in arterial diastolic pressure may not be an accurate measurement of the peripheral vasodilator action of isoprenaline. In these experiments the four drugs produced dose related decreases in arterial diastolic pressure. The dose-response curves were parallel. The relative activities of orciprenaline, salbutamol and isoetharine in comparison to isoprenaline, are given in Table 1.

The order of activity of the four drugs in eliciting the two responses was different (Table 1), although isoprenaline was the most active in each case. Orciprenaline was more active than salbutamol and isoetharine in increasing heart rate but less active in increasing femoral blood flow. There was little difference between the effects of salbutamol and isoetharine in eliciting the two responses. As it has been

TABLE 1. *Effect of the intravenous administration of isoprenaline, orciprenaline, salbutamol and isoetharine on heart rate and diastolic arterial pressure and of the intra-arterial injection of these drugs on femoral blood flow*

	Heart rate		Diastolic pressure		Femoral flow	
Drug	μg/kg	Rel. activity	μg/kg	Rel. activity	μg/kg	Rel. activity
Isoprenaline	0·19	1,000	0·2	1,000	0·035	1,000
Orciprenaline	4·8	39·6	7·6	26·3	1·6	21·9
Salbutamol	40	4·75	3·65	54·8	0·27	130
Isoetharine	100	1·90	4·45	44·9	0·35	100

For explanation see text.

generally assumed that isoprenaline stimulates β_1 and β_2-adrenoceptors to the same extent, the small variation in activity of orciprenaline in comparison to isoprenaline in increasing heart rate and femoral blood flow, 25·5 and 45·66 respectively, would support earlier observations which showed that orciprenaline activated β-adrenoceptors in the heart and peripheral blood vessels to the same extent (Engelhardt *et al.*, 1961; Shanks *et al.*, 1967). As salbutamol and isoetharine were more active than orciprenaline in increasing femoral flow but less active in increasing heart rate, the results were compatible with the concept that the cardiovascular system contains β_1 and β_2-adrenoceptors.

The dose-response curves for decreases in diastolic arterial pressure for all four drugs were parallel. The curves for increases in femoral blood flow were also parallel. The isoprenaline and orciprenaline dose-response curves for increases in heart rate were parallel to each other but the ones for salbutamol and isotharine, although parallel to each other, were much flatter. The parallelism of the dose-response curves for isoprenaline and orciprenaline for the three cardiovascular responses studied is further proof that both drugs are acting on the same receptors. As the dose-response curves for the increases in femoral blood flow and decreases in diastolic arterial pressure for salbutamol and isoetharine were parallel to those for isoprenaline and orciprenaline, it would appear that these four drugs were acting on the same receptors in eliciting these responses. The reason for the lack of parallelism in the heart rate responses for the four drugs is not clear. The contribution of a reflex tachycardia to the increase in heart rate produced by the four drugs has not been evaluated although it may account for the two sets of dose response curves with different slopes (Dunlop & Shanks, 1968).

The results show that isoprenaline and orciprenaline stimulate β_1 (cardiac) and β_2 (vascular)-adrenoceptors and that there is no evidence of selectivity in their effects. Salbutamol and isoetharine also stimulate both β_1 and β_2-adrenoceptors but have relatively greater effect on the latter. This indicates that they show some selectivity of action.

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