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THE ROLE OF β-ADRENOCEPTORS IN THE RESPONSES OF THE HEPATIC ARTERIAL VASCULAR BED OF THE DOG TO PHENYLEPHRINE, ISOPRENALINE, NORADRENALINE AND ADRENALINE

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1 The sympathetically-innervated hepatic arterial vascular bed of the dog was perfused from a femoral artery. Hepatic arterial blood flow and perfusion pressure were recorded continuously, and the hepatic arterial vascular resistance (HAVR) calculated from these measurements.

2 Intra-arterial injections of phenylephrine caused dose-dependent rises in HAVR, indicating hepatic arterial vasoconstriction, at all doses above threshold. No secondary reductions in HAVR followed these responses.

3 Intra-arterial injections of isoprenaline caused only dose-dependent reductions in HAVR at doses above threshold.

4 Intra-arterial injections of noradrenaline typically caused an initial increase in HAVR which was followed at all but the highest doses by a secondary, delayed, reduction in HAVR.

5 Intra-arterial injections of adrenaline, like those of noradrenaline, resulted in hepatic arterial vasoconstriction followed by hepatic arterial vasodilatation.

6 On a molar basis, the most potent hepatic arterial vasoconstrictor was noradrenaline, followed by adrenaline and phenylephrine.

7 The maximum reductions in HAVR caused by adrenaline (mean reduction = 21.9%) and noradrenaline (16.9%) were significantly smaller than those due to isoprenaline ($P < 0.001$).

8 Propranolol attenuated the hepatic arterial vasodilator responses due to isoprenaline, and the secondary falls in HAVR following intra-arterial adrenaline and noradrenaline.

9 Propranolol did not modify the vasoconstrictor responses to phenylephrine.

10 Both adrenaline and noradrenaline were more potent hepatic arterial vasoconstrictors after propranolol than in the absence of β-adrenoceptor blockade. The potentiation of the vasoconstrictor effects of adrenaline was statistically significant.

11 After propranolol, adrenaline was a more potent hepatic arterial vasoconstrictor than noradrenaline.

12 Since the β-adrenoceptors in the hepatic arterial vasculature were not blocked by atenolol, but were stimulated by salbutamol, it is concluded that they are predominantly of the β_2-type.

13 The vasoconstrictor actions of phenylephrine, noradrenaline and adrenaline were all antagonized by the systemic administration of phentolamine, all three dose-response curves being shifted to the right.

14 The results are discussed with regard to the possible control of the hepatic arterial vasculature by naturally-occurring catecholamines.

Introduction

The responses of the hepatic arterial vascular bed to locally and systemically administered catecholamines have yielded information regarding the adrenoceptor population in the hepatic arterial resistance vessels in various species (Greenway & Stark, 1971). In the dog, the presence of α-adrenoceptors mediating hepatic arterial vasoconstriction has been indicated by the responses to locally administered noradrenaline,

adrenaline (Andrews, Hecker, Maegraith & Ritchie, 1955; Green, Hall, Sexton & Deal, 1959; Scholtholt, Lochner, Renn & Shiraishi, 1967; Hanson, 1973; Richardson & Withrington, 1976a and unpublished observations) and phenylephrine (Bender, Larsen & Horvath, 1962). In the cat, the influence of adrenoceptor antagonists on the responses of the hepatic arterial vasculature has also been investigated (Greenway & Lawson, 1969; Ross & Kurrasch, 1969).

The presence of β-adrenoceptors in the hepatic arterial vasculature has been established in the cat (Greenway & Lawson, 1969) and dog (Richardson & Withrington, 1976b,d) but the possible contribution of β-adrenoceptor stimulation to the hepatic arterial vascular responses to locally-administered naturally-occurring catecholamines has not been established quantitatively. In order to demonstrate vasodilatation quantitatively in this vascular bed, it is necessary to retain the sympathetic innervation intact (Richardson & Withrington, 1976d; 1977a); if the periarterial nerves accompanying the hepatic artery are sectioned, catecholamines injected intra-arterially cause only vasoconstriction (Richardson & Withrington, 1976a and unpublished observations); similarly, the administration of α-adrenoceptor antagonist drugs may reduce the hepatic arterial vasoconstrictor tone which is necessary for the quantitative examination of vasodilator responses.

The present experiments were designed to establish the relative properties of intra-arterially injected phenylephrine, isoprenaline, noradrenaline and adrenaline on the hepatic arterial resistance vessels. The contribution of β-adrenoceptor stimulation to these effects was analysed by using the nonselective β-adrenoceptor antagonist, propranolol. These experiments revealed the presence of β-adrenoceptors which were stimulated to cause hepatic arterial vasodilatation by isoprenaline and also by adrenaline and noradrenaline, but not by phenylephrine. Subsequently, the nature of the β-adrenoceptors in this vascular bed was examined by using the selective β_2-adrenoceptor stimulant salbutamol (Daly, Farmer & Levy, 1971) and the selective β_1-adrenoceptor antagonist atenolol (ICI 66082) (Hainsworth, Karim & Stoker, 1974).

Preliminary reports of parts of this investigation have been presented to the British Pharmacological Society (Richardson & Withrington, 1976b; 1977c).

Methods

The technique used in these experiments was the same as that reported previously (Richardson & Withrington, 1976d; 1977a) and only brief details of the preparation are given here.

Seventeen dogs weighing between 10.0 and 17.5 kg (13.2 ± 2.1: mean $\pm$ s.d.) were deprived of food but allowed access to water *ad libitum* for 24 h before the induction of anaesthesia with methohexitone sodium (Brietal, Lilly); anaesthesia was maintained with chloralose and urethane (50 mg/kg and 500 mg/kg, i.v. respectively). Following a midline laparotomy, the common hepatic artery was dissected free from its accompanying sympathetic nerves which were carefully preserved. The animals were heparinized (250 iu/kg and 100 iu/kg hourly, i.v.) and the hepatic artery cannulated and perfused with blood from a femoral artery. The hepatic arterial mean blood flow and mean perfusion pressure were monitored continuously as described previously (Richardson & Withrington, 1976d). In addition, the systemic arterial blood pressure, heart rate, and inferior vena cava pressure were recorded continuously, to monitor the possible systemic effects resulting from large doses of locally administered drugs which may survive passage through the liver and pulmonary vascular bed to enter the systemic circulation.

Presentation of results

Hepatic arterial vascular resistance (HAVR) was calculated as (hepatic arterial mean perfusion pressure; mmHg)/(hepatic arterial mean blood flow; ml/min or ml min^{-1} 100 g^{-1}). Hepatic arterial vascular resistance was therefore expressed in mmHg ml^{-1} min, or mmHg ml^{-1} min 100 g.

Changes in hepatic arterial vascular resistance were calculated as percentage changes from the control value immediately before any drug administration to the peaks of the drug-induced effects, i.e. (change in HAVR $\times$ 100)/(control HAVR).

Liver weight was obtained immediately *post mortem*; all values expressed per 100 g refer to this terminal weight of liver.

Drug administration

Agonist agents. Agonists were injected directly into the hepatic arterial blood stream, in volumes not in excess of 0.5 ml, washed in with 0.9% w/v NaCl solution (saline) to a total injectate volume of 1.5 ml. The drugs used, their molecular weights and the forms in which their weights are subsequently expressed, were as follows: adrenaline bitartrate (Macarthays; mol. wt. 333.3; salt) isoprenaline sulphate (Macarthays; mol. wt. = 556.6; salt), phenylephrine hydrochloride (Boots; mol.wt. = 203.7; salt), noradrenaline acid tartrate (Levophed, Winthrop; mol. wt. of base = 169.0, mol. wt. of salt = 319.3; base), and salbutamol sulphate (Ventolin, Allen & Hanburys; mol. wt. = 288.4; salt).

Antagonist agents. Antagonists were administered by intravenous injection in amounts previously found adequate to attenuate the effects of the corresponding

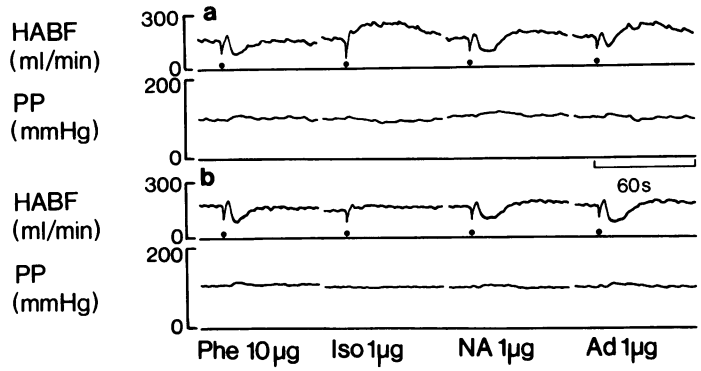

Figure 1 The effects of intra-arterial injections of phenylephrine (Phe, 10 μg), isoprenaline (Iso, 1.0 μg), noradrenaline (NA, 1.0 μg), and adrenaline (Ad, 1.0 μg) on the hepatic arterial blood flow (HABF) and perfusion pressure (PP), (a) before and (b) after the intravenous administration of propranolol (250 μg/kg).

receptor stimulants. The drugs used were: atenolol (Tenormin, ICI), phentolamine mesylate (Rogitine, Ciba) and propranolol hydrochloride (Inderal, ICI).

Drugs were dissolved in, or diluted from ampoules with saline; fresh solutions were prepared for each experiment.

The volume of the external vascular circuit was compensated with a solution of low molecular weight dextran in saline (Rheomacrodex, Pharmacia).

Assessment of drug effects

Agonists were injected intra-arterially in increasing, graded doses to establish the dose-response relationships, and $\log_{10}$ dose-response curves constructed from these data. Where vasodilatation occurred, the maximum change in calculated HAVR was established, and for comparative purposes the ED_{50}, defined as the dose of an agonist which would produce 50% of the established maximum for that agonist, is cited. Where vasoconstriction occurred, the absolute maximum response could not be established without injecting such large doses that pronounced systemic effects would have resulted from the locally-injected agents passing through the liver and entering the systemic circulation. In such cases, for comparative purposes the dose of agonist which produced a doubling in the calculated HAVR (i.e. a 100% increase) is cited.

The influence of antagonists upon the responses to agonists are assessed from dose-response curves obtained in the presence and absence of the antagonists. In addition, the antagonist-induced shift in the ED_{50} where the maximum effect could be established, or the dose-ratio for a doubling in HAVR, i.e. (dose of agonist required to double HAVR in the presence of antagonist)/(dose of agonist required to double HAVR in the absence of antagonist) are cited for comparative purposes.

Except where indicated to the contrary, results are expressed as means ± s.e. mean, and initial control values as means ± 1 s.d. The significance of differences between sets of paired data is assessed using Student's *t*-test.

Results

Control values

Under control conditions, the systemic arterial mean blood pressure was 132.8 ± 16.6 (mean ± s.d.) mmHg and the inferior vena caval pressure 1.5 ± 0.7 mmHg. The hepatic arterial blood flow (HABF) was 202.9 ± 57.6 ml/min and the hepatic arterial perfusion pressure (PP) 118.6 ± 14.6 mmHg, giving a calculated hepatic arterial vascular resistance (HAVR) of 0.64 ± 0.23 mmHg ml^{-1} minute. The livers weighed 292.2 ± 53.8 g, and expressed per unit weight of the livers, the hepatic arterial blood flow was 70.5 ± 17.3 ml min^{-1} 100^{-1} and the HAVR 1.82 ± 0.71 mmHg ml^{-1} min 100 g.

Effects of phenylephrine, isoprenaline, noradrenaline and adrenaline on the hepatic arterial vascular bed

Phenylephrine. Phenylephrine was injected in increasing, graded, doses over the dose range 100 ng to 100 μg or 200 μg in each of 5 experiments. At all doses above the threshold, which was 1.0 μg in all 5 experiments, vasoconstriction, manifest as rises in the calculated hepatic arterial vascular resistance, was the only response of the hepatic arterial vasculature. In no experiment was any increase in hepatic arterial blood flow observed to succeed the initial vasoconstrictor effect after any dose of phenylephrine. This effect was of short duration (Figure 1) and was dose-dependent (Figure 2), the maximum rise in HAVR of

384.1 ± 73.4% occurring with doses between 50 and 200 μg; the mean dose required to double the HAVR was 1.32×10^{-7} mol (Table 2). The effects of the maximal vasoconstrictor doses of phenylephrine on the hepatic arterial blood flow, perfusion pressure and calculated HAVR are shown in Table 1.

Isoprenaline. The responses of the hepatic arterial vasculature to intra-arterial injections of isoprenaline have been described previously (Richardson & Withrington, 1976b, d). In both the present and previous experiments, the only hepatic arterial vascular response resulting from intra-arterial injections of isoprenaline over the dose range 10 ng to 50 μg was a dose-dependent increase in hepatic arterial blood flow at a constant or slightly reduced perfusion pressure (Figure 1). This vasodilatation was of slower onset and longer duration of action than the vasoconstrictor response to intra-arterial phenylephrine (Figure 1). No subsequent vasoconstrictor responses were observed after the intra-arterial injection of isoprenaline in the present experiments; the maximum reduction in the calculated HAVR to isoprenaline was 39.1 ± 2.0% ($n=9$). The effects of maximal vasodilator doses of isoprenaline have been described previously (Richardson & Withrington, 1976d).

Noradrenaline. Noradrenaline was injected into the sympathetically-innervated hepatic arterial vascular bed in graded increasing doses over the dose-range 1 ng to 100 μg to construct dose-response curves in 12 experiments. In contrast to the vascular responses to phenylephrine and isoprenaline, the hepatic arterial vascular responses to intra-arterial noradrenaline were, over most of the dose-range employed, biphasic.

An initial dose-dependent reduction in the hepatic arterial blood flow was followed at all but the highest doses of noradrenaline by a delayed and more sustained increase in flow (Figure 1). The consequent initial rise in calculated HAVR, indicating vasoconstriction, and the subsequent fall in calculated HAVR, indicating vasodilatation, were related to the dose of noradrenaline injected (Figure 2) until doses in excess of 10 or 50 μg in different experiments were injected, when no secondary effect was apparent. The

Table 1 Maximal vasoconstrictor and vasodilator effects of phenylephrine, noradrenaline and adrenaline on hepatic arterial perfusion pressure (PP), hepatic arterial blood flow (HABF) and calculated hepatic arterial vascular resistance (HAVR)

	Phenylephrine	*Noradrenaline*	*Adrenaline*
Vasoconstrictor responses			
Maximal doses (μg)	50–200	50–200	50–200
PP control (mmHg)	108.3 ± 1.7	100.2 ± 3.6	102.5 ± 6.5
PP peak response (mmHg)	121.5 ± 2.3	121.6 ± 4.0	119.9 ± 6.9
HABF control (ml min^{-1} 100 g^{-1})	70.0 ± 6.2	83.6 ± 7.0	64.1 ± 5.8
HABF peak response (ml min^{-1} 100 g^{-1})	17.4 ± 4.4	31.6 ± 5.4	16.4 ± 3.5
HAVR control (mmHg ml^{-1} min 100 g)	1.58 ± 0.12	1.27 ± 0.12	1.74 ± 0.22
HAVR peak response (mmHg ml^{-1} min 100 g)	8.32 ± 1.80	5.81 ± 1.53	10.79 ± 1.90
Vasodilator responses			
Maximal doses (μg)		1–50	0.05–50
PP control (mmHg)		101.8 ± 3.1	110.5 ± 6.2
PP peak response (mmHg)		101.3 ± 3.5	106.3 ± 5.7
HABF control (ml min^{-1} 100 g^{-1})		78.1 ± 6.0	67.7 ± 4.6
HABF peak response (ml min^{-1} 100 g^{-1})		92.0 ± 6.3	82.6 ± 3.6
HAVR control (mmHg ml^{-1} min 100g)		1.36 ± 0.11	1.74 ± 0.21
HAVR peak response (mmHg ml^{-1} min 100 g)		1.13 ± 0.08	1.33 ± 0.13

Values are shown as means ± s.e. means for points immediately before maximal vasoconstrictor and vasodilator doses (control) and at the maximum effect (peak response). The data are derived from the same experiments as in Figure 2; comparable values for isoprenaline have been published previously (Richardson & Withrington, 1976d).

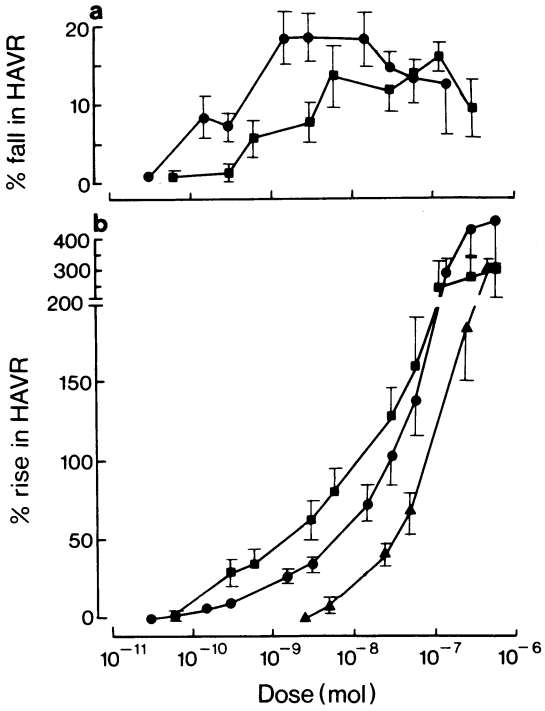

Figure 2 Dose-response curves to the intra-arterial injection of phenylephrine (▲), noradrenaline (■) and adrenaline (●). Abscissa scale: dose, expressed as a fraction of one mole of each agent injected intra-arterially. Ordinate scale: (a) percentage reduction in calculated hepatic arterial vascular resistance (HAVR), (b) percentage increase in HAVR. The symbols represent the means of 5 observations for phenylephrine, 12 for noradrenaline and 13 for adrenaline, and the vertical bars show the s.e. means.

threshold dose for the initial vasoconstriction was between 10 and 100 ng, and in all but one experiment the threshold for the subsequent vasodilator effect was higher (10 ng to 5.0 μg). The time course of the initial vasoconstriction and the subsequent dilatation, characteristic of the response to intra-arterial noradrenaline were similar to the monophasic responses to equipotent doses of phenylephrine and isoprenaline, respectively (Figure 1).

The dose-response curves (Figure 2) reveal that noradrenaline was a more potent vasoconstrictor of the hepatic arterial vasculature than phenylephrine, the mean dose of noradrenaline required to double the HAVR in 5 experiments was 1.34×10^{-8} mol, being significantly smaller than the corresponding dose for phenylephrine in the same experiments ($P < 0.025$). The maximum increase in calculated HAVR to noradrenaline was $335.5 \pm 74.4\%$, representing minimum hepatic arterial blood flows which were not significantly different from those attained at maximal vasoconstriction with phenylephrine ($P > 0.20$: for analysis see Richardson & Withrington, 1976a). The maximum secondary reduction in HAVR to noradrenaline of $16.0 \pm 2.4\%$ was however significantly smaller than the maximum response to isoprenaline of $38.6 \pm 2.9\%$ ($P < 0.001$) where maximum responses to both agents were established in the same 8 preparations.

Adrenaline. Adrenaline was injected into the hepatic artery in increasing graded doses over the range 10 ng–100 μg to obtain dose-response curves in 13 experiments. In five experiments, the injections were 'paired' with equal-weight administrations of noradrenaline and phenylephrine, for analysis of the relative potencies of these agents. The hepatic arterial vascular responses to doses of adrenaline above threshold were, as to noradrenaline but in contrast to phenylephrine and isoprenaline, biphasic (Figure 1). An initial reduction in hepatic arterial blood flow of rapid onset and short duration was succeeded at most doses by a more protracted increase in blood flow. The time courses of the two responses were similar to those of noradrenaline (Figure 1), and both effects were dose-dependent (Figure 2), though at the higher doses (5–50 μg) the secondary vasodilatation was masked by the predominant primary vasoconstriction, as with noradrenaline (Figure 2). The threshold vasoconstrictor doses for adrenaline of 10 ng to 500 ng were slightly higher than for noradrenaline, whilst the threshold vasodilator doses were slightly lower (10–100 ng) than for noradrenaline. In individual experiments, the threshold doses for the two effects of adrenaline were, in contrast to noradrenaline, usually coincident. The dose-response curves (Figure 2) reveal that on a molar basis, adrenaline has a potency as a constrictor of the hepatic arterial vascular bed intermediate between noradrenaline and phenylephrine; the mean dose of adrenaline required to double the calculated HAVR (3.06×10^{-8} mol) was significantly smaller ($P < 0.025$) than that for phenylephrine, though not significantly greater ($P > 0.10$) than for noradrenaline in the same 5 experiments. The maximum rise in HAVR of $485.2 \pm 75.3\%$ was attained with doses of adrenaline which produced minimum hepatic arterial blood flows not significantly different from those attained with maximum vasoconstrictor doses of noradrenaline ($P > 0.20$) or phenylephrine ($P > 0.10$); details of the maximum vasoconstrictor effects of adrenaline are given in Table 1. It is not, therefore possible on the basis of these experiments to establish any particular order of maximum vasoconstrictor potency for phenylephrine, noradrenaline and adrenaline (see Richardson & Withrington, 1976a).

The maximum vasodilator effect of adrenaline ($21.9 \pm 2.9\%$ reduction in the calculated HAVR) whilst slightly greater than that for noradrenaline, was

significantly less than that for isoprenaline ($35.9 \pm 1.9\%$; $P < 0.0025$) in the same 10 experiments where maximal responses to both were established. Adrenaline was, however, a more potent hepatic arterial vasodilator than noradrenaline, as revealed by the significantly smaller ED_{50} dose for adrenaline ($P < 0.005$) (Table 2).

Responses of the hepatic arterial vascular bed to intra-arterial injections of phenylephrine, isoprenaline, noradrenaline and adrenaline after the systemic administration of propranolol

The nonselective β-adrenoceptor antagonist, propranolol, was injected intravenously in a dose previously found adequate to attenuate the positive chronotropic effects of intravenous isoprenaline (5 μg). Propranolol itself caused a transient increase in the calculated hepatic arterial vascular resistance of $13.0 \pm 6.4\%$, when injected intravenously ($P > 0.10$, $n = 5$).

Phenylephrine. The hepatic arterial vascular response to intra-arterial phenylephrine was not significantly modified by pretreatment with propranolol, both the form and position of the dose-response curve to phenylephrine being unaffected (Figure 3). The dose of phenylephrine required to double the calculated HAVR in 3 experiments was 20.9 ± 7.2 μg before propranolol and 14.4 ± 6.0 μg after, the difference between these values being statistically insignificant ($P > 0.50$). The dose-ratio for this effect was 0.79 ± 0.28.

Isoprenaline. Propranolol greatly attenuated the hepatic arterial vasodilator response to isoprenaline in each of 3 experiments, there being a marked parallel shift of the isoprenaline dose-response curve to the right without suppression of the maximum responses compared with the control. The ED_{50} dose of isoprenaline before propranolol in these 3 experiments was 0.10 ± 0.05 μg and after β-adrenoceptor blockade this was significantly increased to 2.93 ± 0.22 μg ($P < 0.05$). After propranolol, isoprenaline did not evoke hepatic arterial vasoconstriction at any dose. However propranolol raised the threshold to isoprenaline, and thereafter doses of isoprenaline overcame the competitive blockade and resulted in hepatic arterial vasodilatation.

Noradrenaline. The biphasic response to intra-arterial noradrenaline was modified by pretreatment with propranolol; the secondary vasodilator response was reduced (Figure 1), whilst the initial vasoconstrictor response was slightly increased over most of the dose-range (Figure 3).

The dose-response curve for the vasoconstrictor effect of noradrenaline was shifted to the left by propranolol, though neither the reduction in the dose of noradrenaline required to double the HAVR from 0.86 ± 0.8 μg to 0.69 ± 0.26 μg ($n = 3$), representing a dose-ratio for this effect similar to that for

Table 2 Relative potencies of phenylephrine, isoprenaline, noradrenaline and adrenaline on the hepatic arterial vascular bed of the dog

Initial vasoconstrictor responses

	Threshold dose (range)	*Dose (± s.e. mean) required to double HAVR*
Phenylephrine	4.9×10^{-9}	$1.32\ (\pm 0.04) \times 10^{-7}$ * **
Noradrenaline	5.9×10^{-11} to 5.9×10^{-10}	$1.34\ (\pm 0.71) \times 10^{-8}$ *
Adrenaline	3.0×10^{-11} to 1.5×10^{-9}	$3.06\ (\pm 0.10) \times 10^{-8}$ **

Vasodilator responses

	Threshold dose (range)	*ED_{50} dose (± s.e. mean)*
Isoprenaline	1.8×10^{-11} to 9.0×10^{-11}	$2.90\ (\pm 0.90) \times 10^{-10}$
Noradrenaline	5.9×10^{-11} to 3.9×10^{-8}	$3.96\ (\pm 0.77) \times 10^{-9}$ ***
Adrenaline	3.0×10^{-11} to 3.0×10^{-10}	$5.52\ (\pm 1.90) \times 10^{-10}$ ***

All doses are expressed as fractions of one mole injected intra-arterially. The ranges for the thresholds are derived from all dose-response curves constructed (phenylephrine = 5, noradrenaline = 12, adrenaline = 13, isoprenaline = 12), and the doses required to double the hepatic arterial vascular resistance (HAVR) and ED_{50} values are derived from 5 experiments in which dose-response curves to all 4 substances were constructed by injecting blocks of the same weight of each substance followed by the next higher dose, again of all four substances in turn. Statistically significant differences between pairs of data obtained in this way are shown by * and ** = $P < 0.025$; *** = $P < 0.005$. Data included in the isoprenaline range are derived from Richardson & Withrington (1976b).

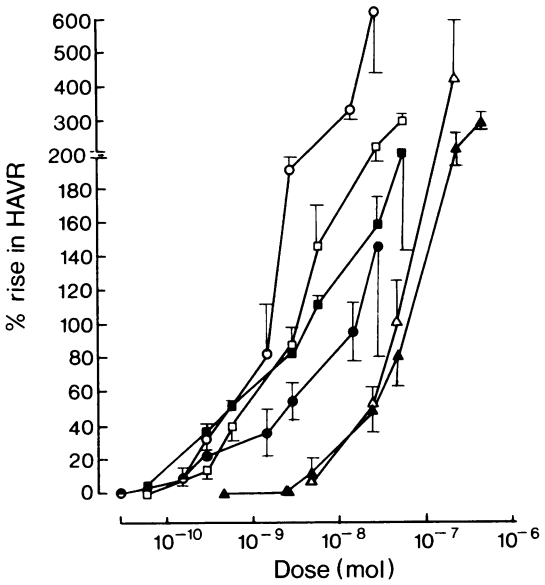

Figure 3 Dose-response curves for the vasoconstrictor actions of phenylephrine, noradrenaline and adrenaline on the canine hepatic arterial vascular bed. The doses are expressed as a fraction of one mole of each substance injected intra-arterially (abscissa scale), and the responses as the percentage increase in the calculated hepatic arterial vascular resistance (HAVR) (ordinate scale). In all cases, the points represent the means of three observations: noradrenaline before propranolol (■); noradrenaline after propranolol (□); adrenaline before propranolol (●); adrenaline after propranolol (○); phenylephrine before propranolol (▲); phenylephrine after propranolol (△). The vertical bars show the s.e. means.

phenylephrine of 0.78 ± 0.24, nor the increases in the vasoconstrictor effects of individual doses of noradrenaline (Figure 3) were statistically significant ($P > 0.05$). Propranolol pretreatment resulted in a marked suppression of the vasodilator effect of all doses of noradrenaline (Figure 1), suggesting that it was due to β-adrenoceptor stimulation.

Adrenaline. As with noradrenaline, the hepatic arterial vascular responses to intra-arterial adrenaline after propranolol were modified over most of the dose-range, the secondary delayed vasodilatation due to adrenaline being markedly attenuated by propranolol (Figure 1). The vasoconstrictor potency of adrenaline was significantly enhanced by propranolol (Figure 3), the dose-response curve being shifted to the left of the control dose-response curve. The dose of adrenaline required to double the HAVR fell significantly ($P < 0.005$) from 11.8 ± 4.4 μg before propranolol to 0.6 ± 0.1 μg after β-adrenoceptor blockade, the dose-ratio for this effect of 0.09 ± 0.06 being very much smaller than the corresponding dose-ratios for noradrenaline and phenylephrine.

Furthermore, after propranolol, the dose-response curve for the vasoconstrictor effect of adrenaline lay to the left of the corresponding curve for noradrenaline in the same three preparations (Figure 3), in contrast to the potency order before propranolol (Figures 2 and 3). After β-adrenoceptor blockade therefore, adrenaline was a more potent hepatic arterial vasoconstrictor than noradrenaline or phenylephrine (Figure 3).

The attenuation of the vasodilator responses to intra-arterial noradrenaline and adrenaline by propranolol (Figure 1) and the consequent potentiation of the hepatic arterial vasoconstrictor responses to these catecholamines, particularly to adrenaline (Figure 3) represents evidence that a component of their normal hepatic arterial vascular actions is mediated through β-adrenoceptors.

Nature of the β-adrenoceptors in the hepatic arterial vascular bed of the dog

β-Adrenoceptors have been demonstrated to be present in the hepatic arterial vasculature of the dog (Richardson & Withrington, 1976b,d) and from the present experiments, appear to contribute to the responses of this vascular bed to intra-arterial injections of the naturally-occurring catecholamines noradrenaline and adrenaline. The nature of the β-adrenoceptor population at this site has been examined further by the use of the selective β_2-adrenoceptor stimulant, salbutamol (Daly, Farmer & Levy, 1971) and the selective β_1-adrenoceptor antagonist atenolol (Hainsworth *et al.*, 1974).

Hepatic arterial vascular responses to isoprenaline and salbutamol. The complete dose-response curve to isoprenaline on this preparation has been published previously (Richardson & Withrington, 1976d). The dose-response relationship for salbutamol was similarly constructed in 3 experiments by the injection of increasing graded doses over the range 10 ng to 50 μg. All doses of salbutamol above the threshold (50–100 ng) caused only dose-dependent hepatic arterial vasodilatation with the maximum effect, a fall in HAVR of $39.5 \pm 2.7\%$, occurring at 20 or 50 μg (Figure 4). These responses and their time courses were similar to the responses to isoprenaline (Richardson & Withrington, 1976d). In two experiments in which paired equal weight doses of the two agonists were administered it was evident that at each weight injected, the hepatic arterial vasodilator response to isoprenaline was greater than that to salbutamol (Figure 4). The greater intrinsic potency of isoprenaline in stimulating β-adrenoceptors was

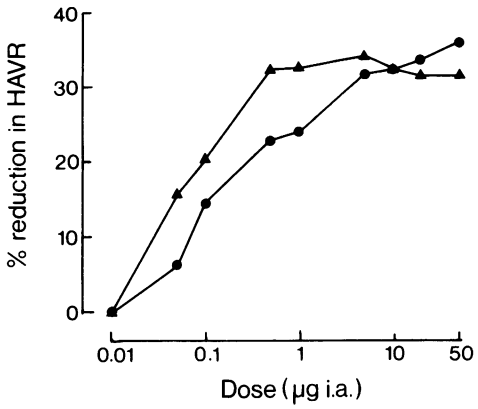

Figure 4 Log-response curve for isoprenaline (▲) and salbutamol (●). The doses are expressed in terms of the weight of each substance injected intra-arterially in the same experiment (abscissa scale) and the response as the percentage reduction in the calculated hepatic arterial vascular resistance (HAVR) (ordinate scale).

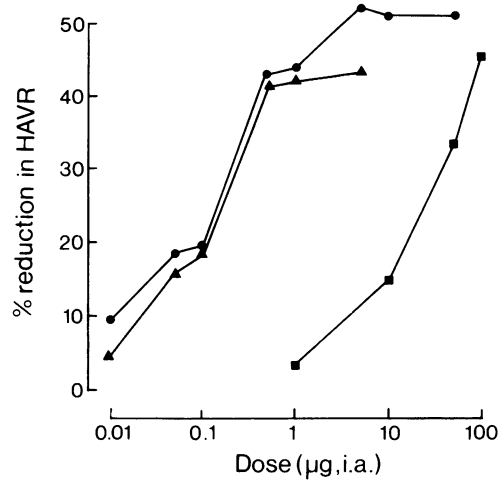

Figure 5 Effects of isoprenaline injected intra-arterially to the canine liver before (▲) and after atenolol, 100 μg/kg (●) and after propranolol, 100 μg/kg (■) in the same experiment. Doses of isoprenaline are expressed in terms of the weight injected (abscissa scale) and the response as the percentage reduction in the calculated hepatic arterial vascular resistance (HAVR) (ordinate scale). The antagonists were injected intravenously.

confirmed when the results were expressed on a molar basis where the mean ED_{50} for isoprenaline (6.3×10^{-10} mol) is less than that for salbutamol (3.5×10^{-9} mol).

Responses of the hepatic arterial vascular bed to isoprenaline in the presence of propranolol or atenolol. In two experiments, dose-response curves to intra-arterial isoprenaline were constructed (i) in the absence of antagonists (ii) after the intravenous administration of atenolol (100 μg/kg), a dose which almost completely blocked the positive chronotropic responses to isoprenaline (5 μg i.v.) and (iii) after propranolol (100 μg/kg).

The dose-response curves obtained in both of these experiments revealed that atenolol caused neither a shift in the position of the dose-response curve nor any suppression of the maximum vasodilator response. In contrast, the intravenous administration of propranolol caused a marked and parallel shift of the dose-response curve to the right with no suppression of the maximum response; characteristics of competitive antagonism. The results from one such experiment are shown in Figure 5.

The observations that salbutamol, a relatively selective β_2-adrenoceptor stimulant, evokes marked hepatic arterial vasodilatation and that the responses of the bed to isoprenaline are not antagonized by atenolol, a β_1-adrenoceptor antagonist, supports the conclusion that the β-adrenoceptors present in the hepatic arterial vascular bed of the dog are predominantly of the β_2 type.

The role of α-adrenoceptors in the responses of the hepatic arterial vascular bed to intra-arterial phenylephrine, isoprenaline, noradrenaline and adrenaline

Dose-response curves to phenylephrine, isoprenaline, noradrenaline and adrenaline were constructed in two experiments before and after the administration of the α-adrenoceptor antagonist, phentolamine (500 μg/kg, i.v.). Phentolamine caused a suppression of the hepatic arterial vasoconstrictor actions of phenylephrine, noradrenaline and adrenaline, the dose-response curves to all three being shifted to the right in a parallel manner without a reduction in the maximum response that could be elicited. However, quantitative comparisons of the potencies of the vasoconstrictor agents before and after phentolamine is complicated by the alteration in the control hepatic arterial vascular resistance caused by the administration of phentolamine (Hanson, 1973).

It is not possible to assess the influence of α-adrenoceptor blockade upon the vasodilator responses to isoprenaline or the secondary falls in HAVR due to adrenaline and noradrenaline because the vasodilator action of phentolamine reduced the vascular tone necessary for the quantitative evaluation of vasodilatation in this bed (Richardson & Withrington, 1976d; 1977a).

Despite the difficulty in making quantitative deductions from these experiments, they demonstrate clearly that the vasoconstrictor responses to phenylephrine, noradrenaline and adrenaline injected intra-arterially into the hepatic artery are dependent upon α-adrenoceptor stimulation.

Discussion

The use of the sympathetically-innervated arterial vascular bed of the dog's liver for the quantitative study of the effects of vasodilator hormones has previously been reported from this laboratory, and the relative molar potencies of a range of naturally-occurring and synthetic vasodilator agents established (Richardson & Withrington, 1976c, d; 1977a). In the present experiments, this preparation has been used to study the vascular effects of noradrenaline and adrenaline, injected intra-arterially, comparing these with the synthetic α- and β-adrenoceptor agonists, phenylephrine and isoprenaline respectively. In this way, the contribution of α- and β-adrenoceptor stimulation to the vascular effects of the naturally-occurring catecholamines on the hepatic arterial vascular bed can be ascertained.

Phenylephrine, injected into the hepatic arterial vasculature, caused reductions in the hepatic arterial blood flow at constant or, with high doses, slightly increased perfusion pressure, changes indicative of hepatic arterial vasoconstriction. These effects were dose-dependent and were not followed at any dose level in any experiment by secondary rises in hepatic arterial blood flow, which would indicate hepatic arterial vasodilatation. It is established that phenylephrine produces vasoconstriction in other vascular beds by direct α-adrenoceptor stimulation, and is devoid of actions on cardiac and other β-adrenoceptors (Aviado, 1959; 1970; Innes & Nickerson, 1975): on these grounds, it is commonly used as a pure α-adrenoceptor stimulant in the analysis and definition of adrenoceptor populations in vascular and non-vascular tissues. In the present experiments the specificity of phenylephrine as an α-adrenoceptor agonist was confirmed by the lack of antagonism by propranolol: a dose of propranolol sufficient to cause a shift of the isoprenaline dose-response curve on the hepatic arterial vasculature to the right, was without any effect on the form or position of the phenylephrine dose-response curve in this vascular bed. Consequently, the response to intra-arterial injections of phenylephrine is due solely to α-adrenoceptor stimulation in the canine hepatic arterial vasculature; this always caused an increase in hepatic arterial vascular resistance.

Isoprenaline, the archetypal β-adrenoceptor stimulant, has previously been shown to produce dose-dependent hepatic arterial vasodilatation (Richardson & Withrington, 1976b, d) manifest as an increase in hepatic arterial blood flow at constant, or with high doses, slightly reduced perfusion pressure. The systemic administration of propranolol caused a parallel shift of the isoprenaline dose-response curve to the right without suppression of the maximum response, but did not reveal a vasoconstrictor action of isoprenaline due to α-adrenoceptor stimulation: after propranolol, high doses of isoprenaline overcame the competitive β-blockade (Figure 4) without revealing any additional effects of the agonist. The responses of the hepatic arterial vascular bed to intra-arterial isoprenaline are therefore taken to represent pure β-adrenoceptor stimulation, an action resulting in a reduction in hepatic arterial vascular resistance.

The actions of the two naturally-occurring catecholamines, noradrenaline and adrenaline, on the hepatic arterial vascular bed, are more complex than those of phenylephrine and isoprenaline: over most of the dose-ranges used, intra-arterial injections of noradrenaline and adrenaline resulted in biphasic effects. Typically, an initial vasoconstriction of rapid onset and short duration was succeeded by a vasodilatation of longer duration, both effects being dose-dependent (Figure 2). Similar vascular effects have been noted previously (Andrews *et al.*, 1955). When equal weights of the two agents were injected, the vasoconstrictor response was of greater magnitude with noradrenaline, and the vasodilator response greater with adrenaline. In the absence of antagonists, the vasoconstrictor potency of noradrenaline was greater than that of adrenaline, and both were significantly more potent hepatic arterial vasoconstrictors than phenylephrine (Figure 2, Table 2); the maximal vasoconstriction attained in these experiments was similar with all three substances, and on the basis of these experiments, they cannot be ranked in any particular order of maximal vasoconstrictor potency. Quantitative comparisons of the vasodilator responses are however rather more difficult since with high doses of both noradrenaline and adrenaline, the secondary vasodilatation was no longer apparent, being masked by the substantial primary vasoconstrictor response. The maximum vasodilator responses to noradrenaline and adrenaline could not therefore be established with certainty. Adrenaline was a more potent hepatic arterial vasodilator than noradrenaline, the molar dose-response curve for the vasodilator action of adrenaline being to the left of the corresponding curve for noradrenaline (Figure 2); further, the ED_{50} for the vasodilator action of adrenaline was smaller than that for noradrenaline, but since the maximum effects may not have been established, this may be a less reliable means of comparison of potencies: the maximum reduction in hepatic arterial vascular resistance with both noradrenaline and adrenaline was significantly smaller than that with isoprenaline in the same experiments.

It is not practicable in this preparation to assess these vasodilator effects in a more rigidly controlled

manner, since the administration of α-adrenoceptor antagonists, which might be expected to uncover maximal vasodilator effects by abolishing the primary vasoconstrictor responses to the catecholamines, *a priori* diminishes the hepatic arterial sympathetic vasoconstrictor tone and results in an alteration in the baseline vascular resistance, making quantitative comparisons difficult.

The systemic administration of propranolol, an established nonselective β-adrenoceptor antagonist was, in contrast to α-adrenoceptor blockade, without sustained effect on the hepatic arterial vascular resistance (see Hanson, 1973). Propranolol competitively antagonized the effects of isoprenaline on this vascular bed, and was without effect on the responses to phenylephrine. It attenuated the secondary vasodilator responses to noradrenaline and adrenaline (Figure 1) and increased the vasoconstrictor responses to adrenaline. The enhancement of the vasoconstrictor effects of adrenaline was particularly marked, and was demonstrated to be statistically significant: after propranolol, adrenaline was, on a molar basis, a more potent hepatic arterial vasoconstrictor than noradrenaline, the reverse of the potency order in the absence of antagonists, and the same as the potency order found in the hepatic portal venous vascular bed in the absence of antagonists (Richardson & Withrington, 1977b).

This attenuation by propranolol of the vasodilator responses to noradrenaline and adrenaline, and the potentiation of the vasoconstrictor responses to adrenaline injected intra-arterially to the liver unequivocally demonstrate the involvement of β-adrenoceptors in the reponses to these catecholamines. This conclusion accords with the results of Greenway & Lawson (1969) in the cat, but is contrary to the views of Green *et al.* (1959) using the dog, who concluded that only α-adrenoceptors are present in hepatic arterial resistance vessels.

The conclusion from these observations is that the initial vasoconstrictor response to noradrenaline and adrenaline is due to α-adrenoceptor stimulation, and the succeeding vasodilatation is due to β-adrenoceptor stimulation. The effects of the four catecholamines studied therefore depend directly upon their relative efficacies as α- and β-adrenoceptor stimulants. It is improbable that the secondary vasodilatation arises as a consequence of the primary vasoconstriction whether mediated by local or systemic mechanisms, since: (i) phenylephrine, which exerted as great a vasoconstrictor effect as the catecholamines noradrenaline and adrenaline did not exhibit secondary vasodilatation; (ii) the systemic effects of all substances were small, and similar for each, probably reflecting the deactivation of the substances in passage through the liver and cardiopulmonary circuit (Vane, 1969); (iii) the larger vasoconstrictor effects were not associated with correspondingly larger secondary vasodilator effects as might be expected in a local 'reactive hyperaemia' response.

The hepatic arterial vasculature dilates in response to intra-arterial injections of both salbutamol and isoprenaline, the dose-response curves being parallel and the maximum responses very similar (Figure 4). On a molar basis salbutamol was 18 times less potent than isoprenaline (comparing ED_{50} values), a value similar to that for the effects on arterial pressure, but in contrast to the value (285–792) for the effects on heart rate after intravenous injection (Daly *et al.*, 1971) in the dog. Conversely, the responses of this vasculature to intra-arterial isoprenaline were not antagonized by a dose of atenolol adequate to suppress the positive chronotropic effects of intravenous isoprenaline. Atenolol is cardioselective in animals (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973; Hainsworth *et al.*, 1974; Harry, Knapp & Linden, 1974) and man (Graham, Littlejohns, Pritchard, Scales & Southorn, 1973; Marlin, Kumana, Kaye, Smith & Turner, 1975), and its lack of effect in the present experiments suggests that if there are β_1-adrenoceptors in this vascular bed, their population is small or they are resistance to blockade. The conclusion from these studies is that the predominant β-adrenoceptor population in the hepatic arterial vascular bed of the dog is of the β_2 type.

The differences in the time courses of the responses to α- and β-adrenoceptor stimulation prompt the question of the possible locations of these receptors. One established physiological action of adrenaline is to stimulate liver parenchymal metabolism by the activation of adenylcyclase (Exton, Mallette, Jefferson, Wong, Friedman & Park, 1970); these metabolic changes, classically described as a β-action of adrenaline (Newton & Hornbrook, 1972), may alter the vascular smooth muscle environment or activity to cause a delayed and prolonged vasodilatation. Whilst it may be that the hepatic arterial vasodilatation is a secondary effect of β-adrenoceptor-mediated stimulation of liver metabolism, the two effects, both mediated by β-adrenoceptors may well have distinct receptor sites. It seems probable that the vasodilator action of adrenaline is of physiological importance in ensuring an adequate blood flow to the hepatic parenchyma in states of stimulated liver metabolism; in this context, the study of the effects of intra-arterial infusions of catecholamines which may more closely mimic the physiological release of suprarenal medullary catecholamines than acute injections, may be of importance, as may the possible interactions between adrenaline and other hormones such as glucagon (Richardson & Withrington, 1976a, c; 1977a).

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