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THE EFFECTS OF GLUCAGON, SECRETIN, PANCREOZYMIN AND PENTAGASTRIN ON THE HEPATIC ARTERIAL VASCULAR BED OF THE DOG

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

2 Commercial preparations of secretin, pancreozymin, glucagon and pentagastrin were administered by intra-arterial (i.a.) injection and infusion.

3 Secretin and pancreozymin by injection caused dose-dependent hepatic arterial vasodilatation, and on a molar basis were both more potent than glucagon or pentagastrin.

4 Intra-arterial infusions of secretin and pancreozymin caused hepatic arterial vasodilatation at calculated blood concentrations close to those measured under physiological conditions by other investigators. The vasodilatation was of the same duration as that of the hormone infusions.

5 Pentagastrin by i.a. injection caused dose-dependent hepatic arterial vasodilatation; by i.a. infusion, vasodilatation occurred but there was marked 'escape' from the effects during the continued infusion.

6 As reported previously, glucagon by injection caused dose-dependent hepatic arterial vasodilatation of long duration; by infusion, glucagon caused vasodilatation that persisted after the cessation of the infusion.

7 Glucagon infused i.a., inhibited the vasoconstrictor effects of i.a. noradrenaline, over the same range of infusions that caused hepatic arterial vasodilatation.

8 Secretin or pancreozymin did not antagonize the effects of noradrenaline on the hepatic arterial vascular bed at any doses used.

9 Pentagastrin did not antagonize the vasoconstrictor effect of noradrenaline whether hepatic arterial vasodilatation resulted from the pentagastrin infusion, or not.

10 These results are discussed with respect to the possible control of the hepatic arterial vascular bed by gastrointestinal hormones.

Introduction

The four gastrointestinal hormones pancreozymin, gastrin, secretin and glucagon are secreted from their sources into the portal vein to enter the liver. After passage through the liver and cardiopulmonary circuit they enter the systemic arterial system where, by distribution to the stomach and pancreas, they exert their established actions as secretagogues. Once again they enter the liver in the arterial supply.

Glucagon and secretin both evoke dose-dependent hepatic arterial vasodilatation (Richardson & Withrington, 1976b), and, in addition, glucagon inhibits the hepatic arterial vasoconstrictor actions of noradrenaline, angiotensin and vasopressin (Richardson & Withrington, 1976a), and of adrenaline and 5-hydroxytryptamine (Richardson & Withrington, unpublished).

The present experiments were carried out with three aims: first, to establish the effects of pancreozymin on the hepatic arterial vascular bed and its molar potency in relation to glucagon and secretin; second, to discover whether the hepatic arterial vasoconstrictor response to noradrenaline is affected by secretin and pancreozymin, as in the case of glucagon; and third, to investigate, in addition to the three naturally-occurring hormones, the vascular actions of a synthetic analogue of gastrin, pentagastrin.

The physiological implications of our findings will be discussed, particularly in relation to the reported blood levels of these hormones.

Methods

Experiments were performed on nine dogs weighing between 10.3 and 16.0 kg (12.3 ± 1.9 kg; mean ± s.d.)

which had not been fed for 24 h prior to the experiments, but had been allowed access to water *ad libitum* throughout this time. Anaesthesia was induced by sodium methohexitone (7.5–10 mg/kg i.v.; Brietal, Lilly) and maintained with α-chloralose (50 mg/kg i.v.; Kuhlmann, Paris) and urethane (500 mg/kg i.v., BDH), supplementary doses of chloralose and urethane, in the same proportions, being given as necessary to maintain a constant level of anaesthesia.

The preparations were essentially as described previously (Richardson & Withrington, 1976b, c). Following a midline laparotomy, the hepatic artery was dissected free from its periarterial sympathetic nerves, which were carefully preserved intact. The animals were heparinized (Weddel Pharmaceuticals; 250 iu/kg i.v., followed by 100 iu/kg hourly) and the common hepatic artery cannulated and perfused with blood from the cannulated left femoral artery. The blood flow in this cannula (hepatic arterial blood flow; HABF) was measured with a cannulated flowprobe and electromagnetic flowmeter (Cardiovascular Instruments) and the hepatic arterial perfusion pressure (PP) was measured from a 'T'-piece in the cannula, close to the point of cannulation of the hepatic artery, with a Consolidated Electrodynamics L221 strain gauge transducer. Additional 'T' pieces in this cannula system were used for the intra-arterial (i.a.) administration of vasoactive agents.

When the surgery was complete, the laparotomy incision was closed, and a thermometer inserted into the abdominal cavity; intra-abdominal temperature was maintained at 37–38°C with table heaters and heating lamps. Stable control variables were recorded for at least 20 min prior to the administration of vasoactive agents.

To monitor possible systemic effects of the vasoactive agents which were administered i.a. to the liver, systemic arterial blood pressure was measured from the cannulated right femoral artery with a Statham P23Gb strain gauge transducer, and the heart rate derived electronically with a Devices 4521 ratemeter.

Recording of variables

Hepatic arterial mean perfusion pressure (PP) was derived electronically with a Devices 3502 averaging circuit with time constants 0.5, 1.0 or 2.0 s selected appropriately. Both mean and phasic perfusion pressures were recorded continuously.

Hepatic arterial mean blood flow (HABF) was derived electronically by passing the phasic waveform through an averaging circuit with a time constant of 0.6 second. Both mean and phasic flow records were displayed continuously.

After appropriate amplification, all recordings were displayed continuously on a Devices M 19 rectilinear recorder.

Calculation of results

Liver weight was obtained immediately after each experiment. Values expressed per 100 g refer to this terminal weight of liver.

Hepatic arterial vascular resistance (HAVR) was calculated as hepatic arterial mean perfusion pressure (mmHg) divided by 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.

Changes in vascular resistance were calculated as percentage changes from the control values immediately prior to any procedure, i.e., change in vascular resistance × 100/control vascular resistance.

When the hormones were injected i.a. to the liver, there were large increases in hepatic arterial blood flow, which were accompanied by small reductions in perfusion pressure. The myogenic and hydrostatic changes in hepatic arterial vascular resistance which occur as a result of the reductions in perfusion pressure (Bayliss, 1902; Folkow, 1964) are insignificant compared with the direct effects of the vasoactive substances in these preparations (Richardson & Withrington, 1976a, 1976b).

Expression of results

Except where indicated to the contrary, results are expressed as means ± s.e. means.

Where the term ED_{50} is used, it indicates the dose of any substance which produced 50% of the maximum effect that could be obtained by progressively increasing i.a. injections of that substance.

The time courses of the responses to i.a. injections of the substances are expressed as the time taken for 50% recovery from the peak effects of selected doses of each substance ($T_{\frac{1}{2}}$). The doses selected for this analysis were just submaximal doses which produced about the same reduction in HAVR for each substance (Richardson & Withrington, 1976b).

The direct effects of i.a. infusions of the agents are expressed as the difference in HAVR during the infusions compared with the mean control vascular resistance before and after each infusion. The control vascular resistances after the infusions did not differ systematically from the control vascular resistances before the infusions, the mean post-infusion vascular resistance being 4.1 ± 2.2% lower than the pre-infusion value.

The influence of i.a. infusions on the responses of the hepatic arterial vasculature to i.a. injections of noradrenaline was also assessed. A test dose of noradrenaline (10.0 μg) which was selected on the basis of previous experiments (Richardson & Withrington, 1976a), produced a repeatable and pronounced increase in HAVR. At least 3 injections of noradrenaline were made before, during and after the infusions of glucagon, secretin, pancreozymin and

pentagastrin, and the mean increases in calculated HAVR due to noradrenaline were calculated for each period. The mean increase in HAVR due to the noradrenaline injections during the infusion was then compared to the mean effect of noradrenaline both before and after infusion, and the percentage difference calculated. A positive sign to this percentage difference indicates that the increase in HAVR due to the i.a. injection of noradrenaline during an infusion was greater than the increase in HAVR due to noradrenaline under control conditions, and *vice versa.*

Vasoactive substances

Since pure forms of the hormones are not available in adequate quantities for this type of investigation, commercially-available preparations have been used throughout. The substances used were: glucagon hydrochloride (Lilly), secretin (natural secretin, Boots), pancreozymin (natural pancreozymin, Boots) and the synthetic gastrin pentapeptide analogue, pentagastrin (Peptavlon, ICI). Doses of glucagon and pentagastrin are expressed in μg or ng, and doses of secretin and pancreozymin in Crick-Harper-Raper Units and milliunits (u, mu) where for secretin 1 unit is equivalent to 62.5 ng and for pancreozymin 1 unit is equivalent to 333 ng (manufacturer's data). Molar concentrations were calculated on the basis of molecular weights supplied by the manufacturers: glucagon, 3485; secretin, 3056; pancreozymin, 3883; pentagastrin 767.9. Test doses of (−)-noradrenaline (10.0 μg base; Levophed, Winthrop), were also used.

All vasoactive substances were dissolved in, or diluted from ampoules with 0.9% w/v NaCl solution (saline). Intra-arterial injections were made at a point between the flowprobe and the hepatic arterial cannula, resulting in an injection artifact which was clearly separable from the subsequent response of the preparation (Richardson & Withrington, 1976a, b). Intra-arterial infusions were from a Watson-Marlow MHRE200 pump precalibrated to deliver 1.0 ml/min against pressures in excess of the hepatic arterial perfusion pressure.

The volume of the external circuit was compensated for by an i.v. injection of a corresponding volume of low molecular weight dextran solution (Rheomacrodex, Pharmacia).

Results

Control values

Under control conditions in 9 animals, the hepatic arterial mean perfusion pressure (PP) was 115.3 ± 6.0 mmHg and the hepatic arterial blood flow (HABF) 195.2 ± 26.0 ml/min, giving a calculated

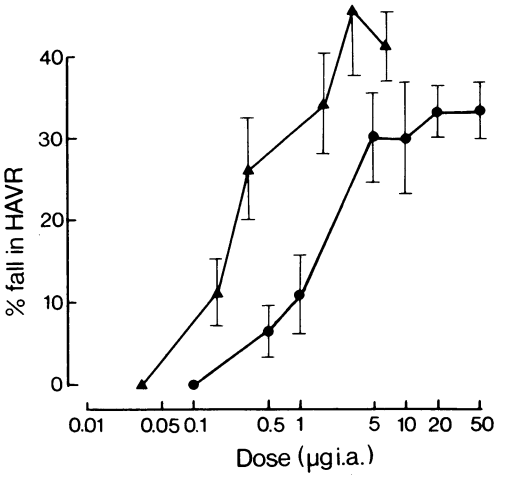

Figure 1 Log_{10}-dose/response curves for pentagastrin (circles) and pancreozymin (triangles). The doses (abscissa) are expressed in terms of the weight of the substances injected i.a. to the liver, and the responses (ordinate) are expressed as the percentage reduction in the calculated hepatic arterial vascular resistance (HAVR). The symbols represent the means of 4 (pancreozymin) or 5 (pentagastrin) injections; the vertical bars represent the s.e. means.

hepatic arterial vascular resistance (HAVR) of 0.68 ± 0.09 mmHg ml^{-1} min. The livers weighed 274.6 ± 43.5 (s.d.) grams, representing 2.23 ± 0.17 (s.d.) % of the weights of the dogs: expressed per 100 g, the HABF was 71.3 ± 9.5 ml min^{-1} 100 g^{-1} and the HAVR 1.83 ± 0.23 mmHg ml^{-1} min 100 g. The heart rate was 180.4 ± 13.4 beats/min. These values are similar to those reported previously for similar preparations (Richardson & Withrington, 1976b,c).

Intra-arterial injections of pancreozymin and pentagastrin

Pancreozymin. Pancreozymin was injected in increasing doses between 100 mu and 20 u on one occasion in each of 4 preparations; dose-dependent vasodilatation was the only effect observed above the threshold, which was 500 mu in each experiment (Figures 1 and 2). The maximum reduction in calculated HAVR of 47.9 ± 7.2% was attained on injection of either 10 or 20 u i.a. to the liver (Table 1). The effects of pancreozymin were rapid in onset, and of shorter duration than glucagon (Table 2).

Pentagastrin. Pentagastrin was injected i.a. to the liver in graded doses between 0.1 and 50.0 μg on one occasion in each of 5 preparations. Dose-dependent vasodilatation of slow onset was the only effect seen at doses above the threshold of 0.5 μg (Table 2). The

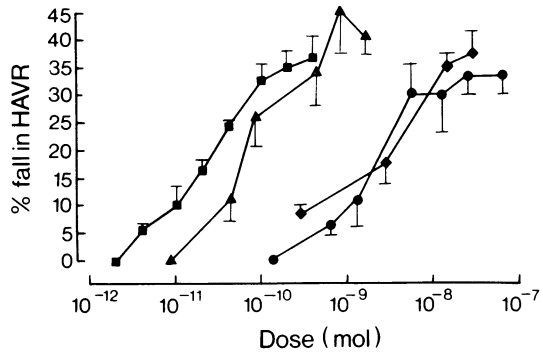

Figure 2 Log_{10}-dose/response curves for glucagon (diamonds), secretin (squares), pancreozymin (triangles) and pentagastrin (circles) injected into the hepatic artery of the anaesthetized dog. The doses are expressed as a fraction of one mole of each substance injected, and the responses are expressed as percentage reductions in calculated hepatic arterial vascular resistance (HAVR). The symbols represent the mean of 4 (glucagon and pancreozymin), 3 (secretin) or 5 (pentagastrin) injections in different experiments, and the vertical bars show the s.e. means.

maximum reduction in calculated HAVR was attained on injection of between 5 and 50 μg in different preparations; at these maximal doses, the HAVR fell by 35.2 ± 3.5%. The changes in PP, HABF and HAVR at maximal vasodilatation are shown in Table 1, and the dose/response relationship summarized in Figures 1 and 2.

Relative vasodilator potency of glucagon, secretin, pancreozymin and pentagastrin

The log_{10}dose/response curves for pancreozymin and pentagastrin, with the doses expressed as a fraction of one mole of the substances are shown in Figure 2, and the ED_{50} values and time courses of the recoveries from selected doses of the agents summarized in Table 2. In addition, the effects of glucagon and secretin, which have been reported previously (Richardson & Withrington, 1976b) are included for comparative purposes.

These results show that secretin and pancreozymin are very much more potent vasodilators of the canine hepatic arterial vascular bed than glucagon or pentagastrin, when administered by i.a. injection.

Intra-arterial infusions of glucagon, secretin, pancreozymin and pentagastrin

All four substances were infused into the hepatic artery in varying doses, to observe whether the resulting vasodilatation was maintained throughout the infusion period or prolonged beyond the cessation of the infusions. The resulting hepatic arterial blood concentrations were calculated by dividing the infusion rate (μg/min or u/min) by the HABF (ml/min), and these calculated blood concentrations are compared with peripheral blood concentrations of the hormones reported to occur physiologically (see Discussion section).

Glucagon. Glucagon was infused into the hepatic artery at rates of 0.5, 1.0, 5.0 and 10.0 μg/min (Table 3). At each dose level, there was a sustained decrease in the calculated HAVR due to a substantial increase in HABF with very small reductions in perfusion pressure. The reductions in HAVR are related to both the infusion rate, and the resulting blood concentrations (Table 3, Figures 4 and 5).

The reduction in HAVR on infusion of glucagon was rapid in onset and of very protracted duration, extending beyond the cessation of the infusions by several min, in contrast to the effects of the other substances (Figure 3).

Table 1 Hepatic arterial perfusion pressure (PP), blood flow (HABF) and calculated hepatic arterial vascular resistance (HAVR) immediately before, and at the peak of responses to maximal vasodilator doses of pancreozymin and pentagastrin

	PP	*PP*	*HABF*	*HABF*	*HAVR*	*HAVR*
	(mmHg)		*(ml min^{-1} 100 g^{-1})*		*(mmHg ml^{-1} min 100 g)*	
	Control	*Peak*	*Control*	*Peak*	*Control*	*Peak*
Pancreozymin	93.3 ± 6.1	82.3 ± 6.3	56.9 ± 15.3	91.0 ± 14.7	2.09 ± 0.63	0.97 ± 0.15
Pentagastrin	100.2 ± 2.9	91.8 ± 3.7	71.3 ± 9.3	98.6 ± 11.6	1.54 ± 0.27	1.00 ± 0.14

Each value is the mean ± s.e. mean immediately before (Control) and at the peak of the responses to maximal vasodilator doses of the two substances. The data were derived from 4 experiments for pancreozymin and 5 for pentagastrin.

Secretin. Secretin was infused i.a. to the liver in doses of 0.5, 1.0 and 5.0 u/min (Table 5). At all doses, there was vasodilatation which was maintained throughout the period of the infusion, and which receded on cessation of the infusions (Figure 3). Even the lowest infusion of secretin, which produced a calculated hepatic arterial blood concentration of 124 pg/ml caused a reduction in HAVR of 2.3%; when 1 u/min was infused, there was a resulting hepatic arterial blood concentration of 382 pg/ml and a statistically significant ($P<0.005$) reduction in HAVR of $11.3 \pm 1.4\%$ (Table 3).

Pancreozymin. Pancreozymin was infused at doses of 0.2, 1.0 and 5.0 u/min to the liver on a total of 5 occasions (Table 3). These infusions resulted in calculated hepatic arterial blood concentrations of between 350 and 9260 pg/ml, and all caused reductions

Table 2 Maximum reductions in hepatic arterial vascular resistance (HAVR), ED_{50} values and times to half recovery from the peak effects of selected doses of each agent ($T_{\frac{1}{2}}$)

Substance	*Maximum fall in HAVR (%)*	*ED_{50} (ng)*	*ED_{50} (mol)*	*Test dose (μg)*	*$T_{\frac{1}{2}}$ to recovery (s)*
a. Glucagon ($n=4$)	37.1 ± 4.3	1120	3.21×10^{-9}	100	219.0 ± 16.3
b. Secretin ($n=3$)	37.1 ± 4.3	80	2.62×10^{-11}	1.25	19.8 ± 3.1
c. Pancreozymin ($n=4$)	47.9 ± 7.2	297	7.63×10^{-11}	1.67	25.3 ± 10.3
d. Pentagastrin ($n=5$)	35.2 ± 3.0	2500	3.25×10^{-9}	20	36.4 ± 2.5

All effects are shown as means ± s.e. means. The number of experiments from which the data were derived is shown in parentheses after the name of each substance. The data in sections a and b are from Richardson & Withrington (1976b).

Table 3 The effects of i.a. infusions of glucagon, secretin, pancreozymin and pentagastrin on hepatic arterial vascular resistance

Substance	*Infusion*	*Number of infusions*	*Blood concentration (ng/ml)*	*Reduction in HAVR (%)*
a. Glucagon	0.5 μg/min	1	1.66	3.2
	1 μg/min	4	6.00 ± 1.80	13.4 ± 4.4*
	5 μg/min	3	31.6 ± 9.0	28.0 ± 8.9*
	10 μg/min	5	59.5 ± 11.6	38.3 ± 7.4***
b. Secretin	0.5 u/min	1	0.12	2.3
	1 u/min	4	0.38 ± 0.10	11.3 ± 1.4***
	5 u/min	4	1.74 ± 0.24	34.0 ± 7.0**
c. Pancreozymin	0.2 u/min	2	0.50 (0.65, 0.35)	7.6 (7.4, 7.8)
	1 u/min	2	1.99 (1.79, 2.18)	18.9 (6.4, 31.3)
	5 u/min	1	9.26	57.7
d. Pentagastrin	1 μg/min	1	5.11	7.1 (1.8 ↗)
	5 μg/min	1	33.9	64.9 (9.6 ↗)
	10 μg/min	2	48.1 (61.2, 34.9)	34.9 (41.9, 27.9) (9.1 ↗ (11.5, 6.7) ↗)

Results are shown as means ± s.e. means for 3 or more infusions; for 2 infusions the mean and individual results are given and for 1 infusion, the single result is given. Because of the lack of maintenance of the effects of pentagastrin (see text), the initial and final (↗) effects are shown.
*=$P<0.05$; **=$P<0.01$; ***=$P<0.005$.

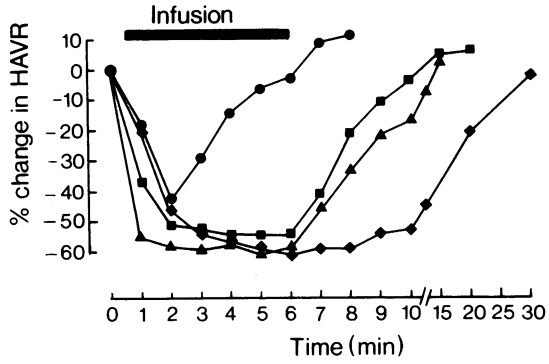

Figure 3 Time courses of the responses of the hepatic arterial vascular bed to i.a. infusions of glucagon (diamonds, 10 μg/min), secretin (squares, 5 u/min), pancreozymin (triangles, 5 u/min) and pentagastrin (circles, 10 μg/min). Abscissa scale: time in min, the period of the infusions is shown by the horizontal bars. Ordinate scale: % reduction in calculated hepatic arterial vascular resistance (HAVR).

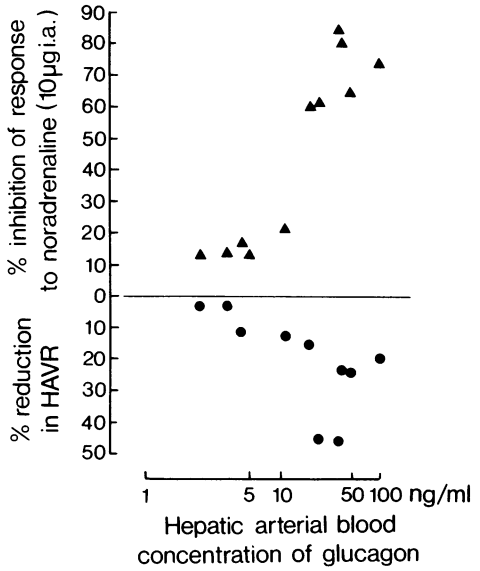

Figure 4 Relationship between calculated hepatic arterial blood concentration of glucagon resulting from i.a. infusions (abscissa scale) and (i) reduction in calculated hepatic arterial vascular resistance (HAVR, lower section) and (ii) % reduction in the vasoconstrictor response to i.a. injections of noradrenaline (upper section). Each point represents one glucagon infusion.

in calculated HAVR which were dose-dependent and maintained for the duration of the infusions (Figure 3).

Pentagastrin. Pentagastrin was infused into the hepatic artery in doses of 1.0, 5.0 and 10.0 μg/min on a total of 4 occasions. Dose-dependent reductions in HAVR occurred with each infusion (Table 3). In contrast to the effects of glucagon, secretin and pancreozymin, however, the hepatic arterial vasodilatation evoked by pentagastrin was not maintained, despite the continued infusions; the HAVR returned towards control levels during all infusions of pentagastrin (Figure 3), and in none of the 4 infusions did the reduction in HAVR at the end of the infusion exceed one third of the reduction attained at the beginning of the infusions (Table 3).

In summary, i.a. infusions of glucagon, secretin, pancreozymin and pentagastrin all evoked reductions in HAVR. The reductions due to secretin and pancreozymin were swift in onset and receded promptly on cessation of the infusions; the effects of glucagon extended beyond the end of the infusion periods, whilst the effects of pentagastrin were evanescent, receding despite the continued infusions.

Influence of intra-arterial infusions of glucagon, secretin, pancreozymin and pentagastrin on the responses to intra-arterial injections of noradrenaline

Intra-arterial injections and infusions of glucagon have previously been shown to antagonize the vasoconstrictor effects of i.a. injected noradrenaline, angiotensin, vasopressin (Richardson & Withrington, 1976a) and adrenaline and 5-hydroxytryptamine (Richardson & Withrington, unpublished observations), but not to antagonize the effects of i.a. administered vasodilator agents (Richardson & Withrington, 1976d). The present investigation was undertaken to examine the relationship between the hepatic arterial blood concentration of glucagon and the extent of the inhibition of the vasoconstrictor effects of i.a. injections of noradrenaline; and also to examine the likelihood of this antagonistic action of glucagon being peculiar to that hormone, or being an effect shared by the other vasodilator hormones secretin and pancreozymin, and by pentagastrin.

Vasoconstrictor effects of noradrenaline. The test dose of noradrenaline (10 μg) was injected i.a. to the liver on 124 occasions under control conditions in 35 groups of injections. These injections caused a mean increase in calculated HAVR of $147.5 \pm 7.7\%$ ($n = 35$), a value similar to that previously reported for the injection of this dose of noradrenaline into the sympathetically-denervated hepatic arterial vascular bed of the dog (Richardson & Withrington, 1967a).

Effect of glucagon on the response to noradrenaline. The relationship between the administered dose and the blood concentration of glucagon and (i) the reduction in HAVR and (ii) the percentage inhibition of the response to i.a. injections of noradrenaline is illustrated in Figures 4 and 5. The effects of the various infusions of glucagon are shown in Table 4: even the lowest infusion of glucagon which resulted in a calculated hepatic arterial blood concentration of 1.66 ng/ml caused inhibition of the response of the hepatic arterial vascular bed to noradrenaline; this inhibition was statistically significant when 1 μg/min was infused to 3 preparations, resulting in hepatic arterial blood concentrations of 5.8 ± 2.0 ng/ml. The inhibition of the vasoconstrictor responses to noradrenaline occurred over the same range of hepatic arterial blood glucagon concentrations as the reductions in HAVR (Figures 4 and 5).

Effect of secretin on the response to noradrenaline. Intra-arterial infusions of secretin, 1.0 and 5.0 u/min, which produced graded reductions in HAVR did not antagonize the effects of i.a. injected noradrenaline. Indeed, the vasoconstrictor effects of noradrenaline were apparently potentiated (Figure 5, Table 4). This effect is probably not a genuine potentiation since the noradrenaline was acting upon a more relaxed hepatic arterial vasculature during the secretin infusion than under control conditions. When 1.0 u/min of secretin was infused i.a., the resulting hepatic arterial blood concentration was 380.0 ± 100.4 pg/ml ($n=4$) and the response to noradrenaline 4.5 ± 4.3% greater than during control conditions.

Effect of pancreozymin on the response to noradrenaline. Intra-arterial infusions of pancreozymin were made at two dose levels to examine their effects on the vasoconstrictor responses of this vascular bed to noradrenaline. Two infusions of 200 mu/min resulted in a mean calculated hepatic arterial blood concentration of 499 pg/ml and an apparent potentiation of the vasoconstrictor response to noradrenaline of 6.4%; two infusions of 1 u/min resulted in a mean blood concentration of 1985 pg/ml

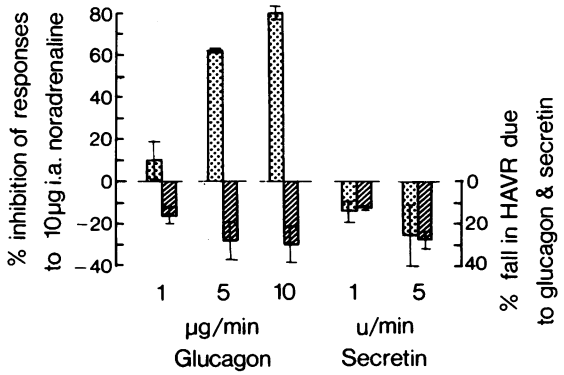

Figure 5 Differences in the response of the hepatic arterial vasculature to i.a. infusions of glucagon and secretin. Hatched bars show the % reduction in calculated hepatic arterial vascular resistance (HAVR) due to the infusions; stippled bars show the influence of the infusions on the response of the hepatic arterial vasculature to i.a. injections of noradrenaline (10 μg). Stippled bars above the baseline indicate that the effect of noradrenaline during the infusion was smaller than that during corresponding control periods. Bars represent the means of 3 infusions at each dose level and the lines within the main bars show the s.e. means

Table 4 The effects of i.a. infusions of glucagon and secretin on the responses of the hepatic arterial vasculature to i.a. injections of noradrenaline

Substance	*Infusion rate*	*Number of observations*	*Change in response to noradrenaline during infusion compared to the response under control conditions*
a. Glucagon	0.5 μg/min	1	−13.0%
	1 μg/min	3	−12.9 ± 3.4%*
	5 μg/min	3	−45.5 ± 11.1%*
	10 μg/min	3	−78.7 ± 5.3%**
b. Secretin	1.0 u/min	4	+ 4.5 ± 4.3%
	5.0 u/min	3	+23.4 ± 11.7%

Values are shown as means ± s.e. means except for individual values; * = $P<0.05$; ** = $P<0.0025$; unmarked values represent changes which are not statically significant ($P>0.05$).

and an apparent potentiation of the effect of noradrenaline of 11.9%. The pattern of the effects of pancreozymin on the vasoconstrictor responses of the hepatic arterial vasculature to noradrenaline is therefore very similar to that for secretin (Figure 5).

Effect of pentagastrin on the response to noradrenaline. Pentagastrin was infused into the hepatic artery in doses of 1, 5 and 10 μg/min and these infusions resulted in hepatic arterial blood concentrations of 5.11, 33.9 and 34.9 ng/ml respectively. When the hepatic arterial vasculature was dilated by pentagastrin (Figure 3), the vasoconstrictor responses to i.a. injections of noradrenaline in these three experiments were potentiated by 1.3, 7.3 and 16.6% respectively. Quantitative results were difficult to obtain with pentagastrin because of the very marked tendency of the direct vasodilator effect to wane despite the continued infusions; the apparent potentiation of the response to noradrenaline was only clear when the hepatic arterial vasculature was demonstrably dilated by the agent.

In summary, the antagonism of the vasoconstrictor effects of test doses of noradrenaline on the hepatic arterial vascular bed of the dog is peculiar to glucagon of the four agents examined. This antagonism by glucagon of the vasoconstrictor effect of noradrenaline has been shown to be dependent upon the hepatic arterial blood concentration (Figure 4) and infused dose (Figure 3) of glucagon.

Discussion

In previous experiments it has been shown that hepatic arterial injections or infusions of glucagon inhibit the hepatic arterial vasoconstriction produced by noradrenaline, angiotensin and vasopressin (Richardson & Withrington, 1976a) and adrenaline (Richardson & Withrington, unpublished). Furthermore the interaction of glucagon with noradrenaline or angiotensin is competitive in nature whilst that between glucagon and vasopressin is non-competitive. In the present series of experiments the dose range over which glucagon evokes hepatic arterial vasodilatation and inhibition of hepatic arterial vasoconstriction by noradrenaline has been examined down to threshold concentrations. There was a clear correlation between the two effects and they were not separable even at the lowest infusion rates of glucagon. The lowest arterial infusion of glucagon in the present experiments produced a calculated arterial blood concentration of 1.66 ng/ml and the test dose of arterial noradrenaline was inhibited by 13%; a calculated arterial concentration of 60.0 ng/ml glucagon caused an inhibition of over 75% in the arterial vasoconstriction to noradrenaline. All arterial concentrations of glucagon which antagonized the hepatic arterial vasoconstrictor effects of noradrenaline also evoked profound vasodilatation of the hepatic arterial bed (Figure 4).

The peripheral plasma concentration of glucagon has been measured by radioimmunoassay (RIA); in man and in monkeys, the normal fasting levels of glucagon in peripheral plasma fall within the range of 25–300 pg/ml (Rehfeld & Heding, 1970; Bloom, Daniel, Johnston, Ogawa & Pratt, 1973; Dudl, Lerner, Ensinck & Williams, 1973; Weir, Turner & Martin, 1973; Tasaka, Sekine, Wakatsuki, Ohgawara & Shizume, 1975) and in dogs, a resting plasma concentration of glucagon in the inferior vena cava of 101 ± 25 pg/ml ($n=4$) has been reported (Santeusanio, Faloona & Unger, 1973). There is, therefore, a clear discrepancy between the plasma levels of glucagon as measured by RIA which occur under physiological conditions, and the hepatic arterial blood concentrations which were calculated in the present experiments (i.e. 1–100 ng/ml) and which produce vasodilatation in the hepatic arterial vascular bed, concomitantly with inhibition of the vasoconstrictor effects of noradrenaline. The disparity between these levels may be due to a number of factors: the calculated concentrations do not take into account either the destruction of the hormone in the blood, or its deactivation or binding to a biologically inert form. A further possibility is that the part of the molecule of glucagon to which an antibody has been raised for purposes of the RIA estimation is not identical with that moiety responsible for the effects observed in the present experiments of hepatic vasodilatation and inhibition of hepatic vasoconstriction evoked by noradrenaline.

The prolonged time-course of action of injections of glucagon on the hepatic arterial bed has been commented on previously (Richardson & Withrington, 1976b); the present experiments reveal the same feature to be present after intra-arterial infusions of the hormone (Figure 3). The cumulative action inherent in this property means that considerably smaller amounts of the hormone may be present under physiological conditions and may cause a significant hepatic vasodilatation. In addition, other substances may be present, like secretin, pancreozymin and prostaglandins; these have profound hepatic arterial vasodilator properties themselves (Richardson & Withrington, 1976b) which are not antagonized by glucagon (Richardson & Withrington, 1976d).

Secretin has been shown to be a vasodilator of various peripheral vascular beds in the cat (Ross, 1970) and, in the dog, to be as potent as prostaglandin E_2 in evoking vasodilatation of the hepatic arterial vascular bed (Richardson & Withrington, 1976b) when administered into the hepatic artery by injection. In the present series, infusions of secretin were given i.a. in doses which, on calculation, produced blood concentrations of 125–2000 pg/ml. Vasodilatation

was observed at all doses and was maintained throughout the period of infusion.

In man, fasting secretin levels range between about 50 and 500 pg/ml of plasma (Bloom & Ogawa, 1973; Boden & Chey, 1973; Bloom, 1974; Henry, Flanagan & Buchanan, 1975). Feeding and the instillation of acid into the duodenum cause these levels to rise in man (Boden & Chey, 1973; Bloom & Ogawa, 1973; Henry *et al.*, 1975) and so it is not unreasonable to assume that if the haematocrit is about 50%, blood concentrations of 25–300 pg/ml of secretin fall within the physiological range. The two lower levels of infusion used in the present series would produce blood concentrations within this range. Therefore significant reductions in HAVR may be expected to accompany the physiological secretion of secretin. In contrast to the hepatic arterial infusions of glucagon, which significantly inhibit the hepatic vasoconstrictor responses to i.a. injections of noradrenaline, arterial infusions of secretin, whilst evoking comparable reductions in HAVR, increase the vasoconstrictor actions of noradrenaline. However, our experiments suggest that this increased responsiveness of the vessels to noradrenaline arises from a reduction in the background hepatic arterial vascular tone induced by the secretin infusion rather than a potentiation of noradrenaline by the secretin.

Pancreozymin was investigated for the first time in the present experiments. Injections into the hepatic artery produced dose-dependent vasodilatation of brief duration; its potency was only slightly less than secretin (Table 2). Infusions into the hepatic artery evoked concentration-dependent hepatic arterial vasodilatation which was maintained throughout the period of infusion and very quickly subsided after cessation of the infusion. Vasoconstrictor responses to i.a. injections of test doses of noradrenaline were increased during the infusion of pancreozymin, in a similar manner to that which occured during the infusions of secretin. Similarly, this increase in response is considered secondary to the vascular smooth muscle relaxing properties which caused the reduction in HAVR. Peripheral serum concentrations of pancreozymin in man have been reported to range between 25–60 pg/ml (Harvey, Dowsett, Hartog & Read, 1973; 1974) and as high as 4 ng/ml (Reeder, Becker, Smith, Rayford & Thompson, 1973). These levels rise to between 9 and 17 ng/ml after food containing fats (Harvey *et al.*, 1973; Reeder *et al.*, 1973). The calculated hepatic arterial blood concentrations in the present series of experiments (0.35–9.26 ng/ml) therefore fall well within the range of peripheral pancreozymin levels determined by RIA in man after meals. It therefore seems probable that significant reductions in HAVR are produced by pancreozymin released in response to physiological stimuli.

The various molecular fractions of gastrin which have been reported are not available in sufficient quantity to be used in this type of experiment. The physiological action of gastrin as a gastric secretogogue resides in the 5 amino acid sequence which is available as the commercial preparation pentagastrin. In the present experiments injections of pentagastrin evoked dose-dependent vasodilatation of the hepatic arterial bed of longer duration than either secretin or pancreozymin; its molar potency was less than either of these and close to that of glucagon (Table 2, Figure 2). In contrast to the three naturally-occurring hormones, the hepatic vasodilator activity of pentagastrin on i.a. infusion was not maintained, a reflection, perhaps, of its rapid deactivation by the liver (Thompson, Reeder, Davidson, Charters, Bruckner, Lemmi & Miller, 1969; Temperley, Stagg & Wyllie, 1971). Because of the rapid decline of the vasodilator response to pentagastrin any alteration of the hepatic arterial vasoconstrictor properties of noradrenaline was difficult to ascertain in these experiments. Nevertheless it was apparent that whilst an hepatic arterial vasodilatation was present due to pentagastrin the vasoconstrictor responses to noradrenaline were increased. This potentiation was as evanescent as the vasodilator response to pentagastrin and supports the conclusions drawn from the secretin and pancreozymin infusions that the effect is a secondary one accompanying a primary action relaxing the vascular smooth muscle.

The question arises of the physiological importance of these observations on the vascular effects of commercial preparations of the gastrointestinal hormones. A comparison of the calculated hepatic arterial blood concentrations of secretin and pancreozymin with the plasma or serum levels determined in other investigations indicates that a significant reduction in vascular resistance in the liver occurs due to their presence in the systemic arterial circulation following physiological stimuli which provoke their release, as in the small intestine (Fara, Rubinstein & Sonnenschein, 1972; Richardson, 1976). During digestion, secretin and pancreozymin would be released concomitantly; previous reports have demonstrated that mesenteric vasodilatation occurs after eating in dogs (Fronek & Stahlgren, 1968), and in cats, the release of gastrointestinal hormones, particularly secretin and pancreozymin, has been strongly implicated in the mediation of mesenteric vasodilatation resulting from the intraduodenal instillation of fat and other substances (Fara *et al.*, 1972).

There was, in the present experiments, a marked similarity between the effects, and the molar potencies, of secretin and pancreozymin. This may reflect an essentially similar vascular action of these structurally-related hormones, but in addition, the possibility that impurities in the preparations may have contributed to their vasoactivity arises. Fara (1975) has noted the possible contamination of Boots secretin with pancreozymin, and since on the isolated portal vein at least, secretin and pancreozymin cause

mutual potentiation of their effects (Fara, 1975), such contamination of one hormone with the other could have contributed to the apparent potency of these two preparations used in the present experiments.

The present experiments represent strong evidence that preparations of natural secretin and pancreozymin cause hepatic arterial vasodilatation which may well occur at physiological concentrations. The concomitant release of pancreatic glucagon might contribute further to hepatic arterial vasodilatation, and in addition, could protect the hepatic arterial vasculature from the vasoconstrictor effects of circulating adrenaline and angiotensin. Therefore, in addition to their well-established primary roles, the gastrointestinal hormones studied in this investigation may, by increasing HABF, participate in ensuring an adequate supply of oxygen to the liver parenchyma for all the biochemical reactions associated with post-absorptive liver function. This would be particularly important due to the greater requirements of the gastrointestinal tract, and it is at this time that the hepatic arterial blood concentrations of these hormones would be greatest.

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