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Effect of β-adrenoceptor blocking compounds (propranolol, practolol and LB 46) on isoprenaline induced changes in regional blood flow in the rat

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Summary

1. The effects of three β-adrenoceptor blocking agents on isoprenaline induced changes in systemic and regional blood flow have been investigated in rats anaesthetized with pentobarbitone. The blood flow to the principal vascular beds was measured by the ^{86}Rb fractionation method.

2. Isoprenaline infusion at a rate of 0·3 (μg/kg)/min intravenously caused an increase in cardiac output and a decrease in peripheral resistance which affected different vascular fields unevenly. The coronary and carcass fractions of cardiac output increased while the cutaneous, renal and splenic fractions decreased.

3. Pretreatment with propranolol (2 mg/kg intravenously) blocked all isoprenaline effects. Practolol (8 mg/kg intravenously) lessened the effect on cardiac output but did not prevent the vascular effects except those on the coronary circulation. LB 46 (0·2 mg/kg) had less marked β-adrenoceptor blocking activity than propranolol (2·0 mg/kg).

Introduction

Most studies on the circulatory effects of agents stimulating or blocking β-adrenoceptors have dealt either exclusively with the overall circulatory effects, such as changes in heart rate, cardiac output, blood pressure and total peripheral resistance, or with the effects on some specially chosen vessels. The most detailed analysis of this kind was given by Green & Kepchar (1959) but at that time β-adrenoceptor blocking agents were not available. More recently, simultaneous measurements have been made on two to four principal vascular beds (Johnson & Parkins, 1966 ; Parratt & Wadsworth, 1970 ; Neely & Windham, 1970 ; Charbon & Reneman, 1970). There is evidence that isoprenaline causes vasodilatation in all vascular beds but that the degree of vasodilatation varies from region to region. Only a few studies have been reported in which the effects of β-adrenoceptor stimulating and blocking agents on the distribution of cardiac output to all the principal vascular beds have been measured simultaneously. Fell (1964) who measured the contribution of various parts of the body in relation to the total body weight could not demonstrate any redistribution of cardiac output after treatment with isoprenaline.

The method using the fractional distribution of indicators (Sapirstein, 1956, 1957, 1958) was of value in estimating regional distribution of the cardiac output and has

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been widely used in our laboratory (Takacs, Kallay & Skolnik, 1962 ; Takacs & Vajda, 1963 ; Takacs, 1965). It was shown that pronethalol (10 mg/kg) had only slight circulatory effects in rats while the effects of dichloroisoprenaline (10 mg/kg) were mostly due to its sympathomimetic activity (Debreczeni & Takacs, 1968).

In the present study the effect of isoprenaline infusion on cardiac output and on regional blood flow has been investigated in normal anaesthetized rats. The modification of this response by three β-adrenoceptor blocking agents, propranolol, practolol and LB 46, was studied.

Methods

Forty-five inbred male rats, weighing 150–190 g, were fasted for 18–24 h and anaesthetized with pentobarbitone sodium (50 mg/kg, intraperitoneally). Arterial blood pressure (1 mmHg$\equiv$1·333 mbar) was measured by a mercury manometer through a cannula introduced into the carotid artery. Blood coagulation was prevented by 2·5 mg heparin in 0·2 ml 0·9% NaCl solution, injected into the tail vein. Cardiac output was estimated by a dye dilution microtechnique, in which 0·3 ml of a solution of Evans blue (15 mg/ml 0·9% NaCl solution) was injected into the femoral vein. Blood samples were taken with a fraction collector from the carotid artery at intervals of 0·67–1 seconds. The dye concentration was measured in samples of 0·02 ml blood diluted to 3·0 ml with 0·9% NaCl solution by determining the absorption at 590 nm in a Beckman spectrophotometer. The proportions of the cardiac output delivered to each organ (organ fraction) were determined by the ^{86}Rb fractionation method (Sapirstein, 1956). The animals were killed 60–90 s after intravenous injection of about 10 μCi ^{86}Rb. The organs were weighed and dissolved in a 20% (w/v) solution of potassium hydroxide. Radioactivity was measured in a NaI scintillation counter. Measurements were made on the heart, kidney, intestines, spleen, skin, lung, liver, the gastrocnemius muscle, and the carcass which was everything left after dissection of the above mentioned organs.

From the values of cardiac output, blood pressure, organ weights and fractions of cardiac output, organ blood flow and vascular resistance were calculated for 100 g tissue ; the total peripheral resistance was also calculated (Sapirstein, 1958 ; Takacs *et al.*, 1962). Alterations in blood pressure and cardiac output in the range occurring in these experiments did not modify the extraction rate of ^{86}Rb from the blood (Takacs, Kallay & Karai, 1964). In organs with a double blood flow (liver, lungs) the contribution made by either blood supply cannot be calculated ; therefore, in these organs only the 'apparent' fractions of the cardiac output are calculated (Takacs *et al.*, 1964).

Isoprenaline solutions were infused at a rate of 0·03 ml/min through a cannula in the jugular vein. The measurements were made after a 5 min infusion period when the blood pressure had reached a steady level. In three groups of animals, infusion of isoprenaline was commenced 7 min after the intravenous injection (0·4 ml in 90 s) of one of the three β-adrenoceptor blocking agents. Isoprenaline was dissolved in a constant volume of saline.

Each treated group was compared with a control group of animals given the same volume of 0·9% NaCl solution as the treated groups. The groups pretreated with β-adrenoceptor blocking agents were compared with the control group and with the isoprenaline infused group. Each group consisted of at least eight animals. As

the within-sample variances did not differ significantly, common standard deviations computed from the pooled sum of squares are given.

The drugs used were: (−)-isoprenaline bitartrate (dihydrate) (Isolevin-Cilag); (±)-propranolol hydrochloride (I.C.I.), practolol (I.C.I.) and (±)-4-(2-hydroxy-3-isopropylaminopropoxy)-indole (LB 46, Sandoz). The doses refer to the salts of isoprenaline and propranolol and to the base of practolol and LB 46.

Results

Effects of isoprenaline infusion

Intravenous infusion of isoprenaline (0·3 μg/kg)/min caused a highly significant decrease in blood pressure from 111·7 to 75·6 mmHg and of total peripheral resistance from 378·6 to 155·1 (10^3 dyn. s/cm^5)/100 g body weight, while cardiac output increased from (24·2 to 42·8 ml/min)/100 g body weight (Table 1). Two principal types of responses were noticed in different vascular fields after isoprenaline. The resistance of the coronary, gastrocnemius and carcass vascular beds were reduced to 27, 36 and 29% of the control values, while those of the kidney, intestine and skin were 62, 57 and 54% of the control values; there was no significant change in the resistance of the splenic circulation.

The organ fractions of cardiac output showed the following changes. The heart fraction increased from 1·5 to 2·1% and the carcass fraction from 40·2 to 57·3% but there were significant decreases in the renal fraction from 14·9 to 9·5%, in the intestinal fraction from 20·8 to 15·0%, in the skin fraction from 9·4 to 7·1% and in the spleen fraction from 0·7 to 0·3%. As a result regional blood flow ((ml/min)/100 g organ weight) increased significantly only to the heart, the gastrocnemius muscle and the carcass.

Effect of β-adrenoceptor blocking agents on the circulatory changes caused by isoprenaline

Propranolol. Pretreatment with propranolol (2 mg/kg) reversed all the circulatory effects of isoprenaline. All the measurements in this group of animals were practically identical with the control group and significantly different from the isoprenaline infused group without pretreatment with propranolol.

Practolol. Pretreatment with practolol (8 mg/kg) did not prevent the decreases in total peripheral resistance and blood pressure caused by isoprenaline. The cardiac output was about mid-way between that of the control group and that of the isoprenaline infused group. The effects of isoprenaline on the regional blood flow in the kidney, intestines, skin and carcass were not influenced by the dose of practolol used in these experiments. The increases in coronary blood flow and coronary fraction of cardiac output caused by isoprenaline were reduced or abolished by pretreatment with practolol. The coronary resistance was less than that of the control group but higher than that of the group infused with isoprenaline.

LB 46. Pretreatment with LB 46 (0·2 mg/kg) blocked the blood pressure-lowering effect of isoprenaline to the same extent as did propranolol (2 mg/kg). The increase in cardiac output was similar to that found in the group pretreated with practolol. The total peripheral resistance was lower than that of the control group but higher than that of the group infused with isoprenaline. The effects of iso-

TABLE 1. *Effect of isoprenaline and β-adrenoceptor blocking agents on the circulation in the rat*

	Controls (9)	Isoprenaline (9) (0·3 μg/kg)/min	Propranolol (10) 2 mg/kg	Practolol (8) 8 mg/kg	LB 46 (9) 0·2 mg/kg	Common standard deviation
Total body						
Blood pressure	111·7	75·6...	103·5^{++}	78·8...	102·2^{++}	18·0
Cardiac output	24·2	42·8...	24·3^{+++}	32·8^{+}	35·1^{+}	9·6
Resistance	378·6	155·1...	356·0^{+++}	199·6...	231·8...$^{+}$	76·8
Heart						
Flow	126·4	311·9...	118·0^{+++}	171·7^{+++}	204·2^{+++}	65·9
Resistance	77·6	20·9...	74·5^{+++}	40·7...$^{+}$	43·0...$^{+}$	19·3
Fraction	1·5	2·1...	1·5^{+++}	1·5^{+++}	1·7^{++}	0·3
Kidney						
Flow	345·2	421·3	335·3	369·2	457·0	122·6
Resistance	27·8	16·5...	25·6^{++}	18·4..	18·7..	6·1
Fraction	14·9	9·5...	15·0^{+++}	11·2..	14·5^{+++}	2·5
Lung						
Fraction	3·2	2·9	2·8	3·1	2·8	—
Liver						
Fraction	8·9	5·4	7·9	5·8	7·1	—
Intestine						
Flow	69·5	85·6	70·8	70·2	77·4	20·6
Resistance	131·6	75·5..	126·8^{++}	98·3	117·0^{+}	37·9
Fraction	20·8	15·0..	20·5^{++}	14·7..	16·0^{+}	3·9
Spleen						
Flow	54·8	51·4	38·7^{+}	41·2	56·1	17·2
Resistance	172·1	133·3	232·6^{++}.	178·8	158·4	62·5
Fraction	0·7	0·3...	0·6^{+++}	0·4...	0·5..$^{++}$	0·1
Skin						
Flow	11·3	14·9	10·8^{+}	13·8	14·7	4·3
Resistance	843·2	452·4...	798·7^{++}	477·7..	662·3	231·4
Fraction	9·4	7·1..	9·1^{+}	8·1	8·2	1·7
Gastrocnemius						
Flow	7·5	17·8...	6·6^{+++}	12·9^{+}	9·0^{++}	5·4
Resistance	1221·8	437·5...	1452·1^{+++}	563·6..	1035·3^{++}	429·6
Fraction	0·4	0·5	0·3^{++}	0·4	0·3^{++}	0·1
Carcass						
Flow	15·1	38·3...	15·3^{+++}	27·1..$^{+}$	26·7..$^{++}$	8·6
Resistance	610·1	176·4...	548·2^{+++}	241·6...	343·3...$^{++}$	121·4
Fraction	40·2	57·3...	42·2^{+++}	54·7...	49·0...$^{+++}$	4·9

Figures in brackets are numbers of observations.
Units of measurement: Cardiac output—(ml/min)/100 g body weight; blood pressure—mmHg; resistance—(10^3 dyn. s/cm^5)/100 g body weight or organ weight; flow—(ml/min)/100 g organ weight; fraction—blood flow of the organ as % of the cardiac output.
Statistical signs: .=difference between treated and control groups; $^{+}$=difference between isoprenaline infused group without pretreatment and with pretreatment with β-adrenoceptor blocking agents.
No sign—$P>0·05$; . or $^{+}$—$P<0·05$; .. or $^{++}$—$P<0·01$; ... or $^{+++}$—$P<0·001$.

prenaline on the blood flow through the gastrocnemius muscle were blocked. The results obtained on the circulation of the carcass and the coronary vessels were intermediate between those of the control and isoprenaline infused groups.

Discussion

In this study an attempt was made to investigate the effect of β-adrenoceptor stimulating and blocking agents in the principal vascular fields simultaneously. This aim seemed to be achieved best by using the method of fractionation of the labelled indicator, ^{86}Rb. The main shortcoming of this method is that it estimates only the circulatory state of the animal at the time of killing and that for a full analysis a large number of experiments would be required.

Isoprenaline decreased the total peripheral resistance and blood pressure while at the same time cardiac output was almost doubled. The coronary, striated muscle and carcass vascular resistances were decreased to a much greater extent than those of the renal, intestinal and cutaneous vascular beds. The fraction of cardiac output to the carcass and coronary circulations increased while the renal, intestinal, cutaneous and splenic fractions decreased. Our results are in agreement with earlier findings based mainly on an analysis of the effects of adrenaline and noradrenaline (Green & Kepchar, 1959 ; Takacs, 1965).

As was anticipated, pretreatment with 2 mg/kg of propranolol blocked all circulatory effects caused by isoprenaline. The block of the decrease in renal vascular resistance is in agreement with the view that the renal vasculature contains β-adrenoceptors (McNay & Goldberg, 1966 ; Fenyvesi & Kallay, 1967 ; Neeley & Windham, 1970) although the role of renal circulatory autoregulation cannot be excluded (Selkurt, 1946).

The isoprenaline induced changes in systemic and regional circulations and their blockade by propranolol served as a reference for comparison with two more β-adrenoceptor blocking agents. The method of measuring the regional distribution of cardiac output is very time consuming, and we were therefore obliged to use only single doses of practolol and LB 46. Quantitative comparison between the three β-adrenoceptor blocking drugs are therefore not possible but, in the dose used, practolol reduced the effects of isoprenaline on cardiac output and on the coronary circulation while those on the other vascular beds were much less affected. It is of interest that practolol, in a dose not affecting the peripheral vascular changes caused by isoprenaline, blocked the effects of isoprenaline on coronary flow and coronary fraction of cardiac output, but only attenuated its effect on coronary vascular resistance. This observation may be explained by the fact that cardiac output was still raised and that this increase in cardiac output might have caused secondary coronary vasodilatation (Hashimoto, Shigei, Imai, Saito, Yago, Uei & Clark, 1960 ; Krasnow, Rolett, Yurchak, Hood & Gorlin, 1964 ; Somani & Laddu, 1969 ; Lioy, 1967).

No quantitative comparisons can be made between LB 46 and the other two β-adrenoceptor blocking agents. The doses of LB 46 and propranolol were chosen on the basis of the relative potencies found by Giudicelli, Schmitt & Boissier (1969). However, in this study 0·2 mg/kg of LB 46 proved to be a less effective β-adrenoceptor blocking agent than was 2 mg/kg of propranolol.

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