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A COMPARISON OF THE EFFECTS OF BRADYKININ, 5-HYDROXYTRYPTAMINE AND HISTAMINE ON THE HEPATIC ARTERIAL AND PORTAL VENOUS VASCULAR BEDS OF THE DOG: HISTAMINE H_1 AND H_2-RECEPTOR POPULATIONS

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1 The hepatic arterial and hepatic portal venous vascular beds of anaesthetized dogs were separately perfused in different experiments.

2 From measurements of perfusion pressures and blood flows in the two series of experiments, hepatic arterial vascular resistance (HAVR) and hepatic portal venous vascular resistance (HPVR) respectively were calculated.

3 Bradykinin, 5-hydroxytryptamine (5-HT) and histamine were injected intra-arterially and intraportally and dose-response curves constructed from these data.

4 Bradykinin injected intra-arterially caused dose-dependent hepatic arterial vasodilatation, and with an ED_{50} of 2.66×10^{-13} mol was more potent than any other vasodilator agent yet examined on this vascular bed.

5 Bradykinin injected intraportally at doses up to 10 times those which were maximal on the arterial circuit did not alter the calculated HPVR.

6 5-HT injected intra-arterially caused weak and variable rises in HAVR, indicating vasoconstriction. The maximum rise in HAVR was much less than that attained with noradrenaline in the same preparations.

7 5-HT injected intraportally caused dose-dependent rises in HPVR indicating portal constriction at doses above 15–100 μg: in some experiments small doses of 5-HT resulted in reductions in calculated HPVR.

8 Histamine has previously been shown to cause hepatic arterial vasodilatation: by intraportal injection, it caused dose-dependent rises in HPVR.

9 In order to examine the receptors responsible for the effects of histamine, dose-response curves were constructed before and after mepyramine and metiamide.

10 On the hepatic arterial vascular bed, metiamide did not antagonize the vasodilator effects of intra-arterial histamine, but these effects were antagonized by mepyramine.

11 Similarly on the hepatic portal bed, the rises in HPVR due to histamine were antagonized by mepyramine but not by metiamide.

12 The effects of histamine on both the hepatic arterial and portal venous vascular beds of the dog are therefore mediated predominantly by histamine H_1-receptors.

Introduction

Injections of noradrenaline and angiotensin into the hepatic artery of the anaesthetized dog cause dose-dependent hepatic arterial vasoconstriction (Andrews, Hecker, Maegraith & Ritchie, 1955; Richardson & Withrington, 1976a); when administered by injection into the hepatic portal vein they cause portal venous vasoconstriction although the responses to angiotensin show marked tachyphylaxis (Richardson & Withrington, 1977). Vasopressin, whilst causing profound dose-dependent vasoconstriction of the hepatic arterial vascular bed (Richardson & Withrington, 1976a) has little effect on the resistance sites of the portal system except with very high doses when it causes a reduction in portal vascular resistance (Richardson & Withrington, 1977). Quantitative and qualitative differences in the

responses of the hepatic arterial and portal vascular beds to naturally-occurring substances are therefore evident.

Bradykinin, 5-hydroxytryptamine (5-HT) and histamine all occur naturally, and whilst not normally present in the systemic circulation in vasoactive amounts, they may enter the liver in the portal vein having been released from the intestine, pancreas or spleen. In addition, they may be synthesized within the liver and thereby reach the sites controlling the hepatic arterial and portal vascular resistance. Furthermore, in various pathological states (e.g. anaphylaxis, shock) they may be released from the gut in such quantities as to enter the systemic circulation in vasoactive amounts. In such circumstances therefore, these substances may enter the liver by both the hepatic arterial and portal venous routes.

In the present experiments bradykinin, 5-HT and histamine were administered in increasing graded doses into the hepatic arterial and hepatic portal venous beds when these were separately perfused, in different experiments. The possible physiological implications of the vascular responses to these substances at both sites in the control and regulation of total liver blood flow are discussed.

In addition, by the use of the selective histamine H_1 and H_2-receptor antagonists, mepyramine and metiamide (Black, Duncan, Durant, Ganellin & Parsons, 1972; Black, Owen & Parsons, 1975), the nature of the receptors responsible for the effects of histamine has been examined.

Methods

Experiments were performed on a total of 17 dogs, weighing between 9.6 and 20.9 kg, which had been deprived of food, but allowed free access to water for 24 h before the induction of anaesthesia by an intravenous injection of methohexitone sodium (Brietal, Lilly: 7.5–12.5 mg/kg). Anaesthesia was maintained with chloralose (Kuhlmann, Paris: 50 mg/kg) and urethane (BDH: 500 mg/kg), intravenously, supplements of chloralose and urethane in the same proportions being given as necessary throughout the experiments to maintain a constant level of anaesthesia.

Two types of preparation were used: one ($n=7$) in which the common hepatic artery was perfused from a femoral artery and the hepatic arterial blood flow and perfusion pressure were monitored ('arterial preparations'), and the other ($n=10$) in which the hepatic portal vein was perfused at constant flow from a reservoir into which drained blood from the superior mesenteric vein; in these preparations ('portal preparations'), the hepatic portal vein perfusion pressure was monitored continuously. These preparations have been described previously (arterial: Richardson & Withrington, 1976b; portal: Richardson & Withrington, 1977) and only brief details of the surgical preparation are given here.

In all experiments, possible systemic effects of large doses of vasoactive agents injected locally to the liver were assessed by measurement of the systemic arterial blood pressure and heart rate. Systemic arterial blood pressure was measured from a cannulated femoral artery with a Statham P23Gb strain gauge transducer; phasic pressure was recorded throughout all experiments, and in some experiments, mean pressure derived with an averaging circuit with selectable time constants (Devices, model 3502). Heart rate was measured with a ratemeter (Devices, model 4521) triggered from the pulsatile systemic arterial pressure waveform.

The inferior vena caval pressure (IVCP) was measured at the level of the hepatic vein from a catheter advanced for a known distance through a cannulated femoral vein, using a Consolidated Electrodynamics L212 strain gauge transducer. The position of the catheter tip was confirmed *post mortem*.

All animals received 250 iu/kg heparin (Weddel Pharmaceuticals) immediately before cannulation of the blood vessels, and supplements of 100 iu/kg were given hourly. All cannula systems were primed with a solution of 10% low molecular weight dextran in normal saline (Rheomacrodex, Pharmacia).

Arterial preparations

Following a midline laparotomy, the common hepatic artery was separated from its periarterial nerves which were carefully preserved. The hepatic artery was cannulated and perfused from a femoral artery. The blood flow in this cannula system (hepatic arterial blood flow; HABF) was measured with a cannulating flow probe and Cardiovascular Instruments square-wave pulsed-field electromagnetic flowmeter (model 3765T), and the hepatic arterial perfusion pressure (PP) was measured from a 'T' piece in the cannula system close to the point of cannulation of the hepatic artery, using a Consolidated Electrodynamics L212 strain gauge transducer.

Hepatic arterial mean blood flow was derived by passing the phasic signal through an averaging circuit with a time constant of 0.6 second. Hepatic arterial mean perfusion pressure was derived by passing the phasic signal through an averaging circuit (Devices, model 3502) with selectable time constants of 0.5, 1 and 2 seconds. Both mean and phasic flow and pressure records were displayed continuously.

Portal preparations

After a midline laparotomy, the hepatic portal, superior mesenteric and splenic veins were cleared

from surrounding tissue. The splenic artery was tied and the splenic nerves stimulated supramaximally to expel stored erythrocytes into the circulation; the splenic vein was then tied and cannulated retrogradely, and the hepatic portal vein tied about 5 mm from the confluence of the superior mesenteric and splenic veins: in this way, the outflow from the superior mesenteric vein was diverted via the cannulated splenic vein into a small reservoir. The portal vein was cannulated towards the liver, and perfused with blood from the reservoir by means of a roller pump (Watson Marlow, MHRE-200). Hepatic portal venous perfusion pressure (HPVP) was recorded from a 'T' piece close to the point of cannulation of the portal vein with a Statham P23V strain gauge transducer. The hepatic portal inflow was monitored with a cannulating flowprobe on the outflow side of the roller pump, and a Cardiovascular Instruments electromagnetic flowmeter. Mean pressures and flows were derived as in the arterial preparations.

Calibrations and recordings

All pressure transducers were calibrated with mercury or water manometers before each experiment; zero positions were taken as those recorded with the catheter tips exposed to air *in situ, post mortem.* Cannulating flow probes were calibrated *in situ* with whole blood at the end of each experiment; occlusive flow zeros were established frequently throughout each experiment, in the portal preparations by diverting the blood flow through a bypass in parallel with the flow probe.

After appropriate amplification, all variables were recorded continuously on a Devices M-19 rectilinear recorder.

Calculations

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

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

Hepatic portal vascular resistance was calculated as (hepatic portal mean perfusion pressure − inferior vena cava pressure: mmHg)/(hepatic portal mean blood flow: ml/min or ml min^{-1} 100 g^{-1}) and expressed in the same units as the hepatic arterial vascular resistance.

Changes in vascular resistance were always calculated as percentage changes from control values to the peak of the effect of the vasoactive substance, i.e. (change in vascular resistance × 100)/(control vascular resistance).

Expression of results

Initial control values are expressed as means ± 1 s.d., and all other values are expressed as means ± s.e. means. The ED_{50} values represent the doses of substances which would produce 50% of the maximum effect attained by progressively increasing administrations of those substances.

In the arterial preparations, the arterial perfusion pressure normally remained constant, and therefore changes in calculated hepatic arterial vascular resistance (HAVR) indicate hepatic arterial vasoconstriction or vasodilatation. However, the injection of large doses of the vasoactive agents led to systemic vascular effects manifest as small changes in systemic arterial mean blood pressure; these were reflected in equivalent small changes in hepatic arterial perfusion pressure accompanying the large drug-induced changes in hepatic arterial blood flow. The myogenic and hydrostatic changes in vascular resistance arising from these changes in intravascular pressure *per se* (Bayliss, 1902; Folkow, 1964) are, in these preparations, negligible compared with the drug-induced changes in HAVR, whether vasoconstrictor (Richardson & Withrington, 1976a) or vasodilator (Richardson & Withrington, 1976b) drugs are administered: consequently, no correction factors have been applied for changes in HAVR resulting from alterations in perfusion pressure *per se.*

The portal preparations were perfused at constant flow, and the inferior vena caval pressure remained constant throughout (see Results section), so that changes in calculated hepatic portal vascular resistance (HPVR) indicate hepatic portal vasoconstriction or vasodilatation.

Drugs

The drugs used were: bradykinin triacetate (Sigma; mol. wt. = 1420.5), 5-hydroxytryptamine creatinine sulphate (5-HT, BDH; mol. wt. = 307.1), mepyramine maleate (M & B; mol. wt. = 401.5), metiamide (SK & F; mol. wt. = 244.4) and noradrenaline acid tartrate (Levophed, Winthrop; mol. wt. of base = 169.0). Doses are expressed in terms of the salts used except for noradrenaline where doses are expressed in terms of the base; molar concentrations were calculated from the molecular weights shown above, which are derived from the manufacturers' data. Bradykinin, 5-HT, histamine and mepyramine were dissolved in 0.9% w/v NaCl solution (saline), and metiamide solutions prepared as described by Black *et al.* (1975). All solutions were prepared fresh for each experiment.

Agonist drugs were injected directly into the cannula system, either intra-arterially (i.a.) or into the portal vein (intraportally). To construct dose-response curves, increasing doses of agonists were given, separated by at least 1 min from complete recovery from the effects of the preceding dose. Antagonists were administered intravenously (i.v.), mepyramine by injection, but metiamide, because of its comparatively short plasma half-life in the dog of 40–60 min (Black *et al.*, 1975; manufacturer's data), was infused intravenously for at least 10 min before any subsequent histamine administrations, and the infusion was continued throughout the period of the subsequent histamine administrations.

Results

Control values

(a) Hepatic arterial preparations. Seven dogs weighing between 10.3 and 15.3 kg (12.4 ± 3.2 kg) were used in these experiments; *post mortem*, the livers weighed 288.9 ± 54.2 grams. Under control conditions, the systemic arterial mean blood pressure was 126.3 ± 13.4 mmHg and the inferior vena caval pressure (IVCP) 1.8 ± 0.5 mmHg. The hepatic arterial blood flow was 219.1 ± 49.9 ml/min, or 77.1 ± 20.3 ml min^{-1} 100 g^{-1}; the hepatic arterial mean perfusion pressure (PP) was 114.9 ± 14.2 mmHg, giving a calculated hepatic arterial vascular resistance (HAVR) of 0.55 ± 0.13 mmHg ml^{-1} min, or 1.55 ± 0.34 mmHg ml^{-1} min 100 grams. These values are similar to those found previously for the sympathetically-innervated hepatic arterial vascular bed (Richardson & Withrington, 1976b,c,d).

(b) Hepatic portal preparations. The 10 dogs used in these investigations weighed between 9.6 and 20.9 kg (16.0 ± 4.3 kg), the livers weighing 328.1 ± 80.1 grams. Under control conditions, the systemic arterial mean pressure was 135.5 ± 13.0 mmHg and the inferior vena caval pressure 1.7 ± 0.98 mmHg. The hepatic portal inflow was 259.1 ± 83.7 ml/min or 78.7 ± 13.0 ml min^{-1} 100 g^{-1}, and at this inflow the hepatic portal perfusion pressure was 5.6 ± 1.86 mmHg. The calculated hepatic portal venous vascular resistance was 0.016 ± 0.007 mmHg ml^{-1} min, or 0.056 ± 0.022 mmHg ml^{-1} min 100 grams. These values are similar to those reported previously for this preparation from laboratory (Richardson & Withrington, 1977).

Effects of injections of bradykinin

(a) Hepatic arterial system. Bradykinin was injected into the hepatic artery over the dose range 10 pg to 100 ng on 6 occasions in 3 preparations. The only effect observed with doses above threshold was an increase in hepatic arterial blood flow of short duration. This response was graded and increased with increasing doses; very high doses of bradykinin were accompanied by small reductions in perfusion pressure. These increases in hepatic arterial blood flow represent dose-dependent reductions in calculated hepatic arterial vascular resistance and signify hepatic arterial vasodilatation.

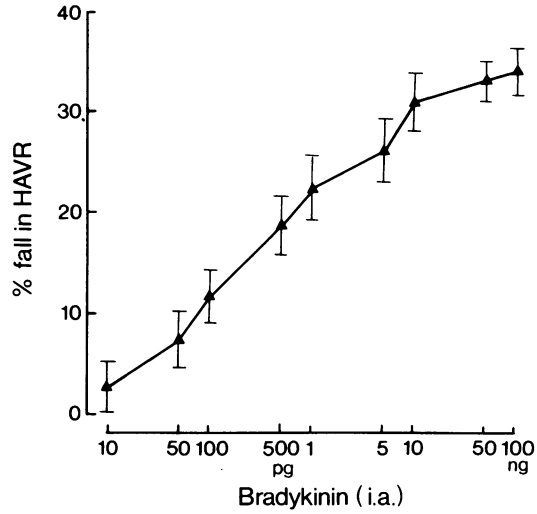

Figure 1 Log_{10} dose-response curve for bradykinin on the hepatic arterial vascular bed of the dog. The dose is expressed in terms of the weight of the salt injected intra-arterially (abscissa scale) and the response as the percentage fall in the calculated hepatic arterial vascular resistance (HAVR) (ordinate scale). The points represent the means of 6 observations and the vertical bars show the s.e. means.

The threshold dose of bradykinin when injected i.a. was either 10 or 50 pg, and the maximum reduction in calculated hepatic arterial resistance which occurred on injection of either 50 or 100 ng was 34.4 ± 2.4%; a value similar to the maximum reduction in hepatic arterial vascular resistance reported previously (Richardson & Withrington, 1976b) for the intra-arterial injection of a series of other vasodilator substances.

The log_{10} dose-response curve for intra-arterial injection of bradykinin on the hepatic arterial vascular bed is shown in Figure 1; the mean ED_{50} is 330 pg or 2.66×10^{-13} mol. The effects of maximal vasodilator doses of bradykinin on the hepatic arterial blood flow, hepatic arterial perfusion pressure and the calculated hepatic arterial vascular resistance are shown in Table 1.

(b) Hepatic portal venous vascular system. Bradykinin was injected intraportally in

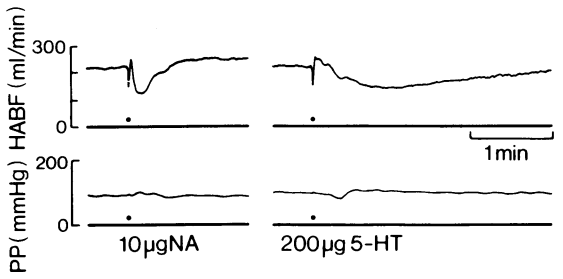

Figure 2 Time courses of the responses of the hepatic arterial vascular bed to intra-arterial injections of 5-hydroxytryptamine (5-HT) and noradrenaline (NA). HABF = hepatic arterial blood flow, PP = hepatic arterial mean perfusion pressure.

doses between 1.0 ng and 10 μg once in each of 3 experiments. No detectable change in hepatic portal perfusion pressure resulted from any of these injections (Figure 4); the hepatic portal blood flow and IVCP remained constant. There was, therefore, no change in the calculated portal vascular resistance to any of the doses of bradykinin used in this series. Doses in excess of 500 ng caused reductions in systemic arterial pressure, presumably due, at least in part, to systemic vasodilatation; these observations confirmed the biological activity of the solution used.

Effects of injections of 5-hydroxytryptamine

(a) Hepatic arterial system. 5-HT was injected intra-arterially over the dose range 500 ng to 500 μg to construct 6 dose-response curves in 5 preparations: at doses above the threshold, there were usually decreases in hepatic arterial blood flow of long duration. The hepatic arterial perfusion pressure either remained constant or increased slightly with the higher doses of 5-HT, and consequently the reductions in hepatic arterial blood flow represent increases in the calculated hepatic arterial vascular resistance, and hepatic arterial vasoconstriction. The threshold varied between 0.5 and 10 μg in different preparations, and the maximum rise in calculated HAVR occurred at doses between 50 and 500 μg: a typical response is shown in Figure 2 and the results are summarized in Figure 3. The effects of maximal vasoconstrictor doses of 5-HT on hepatic arterial perfusion pressure, blood flow, and calculated HAVR are shown in Table 1.

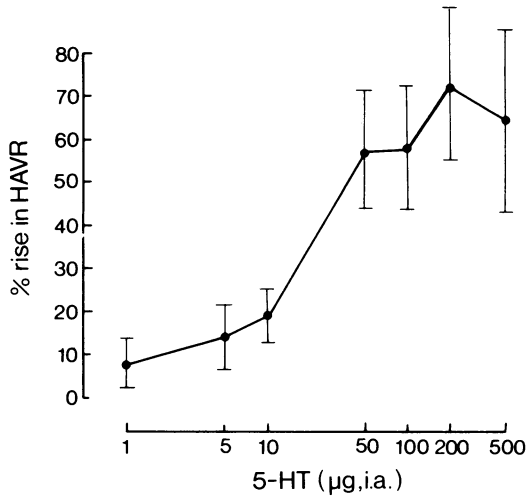

Figure 3 Log_{10} dose-response curve for 5-hydroxytryptamine (5-HT) on the hepatic arterial vascular bed of the dog. The dose is expressed in terms of the weight of the salt injected intra-arterially (abscissa scale), and the response as the percentage rise in the calculated hepatic arterial vascular resistance (HAVR) (ordinate scale). The points represent the means of 5 observations, and the vertical bars show the s.e. means.

In 2 experiments there was an apparent small

Table 1 Hepatic arterial perfusion pressure (PP), blood flow (HABF) and calculated hepatic arterial vascular resistance (HAVR) of the dog immediately before, and at the peak of responses to maximal doses of bradykinin and 5-hydroxytryptamine

PP (mmHg)		*HABF (ml min^{-1} 100 g^{-1})*		*HAVR (mmHg ml^{-1} min 100 g)*	
Control	*Peak*	*Control*	*Peak*	*Control*	*Peak*
Bradykinin (n = 6)					
111.8 ± 4.2	104.3 ± 4.0	56.0 ± 4.6	79.6 ± 5.6	2.06 ± 0.18	1.35 ± 0.13
5-hydroxytryptamine (n = 5)					
94.6 ± 6.7	97.2 ± 8.1	76.1 ± 11.1	45.0 ± 8.5	1.38 ± 0.27	2.57 ± 0.64

Each value is the mean ± s.e. mean immediately before (Control) and at the peak of the responses to maximally effective doses of the two substances (Peak). The number of dose-response curves from which the data were derived is shown in parentheses.

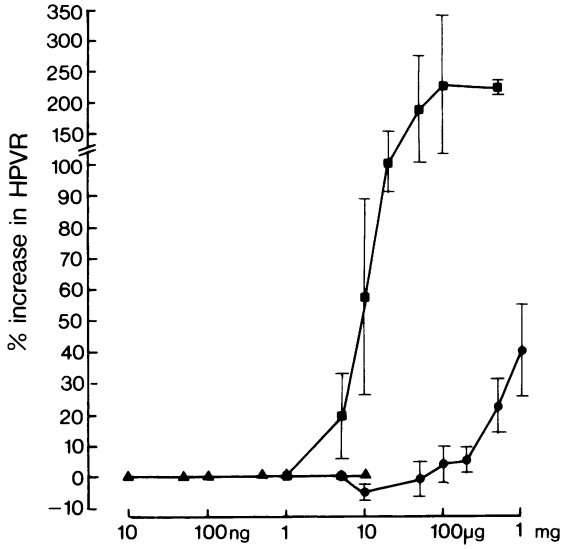

Figure 4 Log_{10} dose-response curves for bradykinin (▲), histamine (■) and 5-hydroxytryptamine (●) on the hepatic portal venous vascular bed of the dog. The doses are expressed in terms of the weight of each substance injected intraportally, and the response as the percentage rise in the calculated hepatic portal vascular resistance (HPVR). The symbols represent the mean observations (for bradykinin, $n=3$; for histamine, $n=5$; for 5-HT, $n=5$), and the vertical bars show the s.e. means.

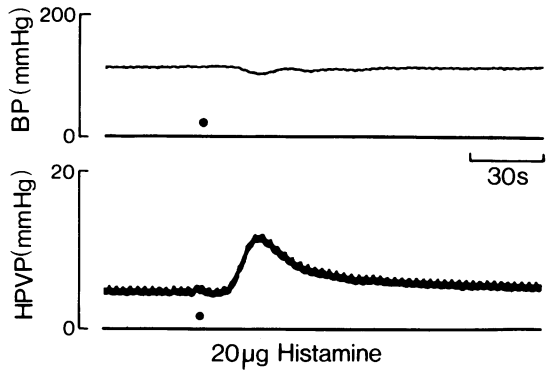

Figure 5 Response of the hepatic portal vascular bed to an intraportal injection of histamine. BP = mean systemic arterial blood pressure. HPVP = hepatic portal venous perfusion pressure. Point of injection shown by circles. Portal venous vascular bed perfused at constant blood flow (264 ml/min) and constant inferior vena caval pressure (1.5 mmHg).

transient increase in hepatic arterial blood flow prior to the main dose-dependent decrease; this initial increase in blood flow was not clearly separable from the injection artifact, and was not dose-dependent.

Hepatic arterial vasoconstriction to 5-HT was weak, and variable between experiments: the maximum increase in HAVR of $80.6 \pm 13.7\%$ was much smaller than the maximum increase ($365.4 \pm 120.9\%$) in HAVR to noradrenaline in the same preparations. The form and time-course of responses to 5-HT also differed from that of other vasoconstrictor agents in that the effect was of slow onset and long duration compared with that of noradrenaline (Figure 2), adrenaline, angiotensin and vasopressin (Richardson & Withrington, 1976a & unpublished observations).

(b) Hepatic portal venous vascular system. 5-HT was injected into the portal vein in doses from 1.0 μg to 1.0 mg on one occasion in each of 6 preparations. In 4/6 preparations, the lower doses within this range caused very small decreases in calculated hepatic portal vascular resistance (HPVR) and higher doses caused increases in HPVR. In the other 2 preparations, only increases in HPVR were seen with doses above threshold. Over the 6 experiments, the lowest dose to cause an increase in hepatic portal vascular resistance was either 50 or 100 μg. The mean effects from all six preparations are shown in Figure 4 which reveals a dose-dependent effect of reductions in HPVR at low doses and increases in HPVR at high doses of 5-HT. The time course of the effects was similar to that of other agents which increase the hepatic portal vascular resistance (Richardson & Withrington, 1977).

Effects of injections of histamine

(a) Hepatic arterial system. By intra-arterial injection, histamine causes dose-dependent reductions in the calculated hepatic arterial vascular resistance (Figure 6); the time course of these effects and relative molar potency of histamine compared with other vasodilator agents have been published previously (Richardson & Withrington, 1976b).

(b) Hepatic portal venous vascular system. Intraportal injections of between 1 and 500 μg of histamine were made once in each of 5 preparations in the absence of antagonists. All doses of histamine above the threshold caused rises in hepatic portal perfusion pressure, which at constant flow and IVCP represent increases in calculated hepatic portal vascular resistance. These effects were dose-dependent, and the effects of the smaller doses were without significant systemic effects though at higher doses, reductions in systemic arterial blood pressure and rises in heart rate occurred due to histamine passing through the liver into the systemic circulation in vasoactive amounts: in these cases, the changes in portal perfusion pressure

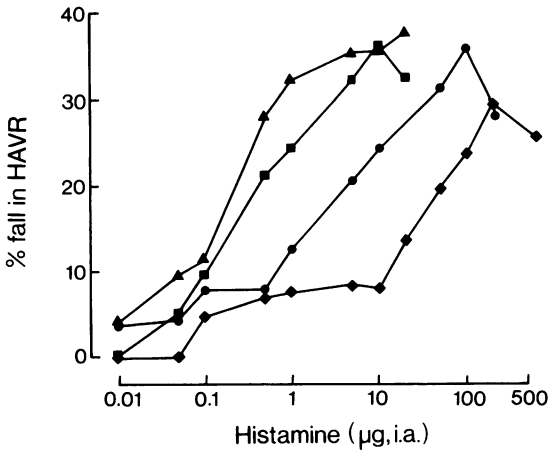

Figure 6 Log_{10} dose-response curves for histamine on the hepatic arterial vascular bed of the dog. The doses are expressed in terms of the weight of the salt injected intra-arterially, and the responses as the percentage fall in calculated hepatic arterial vascular resistance (HAVR). (■) Effects of histamine alone, in the absence of antagonists; (▲) during the infusion of 2.0×10^{-6} mol kg^{-1} min^{-1} metiamide, i.v.; (●) after mepyramine, 2.5×10^{-6} mol/kg, i.v.; (◆) after mepyramine, 5.0×10^{-6} mol/kg, i.v.

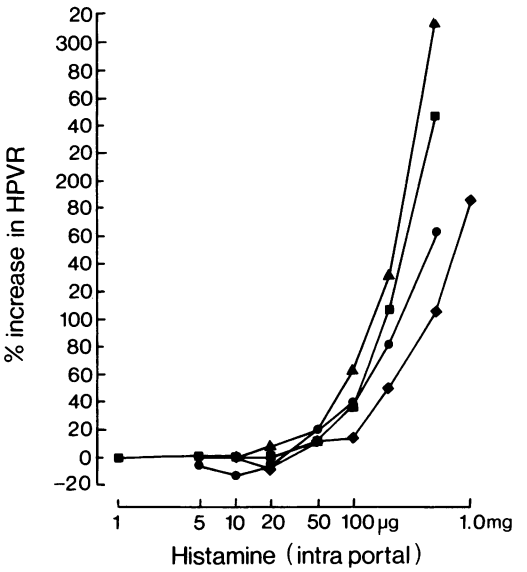

Figure 7 Log_{10} dose-response curves for histamine on the hepatic portal venous vascular bed of the dog. The doses are expressed in terms of the weight of the salt injected intraportally, and the responses as the percentage rise in the calculated hepatic portal venous vascular resistance (HPVR). Symbols and doses of antagonists as in Figure 6.

preceded the systemic effects (Figure 5). The complete dose-response curve is shown in Figure 4.

Histamine receptors in the canine liver vasculature

Dose-response curves to locally administered histamine were constructed in separate arterial and portal preparations in the absence of antagonists and subsequently both during metiamide infusions and after injections of mepyramine. The antagonists were administered in both orders in different preparations, the doses being selected from the observations of Black *et al.* (1975) which preliminary experiments showed to be effective.

(a) Hepatic arterial system. In one experiment, the intravenous infusion of metiamide, 2.0×10^{-6} mol kg^{-1} min^{-1}, which was without effect on the calculated hepatic arterial vascular resistance did not antagonize the effects of i.a. histamine on this vascular bed; indeed, during the metiamide infusion, there was a slight shift of the histamine dose-response curve to the left, with no effect on the maximum response (Figure 6). Mepyramine was then injected in a dose of 2.5×10^{-6} mol/kg (1 mg/kg) i.v., similarly resulting in no change in the calculated HAVR, but the subsequent dose-response curve to histamine showed a marked parallel shift to the right without suppression of the maximum response. A second i.v. injection of the same dose of mepyramine caused a further shift of the histamine dose-response curve to the right, though at this dose of mepyramine (total 5.0×10^{-6} mol/kg, i.v.), there was some suppression of the maximum response to histamine (Figure 6).

Paired analysis of the effects of the various doses of histamine injected in this procedure revealed that, over the whole dose ranges used, the increased effect of histamine after metiamide ($P < 0.005$), and the reduced effects of histamine after 2.5×10^{-6} mol/kg mepyramine ($P < 0.05$) and 5.0×10^{-6} mol/kg mepyramine ($P < 0.02$) were all statistically significant. In contrast, in the absence of antagonists, variations in the form of position of the dose-response curves to histamine in individual experiments were very small, and identical analysis to that used above revealed that the differences between dose-response curves with the same time interval were not statistically significant ($P > 0.70$; Richardson & Withrington, unpublished observations).

In another experiment, mepyramine 2.5×10^{-6} mol/kg was injected i.v. between the construction of two histamine dose-response curves, causing a marked parallel shift of the histamine dose-response curve to the right without suppression of the maximum response. The ED_{50} before mepyramine was 4.6×10^{-9} mol (1.4 μg) and after mepyramine was

increased more than tenfold to 6.5×10^{-8} mol (20.0 μg). In the presence of mepyramine, a subsequent infusion of metiamide (2.0×10^{-6} mol kg^{-1} min^{-1}, i.v.) caused no further antagonism of the effects of histamine; indeed, there was a small parallel shift of the histamine dose-response curve to the left ($ED_{50} = 3.3 \times 10^{-8}$ mol or 10.1 μg). Paired analysis of the effects of the various doses of histamine used in this procedure revealed that the antagonism by mepyramine significantly attenuated the vasodilator effects of histamine ($P < 0.005$), but that the subsequent effect of metiamide did not significantly affect the responses to histamine ($P > 0.05$).

(b) Hepatic portal venous vascular system. Identical procedures to those used in the arterial preparations were repeated for the portal preparations. Metiamide, 2.0×10^{-6} mol kg^{-1} min^{-1}, i.v., caused a small parallel shift of the histamine dose-response curve to the left, whilst subsequent injections of mepyramine i.v. to total doses of 2.5 and 5.0×10^{-6} mol/kg resulted in successive parallel shifts of the histamine dose-response curve to the right (Figure 7). In a further experiment, mepyramine in the absence of metiamide caused a parallel shift of the histamine dose-response curve to the right, and a subsequent infusion of metiamide (2.0×10^{-6} mol kg^{-1} min^{-1}, i.v.) caused no further shift to the right of the histamine dose-response curve.

The effects of histamine in causing reductions in hepatic arterial vascular resistance and increases in hepatic portal venous vascular resistance are therefore attenuated by mepyramine but not by metiamide.

Discussion

The two preparations used in the present series of experiments were identical to those described previously from this laboratory for the investigation of the responses of both the hepatic arterial and hepatic portal venous vascular beds of the dog to injections of a range of vasoactive compounds (Richardson & Withrington, 1976b,c; 1977). The control values for hepatic arterial and hepatic portal vascular resistances agree well with those figures previously reported. In the present paper the effects of three naturally-occurring autacoids, bradykinin, 5-HT and histamine, administered in different preparations to the hepatic arterial and portal vascular territories were examined.

All intra-arterial doses of bradykinin above the threshold caused a dose-dependent reduction in hepatic arterial vascular resistance. On a molar basis, it was the most potent dilator of the hepatic arterial vascular bed yet examined; the ED_{50} was 2.66×10^{-13} mol compared with 2.62×10^{-11} and 5.82×10^{-11} mol for secretin and prostaglandin E_2 respectively which were the most potent agents previously examined (Richardson & Withrington, 1976b). In contrast to its action on the arterial bed, bradykinin was without any direct action on the hepatic portal vascular bed even at a dose 100 times that which was maximal on the arterial system. These experiments confirm and extend the previous observations of Scholtholt & Shiraishi (1968) that intra-arterial or intraportal infusions of bradykinin cause increases in canine hepatic arterial blood flow, but are without measurable effect on the portal venous vascular bed.

Under normal circumstances, bradykinin is unlikely to attain vasoactive levels in the systemic arterial circulation because of its rapid inactivation in the lungs (Vane, 1969) and blood (Douglas, 1975). However, its synthesis and release (Seki, Nakajima & Erdös, 1972) is suspected to occur in many pathological conditions including gastrointestinal tract disorders such as the carcinoid and dumping syndromes, septic shock, pancreatitis and may explain some of the symptoms following regional hypotension consequent to occlusion of the superior mesenteric artery (Berry, Collier & Vane, 1970; Haglund, Hultén, Åhren & Lundgren, 1975; Lundgren, Hagland, Isaksson & Abe, 1976).

Under these circumstances, with high circulating levels of the polypeptide, both hepatic inflow circuits would be influenced, since in addition to hepatic arterial vasodilatation, bradykinin could increase intestinal (Fasth & Hultén, 1973), gastric (Fasth & Martinson, 1973) and splenic (Moerman, Scapagini & de Schaepdryver, 1969; B.N. Davies & P.G. Withrington, unpublished observations) blood flow resulting in a substantial increase in the inflow into the portal vein.

The bulk of the body's store of 5-HT is in the enterochromaffin and related cells of the gastro-intestinal tract (Erspamer, 1954; Gabella & Juorio, 1973; Douglas, 1975) and it is known that the dog small intestine releases 5-HT into the portal vein in response to a range of injurious stimuli (Zweifach, 1962; Burks & Long, 1966). Pathologically, 5-HT together with other hormones, may enter the portal vein, and therefore the liver, from secreting tumours of the pancreas and gastro-intestinal tract (Janoff, Nagler, Baez & Zweifach, 1961; Owman, Hakanson & Sundler, 1973; Murray-Lyon, Rake, Marshall & Williams, 1973); the avidity with which the lungs take up 5-HT is so great that it is unlikely that any enters the systemic circulation from such sources in amounts to cause cardiovascular effects. In addition, amine-secreting tumours are found outside the gastro-intestinal tract and 5-HT may be liberated in large amounts into the general circulation. The present experiments establish the complete dose-response relationship for 5-HT on the hepatic arterial vascular bed and demonstrate that the amine is weakly vasoconstrictor. Similarly the predominant action of 5-HT on the portal vascular bed was weakly vasoconstrictor; but the lower doses (< 100 μg) in some experiments caused a reduction in the portal

vascular resistance. 5-HT was considerably less potent in increasing portal vascular resistance than adrenaline, noradrenaline (Richardson & Withrington, 1977) or histamine but more potent than bradykinin. Confirming previous observations (Andrews & Butterworth, 1958), the responses to 5-HT of both the arterial and portal beds were more variable than those to any substance hitherto investigated. This variability in response to 5-HT may depend upon the prevailing sympathetic vasoconstrictor tone (Page, 1957; Haddy, Gordon & Emmanuel, 1959; McCubbin, Kaneko & Page, 1962; Davies & Withrington, 1973) and the route by which the amine is administered (Biber, Fara & Lundgren, 1973; Richardson, 1974).

Histamine has a wide distribution throughout the gastrointestinal tract, its associated organs and liver (Best, Dale, Dudley & Thorpe, 1927). It is the only substance investigated to date that has profound and qualitatively different effects on the hepatic arterial and hepatic portal vascular resistance. The molar potency and time-course of the vasodilator action of histamine on the hepatic arterial bed have been discussed previously (Richardson & Withrington, 1976b). The present experiments confirm previous observations of the effect of histamine in increasing portal vascular resistance (Greenway & Oshiro, 1973) and reveal that quantitatively it is as potent as adrenaline and noradrenaline at this site (Richardson & Withrington, 1977). The conclusion of Greenway & Oshiro (1973) that the main site of action of histamine when causing a rise in portal pressure in the cat, was by outflow block from the liver is supported by the results of work on isolated preparations of portal or mesenteric vein (Hughes & Vane, 1967; Northover, 1967; Greenway & Oshiro, 1973) and hepatic vein (Greenway & Oshiro, 1973); these reveal that the contraction of the portal vein by histamine is weak compared with that induced by noradrenaline, whilst the two substances are about equipotent on the hepatic vein. It is not possible, using the preparations described in this paper, to ascribe the effects of histamine to any particular part of the series-coupled intra- or extra-hepatic circulation, but the potency of histamine in elevating the portal venous pressure, possibly by outflow block, raises the possibility that histamine when injected intra-arterially may reach both the inflow and outflow resistance sites. This would lead not only to relaxation of the arterial resistance vessels, but also to an increase in outflow resistance. The net effect of such actions would be to decrease the pressure drop along the arterial vascular circuit, thereby reducing the fall in calculated hepatic arterial vascular resistance resulting from the intra-arterial injection of histamine.

Since histamine is destroyed by circulating histaminases it is unlikely to reach vasoactive concentrations in the systemic circulation under normal physiological conditions. However, in conditions of shock and anaphylaxis (Kahlson & Rosengren, 1971) systemic histamine levels could rise and provoke cardiovascular reactions. Hepatic arterial vasodilatation would be accompanied by vasodilatation of the gastric and intestinal (Texter, Chou, Merrill, Laureta & Frolich, 1964) and splenic beds (Davies & Withrington, 1973) leading to increased portal inflow; if histamine also induced an increase in hepatic outflow resistance this might lead to hepatic congestion and a rise in portal pressure.

The existence of two populations of histamine receptors, H_1 and H_2, in the peripheral vasculature of dogs and cats has been recognized for some time (Folkow, Haeger & Kahlson, 1948; Black *et al.*, 1972; Black *et al.*, 1975; Owen, 1975) and in the present experiments we have examined the histamine receptors that mediate the different vascular responses to histamine in the hepatic arterial and hepatic portal venous beds of the dog. In the dog, metiamide, in the absence of mepyramine, does not antagonize the depressor responses to i.v. histamine although if given after mepyramine it causes a further antagonism of these responses (Black *et al.*, 1975). A similar pattern of antagonism to the vasodilator effects of histamine has been reported in the femoral and superior mesenteric arterial vascular beds of the cat (Flynn & Owen, 1975).

In the present series of experiments, we have used the same doses of antagonists as Black *et al.* (1975) found effective in the dog, and we always observed a parallel and significant shift of the histamine dose-response curve to the right after mepyramine for both hepatic arterial vasodilation and hepatic portal vasoconstriction, without suppression of the maxima. This represents strong evidence for the existence of a population of histamine H_1-receptors mediating the effects in the two hepatic vascular beds. However, we found no displacement to the right of the histamine dose-response curves of the hepatic arterial or portal vascular beds during infusions of metiamide, whether these were made before or after mepyramine. In contrast, at both sites, we observed small shifts of the histamine dose-response curves to the left of the controls which might be attributable to the existence of histamine H_2-receptors in both vascular beds which, on stimulation, oppose the actions of H_1-receptor activation. The conclusion from the present experiments is that, as in the saphenous vein of the dog (Powell & Brody, 1976), in both the hepatic arterial and portal venous vascular beds of the dog, histamine H_1-receptors are predominantly responsible for the vascular effects of histamine, which are vasodilatation in the hepatic arterial bed, and vasoconstriction in the hepatic portal vascular bed.

In the present experiments the separate effects of bradykinin, 5-HT and histamine on the hepatic arterial and portal vascular beds have been examined. The evidence suggests that their independent physiological release would not significantly influence the total blood flow or its distribution through the liver.

However, elevated portal or systemic levels of histamine, bradykinin and 5-HT could by themselves evoke marked alterations in liver blood flow and its distribution between the arterial and portal circuits. In pathological circumstances it is unlikely that these substances are released in isolation; more probably they are released as a group together with other vasoactive substances (Lefer, 1973; Douglas, 1975). Interactions between bradykinin, 5-HT and histamine and other vasoactive materials released concomitantly (e.g. prostaglandins, glucagon, catecholamines) which are known to be highly active on the liver vasculature (Richardson & Withrington, 1976a,b,c,d; 1977) could lead to significant changes in liver perfusion.

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