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THE INHIBITION BY GLUCAGON OF THE VASO-CONSTRICTOR ACTIONS OF NORADRENALINE, ANGIOTENSIN AND VASOPRESSIN ON THE HEPATIC ARTERIAL VASCULAR BED OF THE DOG

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1 The hepatic artery of the anaesthetized dog was cannulated and perfused from a femoral artery, the blood flow and perfusion pressure being monitored continuously. The sympathetic periarterial nerves were divided.

2 Dose-dependent increases in hepatic arterial vascular resistance (HAVR) resulted from intra-arterial injections of noradrenaline, angiotensin and vasopressin.

3 Single injections of glucagon (100 μg, i.a.) caused a transient significant fall in HAVR of 19.9 ± 3.2%, and infusions of 25 μg/min of glucagon intra-arterially caused maintained reductions in HAVR of 16.9 ± 4.2%.

4 After single injections of 100 μg glucagon intra-arterially the vasoconstrictor responses to noradrenaline, angiotensin, and vasopressin were reduced by about 85–95%. Recovery occurred in 8–10 minutes.

5 Intra-arterial infusions of glucagon, 2.5–50.0 μg/min, reduced the effects of test doses of noradrenaline, angiotensin and vasopressin throughout the period of the infusions.

6 Dose-response curves to the constrictor agents were constructed before, during and after intra-arterial infusions of 25 μg/min of glucagon. Glucagon caused a parallel shift of the curves for noradrenaline and angiotensin to the right, with no suppression of the maximum response.

7 Infusions of glucagon shifted the dose-response curve for vasopressin to the right, but, in contrast to noradrenaline and angiotensin, the shift was nonparallel and there was a suppression of the maximum response by about one-half.

8 A large dose of insulin, 10 iu, transiently reduced HAVR and caused a weak and very transient inhibition of the effect of test doses of noradrenaline. The characteristics of these effects were quite different from those of glucagon.

9 It is possible that the antagonism by glucagon of the vasoconstrictor responses of the hepatic arterial vasculature may be important in protecting this vascular bed from the effects of concomitantly released vasoconstrictor agents.

Introduction

Pancreatic glucagon is secreted during hypoglycaemia to break down hepatic glycogen and mobilize glucose. It has weak vasodilator properties when introduced into the dog's liver by either hepatic arterial (Bashour, Geumei, Nafrawi & Downey, 1973) or portal venous (Kock, Roding, Hahnloser, Tibblin & Schenk, 1970) routes. In addition, it is reported to antagonize the vasoconstrictor actions of adrenaline, noradrenaline and sympathetic nerve stimulation on the superior mesenteric arterial vascular bed (Kock, Tibblin & Schenk, 1971).

The present experiments were performed to investigate the extent and nature of the antagonism of noradrenaline by glucagon on the hepatic arterial bed in the dog, and in addition to examine whether this antagonism was specific to catecholamines by studying the vasoconstrictor properties of the polypeptides, angiotensin and vasopressin, in the hepatic arterial vasculature and subsequently determining the extent and characteristics of any inhibition by intra-arterial administration of glucagon.

A preliminary report of part of the results of this investigation has been published previously (Richardson & Withrington, 1975).

Methods

Twelve dogs (13.4 ± 2.9 kg: mean ± s.d., range 10.4–19.0 kg) were starved for 24 h before induction of anaesthesia with 7.5–10.0 mg/kg methohexitone sodium (Brietal, Lilly), intravenously. Anaesthesia was maintained with chloralose (Kuhlmann, Paris: 50 mg/kg) and urethane (BDH; 500 mg/kg) intravenous supplements being given when necessary to maintain a constant level of anaesthesia.

After a midline laparotomy, the hepatic artery was separated from its periarterial postganglionic nerves which were divided. Blood coagulation was prevented with intravenous heparin 200 iu/kg (Weddel Pharmaceuticals) followed by hourly supplements of 100 iu/kg. The left femoral artery was cannulated, and blood diverted from this artery to perfuse the hepatic arterial vascular bed through the cannulated common hepatic artery; the blood flow through this cannula to the hepatic artery (hepatic arterial blood flow; HABF) was measured with a cannulated flowhead and electromagnetic flowmeter (CardioVascular Instruments Ltd.), occlusive flow zeros being established regularly throughout the course of each experiment. 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 Bell & Howell L220 strain gauge transducer. To minimize any pressure drop in the cannula system, wide-bore tubing (5.0 mm i.d.) was used, and the length of the tubing kept to a minimum. The cannula system carried additional 'T' pieces for the intra-arterial injection and infusion of vasoactive agents.

To ensure that the blood flow measured was the arterial inflow to the liver only, the extrahepatic branches of the common hepatic artery (gastroduodenal and right gastric arteries) were occluded. The abdominal incision was then closed, a thermometer inserted into the abdomen, and the intra-abdominal temperature maintained at 37–38°C. Drug administration was started after stable control variables had been recorded for 20 minutes.

To monitor possible systemic effects of vasoactive agents administered intra-arterially to the liver, phasic systemic arterial pressure was measured with a Statham P23Gb strain gauge transducer from a cannulated common carotid artery, and mean systemic arterial pressure and heart rate were derived electronically from this measurement. After appropriate amplification, all variables were displayed on a Devices M-19 recorder.

Calculation of results

Hepatic arterial mean perfusion pressure (PP) was calculated as diastolic plus one-third of the pulse pressure; this calculated mean pressure was in agreement with the value obtained by switching the phasic output through an averaging circuit with a time constant of about 3 seconds.

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 s; mean and phasic blood flows were displayed simultaneously.

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

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

Liver weight was obtained immediately after each experiment. Values for blood flow and vascular resistance expressed per 100 g refer to this terminal weight of liver.

Expression of results

Except where indicated to the contrary, values are expressed as means ± s.e. means. The significance of differences between paired data was assessed using Student's *t* test.

Drugs

The drugs used were: angiotensin amide (Hypertensin, Ciba), glucagon hydrochloride (Lilly), neutral insulin (Nuso, Wellcome), noradrenaline acid tartrate (Levophed, Winthrop) and vasopressin (Pitressin for i.v. injection, Parke-Davis). Doses of angiotensin are expressed in terms of the salt, of noradrenaline and glucagon in terms of the bases, and of insulin and vasopressin in international units of activity. For vasopressin, 1 unit is equivalent to 0.5 mg (manufacturer's data).

Drugs were dissolved in, or diluted with 0.9% w/v NaCl solution (saline). Intra-arterial injections were made at a point between the flow probe and the hepatic arterial cannula in volumes not exceeding 0.5 ml, washed in with saline to a total injectate volume of 1.5 ml. This resulted in a constant injection artifact visible on both the flow and pressure records (Figures 2 and 4) and clearly distinguishable from the subsequent drug-induced changes in hepatic arterial blood flow.

Intra-arterial infusions were at a rate of 1.0 ml/min from a precalibrated roller pump (Watson-Marlow MHRE-200).

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

Results

Control values

The livers weighed 331.7 ± 75.8 (s.d.) g, representing 2.53 ± 0.56 (s.d.) % of the weight of the dogs. Under control conditions, the hepatic arterial blood flow was 45.9 ± 4.5 ml min^{-1} 100 g^{-1}, being positively and significantly correlated with the liver weights ($r = +0.299$; $P < 0.05$). The hepatic arterial perfusion pressure was 121.6 ± 6.6 mmHg, and the calculated hepatic arterial vascular resistance, 2.88 ± 0.28 mmHg ml^{-1} min 100 g, or 0.90 ± 0.10 mmHg ml^{-1} min ($n = 11$).

The effects of noradrenaline, angiotensin and vasopressin on the hepatic arterial vascular resistance

Noradrenaline, angiotensin and vasopressin were injected into the hepatic artery in increasing doses to establish the dose-response relationship. The results from 9 experiments are summarized in Figure 1, where the increase in hepatic arterial vascular resistance (HAVR) is expressed as a percentage of the maximum increase attained in individual experiments. The changes in perfusion pressure, hepatic arterial blood flow and calculated hepatic arterial vascular resistance at maximum vasoconstriction due to noradrenaline, angiotensin and vasopressin are shown in Table 1.

Although the maximum rises in HAVR appear to be different for the three vasoconstrictors, small differences in low blood flows attained at maximal vasoconstriction may give rise to very large differences in calculated vascular resistance. In experiments where complete dose-response curves were constructed for two different vasoconstrictors, paired analysis revealed no significant differences between the minimum hepatic arterial blood flows attained at maximal vasoconstriction ($P > 0.10$). On the basis of these experiments alone, it is not therefore justifiable to rank the three vasoconstrictor agents in any order of maximum vasoconstrictor potency.

The increases in calculated HAVR, when large doses of the vasoconstrictor agents were injected, arose from very substantial reductions in hepatic arterial blood flow which were, however, inevitably accompanied by small rises in perfusion pressure (PP). A rise in PP itself leads to an increase in HAVR due to a myogenic reaction (Bayliss, 1902; Folkow, 1964; Mellander & Johansson, 1968). A correction needs to be applied to determine whether this contribution to the changes in HAVR is significant. The pressure-flow curve for the canine hepatic arterial vascular bed has been established in similar preparations (Torrance, 1961) and calculations from these data reveal that, in the present experiments, the proportion of the total calculated rises in HAVR due to noradrenaline which is attributable to the myogenic response to increases in PP is 3.0 ± 1.0%. The corresponding proportions for

Table 1 The effects of doses of noradrenaline, angiotensin and vasopressin which in each experiment produced maximum vasoconstriction, upon hepatic arterial perfusion pressure, blood flow, and calculated vascular resistance

	PP (mmHg)		*HABF (ml min^{-1} 100 g^{-1})*		*HAVR (mmHg ml^{-1} min 100 g)*	
	Control	*Max*	*Control*	*Max*	*Control*	*Max*
a. Noradrenaline						
	104.8 ± 9.0	125.5 ± 9.7	36.9 ±8.5	6.9 ±3.1	4.09 ±1.28	66.74 ±41.10
b. Angiotensin						
	110.2 ± 4.9	135.6 ± 4.1	61.7 ±7.9	29.4 ±6.6	1.88 ±0.20	5.26 ±0.79
c. Vasopressin						
	113.0 ± 8.4	134.5 ± 7.3	53.4 ±7.4	19.5 ±5.2	2.38 ±0.40	7.53 ±1.25

All values are means ± s.e. means for values immediately before (Control) and at the peak of maximum vasoconstriction (Max) for mean perfusion pressure (PP), mean hepatic arterial blood flow (HABF) and calculated vascular resistance (HAVR). For noradrenaline and vasopressin, $n = 6$, and for angiotensin, $n = 5$.

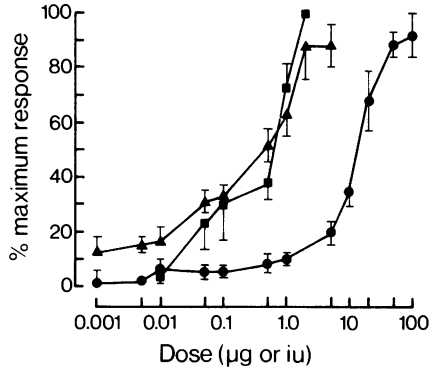

Figure 1 Dose-response curves for increase in hepatic arterial vascular resistance to intra-arterially administered noradrenaline (●), angiotensin (▲), and vasopressin (■). Abscissa scale: $\log_{10}$ dose of drug in μg (noradrenaline, angiotensin) or international units (vasopressin). Ordinate scale: percentage of the maximum increase in hepatic arterial vascular resistance found in each experiment. Points represent the means of 6 experiments (5 for angiotensin). Vertical bars show the s.e. means.

angiotensin and vasopressin were $9.0 \pm 0.6\%$ and $6.2 \pm 1.9\%$ respectively. These small effects have no influence on the form or position of the curves, expressed as percentages of the maxima (Figure 1).

The effect of intra-arterial glucagon on the hepatic arterial vascular bed

Injection. Glucagon was injected in a dose of 100 μg base intra-arterially on 14 occasions to 5 preparations, resulting in a transient and significant ($P < 0.001$) reduction in hepatic arterial vascular resistance of $19.9 \pm 3.2\%$ (Figure 2). The hepatic arterial vascular resistance returned to the control values 183 ± 36 s after the injection of glucagon.

Infusion. Glucagon was infused at a rate of 25 μg/min intra-arterially on 6 occasions in 5 preparations, resulting in a significant ($P < 0.02$) reduction in HAVR of $16.9 \pm 4.2\%$ (Figure 4). This reduction in HAVR persisted throughout the period of the infusion of glucagon.

Effects of single intra-arterial injections of glucagon on the responses of the hepatic arterial bed to noradrenaline, angiotensin and vasopressin

Single doses of noradrenaline (10 μg) angiotensin (0.5 μg) and vasopressin (0.1 or 0.2 iu) were chosen that lay on the linear part of the dose-response curve and produced about 50% maximal vasoconstriction (Figure 1). The test injections were made before, and at set intervals after single intra-arterial injections of glucagon (100 μg) to allow the time-course of both the onset and the recovery of any changes in the hepatic vasoconstrictor test responses to be clearly ascertained.

Noradrenaline. The test dose of noradrenaline (10 μg) was injected into the hepatic artery on 52 occasions in 9 preparations, resulting in a rise in HAVR of $229.4 \pm 27.5\%$. One minute after the single arterial dose of glucagon (100 μg), this hepatic arterial vasoconstrictor response to noradrenaline was reduced to $14.9 \pm 4.2\%$ of the control ($n = 6$). The vasoconstrictor response to noradrenaline returned to the control level 8–10 min after the injection of glucagon (Figure 3).

Angiotensin. The test dose of angiotensin (0.5 μg) was injected intra-arterially on 27 occasions to 7 preparations, resulting in an increase in HAVR of $118.7 \pm 13.4\%$. One minute after the injection of glucagon, the increase in HAVR in response to the test dose of angiotensin was reduced to $5.1 \pm 2.4\%$ of the control in 4 tests. The vasoconstrictor response to the test dose of angiotensin returned to control within 8–10 min (Figure 3).

Vasopressin. The test dose of vasopressin which was used varied between experiments according to the sensitivity of the preparations; the test dose was either 0.1 or 0.2 iu, the dose being that which caused approximately 50% of the maximum vasoconstriction. The test dose of vasopressin was administered on 26 occasions in 7 preparations and provoked an increase in HAVR of $66.4 \pm 9.2\%$. Within 1 min of the glucagon (100 μg) injection, the response to vasopressin was reduced (Figure 2), and in 4 tests, the mean reduction was to $11.0 \pm 4.5\%$ of the control. The time course of the recovery was similar to the other test vasoconstrictors (Figure 3).

In summary the hepatic arterial vasoconstrictor responses to all three substances, noradrenaline, angiotensin, and vasopressin, were significantly but transiently reduced by single intra-arterial injections of glucagon.

Effects of hepatic arterial infusions of glucagon on the hepatic vasoconstrictor responses to noradrenaline, angiotensin and vasopressin

To determine whether the reduction of the vasoconstrictor responses to noradrenaline, angiotensin and vasopressin was transient, even in the continued presence of glucagon, the test doses of each vasoconstrictor were injected into the hepatic arterial circulation during a maintained infusion of glucagon (2.5 to 50.0 μg/min) into the hepatic artery.

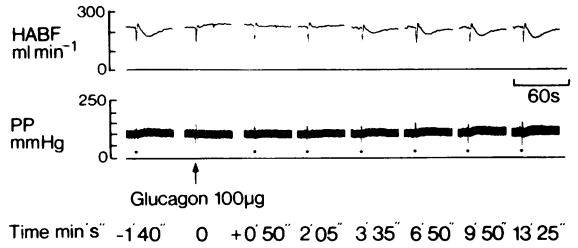

Figure 2 The effects of intra-arterial injections of 0.1 unit of vasopressin (at dots) on hepatic arterial blood flow (HABF) and perfusion pressure (PP) before and at intervals after the intra-arterial injection of 100 μg glucagon. The times of the injections of vasopressin relative to the injection of glucagon are shown (min' s") below the records.

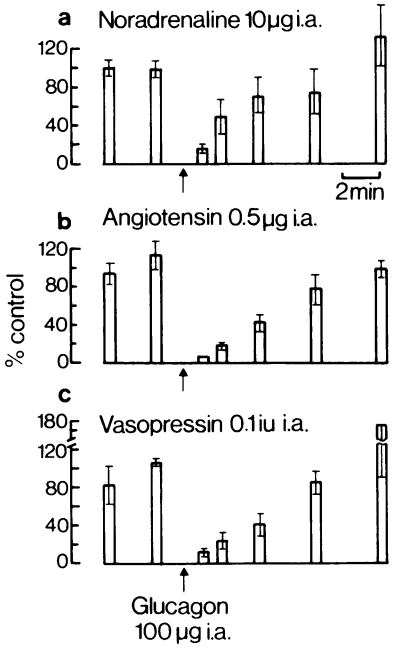

Figure 3 The effects of single injections of 100 μg of glucagon intra-arterially upon the responses of the hepatic arterial vasculature to (a) noradrenaline (10 μg i.a. injections), (b) angiotensin (0.5 μg i.a. injections) and (c) vasopressin (0.1 iu i.a. injections). Abscissa scales: time in min; ordinate scales: percentage of the initial control increase in HAVR produced by the test doses of each drug. Columns represent the means from 4 preparations and the vertical lines show the s.e. means.

Suppression of the hepatic vasoconstrictor responses to the test doses was observed (Figure 4) and persisted throughout the infusion periods of 12–15 minutes. There was no evidence of the antagonist action of glucagon to any of the vasoconstrictors either increasing or decreasing during the course of the infusions. When the infusion of glucagon was stopped, the responses to the test doses returned to control values within 3 min, although in some experiments the recovery of the vasoconstrictor effects of vasopressin was slightly slower.

In any single experiment, the suppression of the vasoconstrictor responses was related to the dose of glucagon infused (from 2.5 to 50.0 μg/min) and therefore to the resultant hepatic arterial blood concentration. However, the dose of glucagon required to induce suppression of vasoconstrictor responses varied between experiments, and without considerable further experimentation, no quantitative analysis can be made to relate blood concentration of glucagon to the extent of suppression of the responses to each of the vasoconstrictor test substances. This variation in the dose of glucagon required for suppression may be related to variations in the hepatic arterial blood distribution, and in the transport and fate of glucagon in the blood and within the liver.

The nature of the antagonism between glucagon and noradrenaline, angiotensin, and vasopressin in the hepatic arterial vascular bed

The magnitude of the antagonism of the hepatic vasoconstrictor responses to the test substances by intra-arterial glucagon whether injected (Figure 3) or infused (Figure 4) was very similar. In addition, the time-course of the onset and recovery from the antagonism was qualitatively the same for each vasoconstrictor.

To determine whether the nature of the antagonism

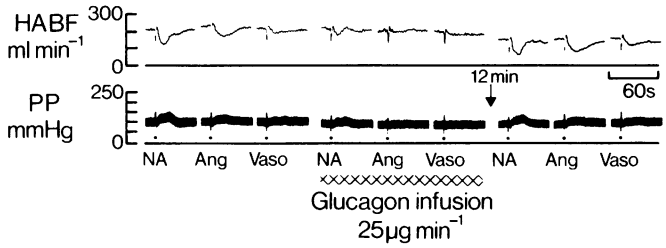

Figure 4 Effect of an intra-arterial infusion of glucagon 25 μg min^{-1} on the change in hepatic arterial blood flow (HABF) and perfusion pressure (PP) brought about by intra-arterial injections of noradrenaline (NA 10 μg), angiotensin (Ang 0.5 μg), and vasopressin (Vaso 0.1 iu). The period of the infusion is shown by the horizontal hatched bar; during this period the changes due to all three constrictor agents are attenuated compared with the control responses before and after the infusion.

was the same for noradrenaline, angiotensin and vasopressin, the characteristics of the interaction between intra-arterial glucagon and each of the vasoconstrictors was assessed. Complete dose-response curves to each substance were determined by increasing intra-arterial injections before, during, and after the intra-arterial infusion of glucagon (25 μg/minute).

Noradrenaline. In 3 experiments, graded doses of noradrenaline (0.01 to 50 μg) were injected into the hepatic artery before, and after the glucagon infusion to establish the control relationship between dose and change in HAVR. There was no significant alteration in the responses due to the interposed infusion of glucagon. The infusion of glucagon caused a parallel and marked shift of the noradrenaline dose-response curve to the right (Figure 5a) but increasing the dose of noradrenaline revealed that the maximum increase in HAVR due to noradrenaline during the infusion of glucagon was not suppressed although there was a considerable increase in the dose of noradrenaline required to evoke this maximum response (Table 2).

Angiotensin. An identical procedure to that used for noradrenaline was adopted to examine the antagonism of glucagon to angiotensin. The result of one experiment (Figure 5b) clearly showed a parallel shift of the dose-response curve to the right with no suppression of the maximum increase in HAVR, although the dose at which the maximum increase in HAVR was attained was higher than that in the absence of glucagon (Table 2).

Vasopressin. Graded doses of vasopressin were injected into the hepatic artery to establish the dose-

Table 2 Hepatic arterial vascular resistance increases resulting from the intra-arterial injection of doses of noradrenaline, angiotensin, and vasopressin causing maximal vasoconstriction before and during the infusion of 25 μg/min glucagon

	Dose causing maximal vasoconstriction	*HAVR control (mmHg ml^{-1} min)*	*HAVR maximum (mmHg ml^{-1} min)*
a. Noradrenaline			
Control	20 μg	0.82	19.20
During glucagon	50 μg	0.66	14.89
b. Angiotensin			
Control	2 μg	0.60	1.68
During glucagon	5 μg	0.61	1.72
c. Vasopressin			
Control	1 u	0.63	1.80
During glucagon	2 u	0.54	0.87

These values are from the same experiments as are illustrated in Figure 5a, 5b and 5c.

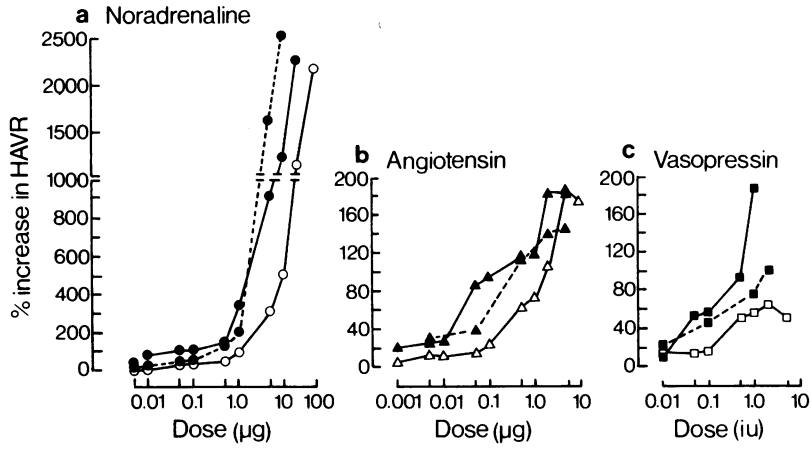

Figure 5 Dose-response curves obtained in different experiments to (a) noradrenaline, (b) angiotensin and (c) vasopressin. In each case the abscissa scale is $\log_{10}$ dose and the ordinate scale the absolute percentage increase in HAVR. The solid symbols show the dose-response curves before (solid lines) and after (broken lines) the i.a. infusion of 25 μg min^{-1} glucagon, and the open symbols show the corresponding dose-response curves during the glucagon infusions.

response curve. The infusion of glucagon (25 μg/min) produced a non-parallel shift of the dose-response curve to the right of the control dose-response curves determined both before and after the glucagon infusion (Figure 5c). In addition, the intra-arterial infusion of glucagon caused a substantial suppression of the maximum increase in HAVR achieved in response to arterial vasopressin in each of three experiments. During the infusion of glucagon, the maximum increase in HAVR attained with vasopressin was 45.4 ± 15.3% of the control maxima (Table 2).

These observations suggest a fundamental difference in the nature of the antagonism between glucagon and vasopressin, and that between glucagon and both noradrenaline and angiotensin.

Insulin

Insulin was injected intra-arterially to the liver in the large dose of 10 iu on 5 occasions to 4 preparations. This caused a variable and insignificant ($P > 0.05$) rise in hepatic arterial vascular resistance of 32.9 ± 13.5%. One minute after these injections of insulin, the vasoconstrictor effects of test doses of noradrenaline (10 μg, i.a.) were reduced to 69.1 ± 7.8% of the effects before the injections of insulin ($P < 0.05$) but the effect of noradrenaline had returned to 98.9 ± 11.8% of the initial control effect 2 min after the insulin injections, in contrast to the more marked and persistent attenuation of the effects of the vasoconstrictors by glucagon.

Discussion

The control values for hepatic arterial blood flow, hepatic arterial vascular resistance and liver weight reported in these experiments accord well with previously published data from the same species (Green, Hall, Sexton & Deal, 1959; Torrance, 1961). At the end of 3 to 4 h of perfusion, the livers were of good colour and showed no obvious signs of swelling or the 'blueness' reported, after more extensive operative procedures, by other investigators (Andrews, Hecker, Maegraith & Ritchie, 1955).

The vasoconstrictor properties of single doses of noradrenaline on the hepatic arterial vascular bed have been established for some time (Grayson & Johnson, 1953; Andrews *et al.*, 1955; Green *et al.*, 1959), and the results presented in this paper establish the complete dose-response relationship. Vasoconstriction was the only effect seen, with no evidence of vasodilatation in any experiment, at even the lowest doses. The maximum increase in HAVR attained with the largest intra-arterial doses of noradrenaline showed a very wide range between experiments (from 270 to 2250%), indicating a considerable variation of sensitivity to the natural transmitter. The highest increases in HAVR represented almost complete cessation of hepatic arterial blood flow (Table 1). Reasons for the wide variation in the absolute maximum response may include variations in uptake processes associated with the sympathetic nerve terminals, variations in degradative enzyme activity within the liver, and

structural variations in the hepatic arterial vascular bed; all of these factors may depend on the age and sex of the animals, which varied.

The effects of other naturally-occurring vasoactive agents on the hepatic vascular bed has been less well established. The present experiments establish the complete dose-response curve to arterial administration of angiotensin; single large (10 μg i.a.) injections have previously been shown to cause vasoconstriction in the bovine liver (Kelly & Nyhus, 1966). In the present experiments vasoconstriction was observed at all doses, and there was no sign of tachyphylaxis to repeated injections. Angiotensin tachyphylaxis varies between species (Khairallah, Page, Bumpus & Turker, 1966) and between vascular beds in the same species (Jonsson, Svanvik & Vikgren, 1967), and may be related to the presence or absence of degradative enzymes particularly angiotensinase-A (Khairallah *et al.*, 1966). The variation in the maximum increase in HAVR in response to angiotensin was not great in the present experiments (109–280%).

Previous reports of the effects of vasopressin on the hepatic arterial vascular bed have suggested a small and variable vasoconstriction (Heimberger, Teramoto & Schumacker, 1960; Greenway & Stark, 1971); the present experiments have established the complete dose-response curve for vasopressin, and hepatic arterial vasoconstriction was the only effect observed. The maximum rises in HAVR in different experiments were between 145 and 630%, values intermediate between the maxima for noradrenaline and angiotensin.

Despite the variations in the maximum increases in HAVR found with the three vasoconstrictors, in experiments where two complete dose-response curves were completed, paired analysis revealed no significant differences between the minimum hepatic arterial blood flows at maximum vasoconstriction. The present experiments do not therefore constitute evidence for ranking the three vasoconstrictor agents in any order of maximum vasoconstrictor potency on the basis of the observed increases in hepatic arterial vascular resistance.

Glucagon caused a reduction in hepatic arterial vascular resistance both when injected and when infused into the hepatic artery, an effect similar in character and magnitude to that observed by Bashour *et al.* (1973) and which has been shown to be present in only certain vascular beds (Kock, Tibblin & Schenk, 1970). In the superior mesenteric arterial vascular bed, the vasodilatation due to glucagon has been shown not to be the result of either α-adrenoceptor blockade, or β-adrenoceptor stimulation (Tibblin, Kock & Schenk, 1970).

The present experiments confirm in principle, and extend the observations that glucagon attenuates the effects of α-adrenoceptor stimulation by adrenaline, noradrenaline or sympathetic nerve stimulation. These observations were made in the superior mesenteric arterial bed of the dog (Kock *et al.*, 1971) where, in addition, the reflex vasoconstrictor effects of haemorrhage were reduced by glucagon.

In the present paper the observations have been deliberately confined to the hepatic arterial bed, where the antagonism of noradrenaline by arterial injections of glucagon has been shown to be transient yet highly significant, and furthermore, by determination of the complete dose response curves before, during and after glucagon, the antagonism has been shown to be of the competitive variety, exhibiting the features of a parallel shift of the dose-response curve to the right without a suppression of the maximum response (Arunlakshana & Schild, 1959).

However, the present experiments reveal that the effect of glucagon in attenuating the vasoconstrictor responses of the hepatic arterial vascular bed is not restricted to α-adrenoceptor stimulation by catecholamines, since the antagonism occurred towards the increases in hepatic arterial vascular resistance caused by the arterial injections of the vasoactive polypeptides angiotensin and vasopressin. The attenuation of the vasoconstrictor responses to the test doses of noradrenaline, angiotensin and vasopressin was similar in extent and time-course, whether the glucagon was injected close-arterially in a single dose or infused intra-arterially (Figures 2–4). The attenuation of the responses was highly significant and was present even at the lowest glucagon infusion used (2.5 μg/min) which would, on calculation, have resulted in a maximum hepatic arterial blood concentration of 9.1 ng/ml.

The nature of the antagonism between glucagon and arterial angiotensin was similar to that for noradrenaline/glucagon: a competitive relationship. The interaction between glucagon and specific angiotensin receptors may conform to this pattern, but in addition there is the observation that angiotensin interacts with adrenergic nerve terminals, enhancing and possibly even stimulating the release of the transmitter (Distler, Liebau & Wolff, 1965; Regoli, Park & Rioux, 1974). The interaction of angiotensin and glucagon might, therefore be expected to conform closely to the characteristics of noradrenaline/glucagon interaction if a major component of the action of angiotensin involves an effect on the adrenergic nerve terminal.

The nature of the interaction between vasopressin and glucagon is different: the duration of antagonism following infusions of glucagon was longer than for either noradrenaline or angiotensin, recovery of the vasoconstrictor effects of vasopressin often being slow and somewhat incomplete (Figure 5c). The characteristics of the antagonism, obtained by determination of the complete dose-response curves before, during, and after an infusion of glucagon revealed in each of three experiments a

'noncompetitive' antagonism since there was a nonparallel shift of the dose-response curve to the right with a very marked suppression of the maximum response (Arunlakshana & Schild, 1959).

It is difficult at present to propose mechanisms at the cellular level which might explain these effects and interactions since it is generally necessary to extrapolate observations made in non-vascular cells to the vascular smooth muscle. Glucagon hyperpolarizes the hepatic parenchymal cells with a consequent raising of the excitation threshold (Peterson, 1974). If a similar effect occurred in the vascular smooth muscle, it might cause relaxation of arteriolar tone with vasodilatation. This hyperpolarization could also explain the antagonism to vascular smooth muscle stimulants, all three of which, noradrenaline (Keatinge, 1964), angiotensin (Keatinge, 1966) and vasopressin (Steedman, 1966), depolarize various excitable tissues and might therefore be antagonized by a hyperpolarizing substance.

A simple link between hormones with effects on blood sugar levels and their effects on the hepatic arterial vasculature is improbable on the basis of the results obtained with insulin. A dose of insulin injected acutely to the liver, which was of the same order as the daily requirement of a pancreatectomized man (i.e. about 0.5 iu kg^{-1}: Goldner & Clark, 1944) produced only a modest and very transient hepatic arterial vasoconstriction, and an attenuation of the effects of noradrenaline which was very much smaller in extent and of very much shorter duration of action than that of glucagon.

In states where high circulating levels of vasoconstrictor hormones might be attained, it would be undesirable for there to be hepatic arterial vasoconstriction which would limit the blood flow to the liver at a time when mobilization of hepatic glycogen would be vital. On these grounds, it is reasonable to postulate that the demonstrated antagonism of the vasoconstrictor actions of noradrenaline, angiotensin and vasopressin by glucagon could represent a physiological mechanism for the 'protection' of the hepatic arterial vasculature from the effects of circulating vasoconstrictor substances.

In the present experiments, glucagon and the vasoconstrictor agents were administered close-arterially, whereas under physiological conditions, glucagon enters the liver predominantly by the portal circulation. The interaction between the portal and systemic circulations, and substances which they may contain has not been studied, and is a point requiring further elucidation.

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