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Effects of infusions of catecholamines, angiotensin, vasopressin and histamine on hepatic blood volume in the anaesthetized cat

C. V. GREENWAY AND W. W. LAUTT

Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada

Summary

1. Hepatic volume was recorded by a plethysmographic technique in cats anaesthetized with pentobarbitone ; the hepatic artery and portal vein remained intact. Dose-response curves were obtained for intravenous infusions of adrenaline, noradrenaline, angiotensin, vasopressin and histamine.

2. Adrenaline and noradrenaline decreased hepatic blood volume and did not differ significantly in potency. Up to 40% of the hepatic blood volume was expelled by doses within the range secreted by the adrenal medullae.

3. Isoprenaline, infused into the hepatic artery, had no significant effect on hepatic blood volume in doses which caused maximal vasodilatation of the hepatic arterial bed. Relaxation of hepatic capacitance vessels mediated by β-adrenoceptors did not occur.

4. Angiotensin infusions in doses previously shown to cause intestinal and splenic vasoconstriction, decreased hepatic blood volume and on a molar or microgramme basis, angiotensin was the most potent of the agents tested. Doses within the probable physiological range of endogenous production decreased hepatic blood volume by up to 20%. The responses were not significantly different when the hepatic nerves were intact or sectioned.

5. Vasopressin infusions produced only small decreases in hepatic blood volume. Doses within the range secreted by the posterior pituitary which constrict the intestinal and splenic resistance vessels, did not decrease hepatic blood volume by more than 10%.

6. Histamine produced no change in hepatic blood volume in doses which readily produce outflow block in dogs. Either the specific hepatic venous smooth muscle involved in outflow block is absent in the cat or it has no histamine receptors.

7. After the rapid change in hepatic blood volume at the onset of the infusion, hepatic volume remained steady for the duration of each infusion. There was no evidence that these agents caused net transsinusoidal fluid movements.

Introduction

Changes in hepatic blood volume are of considerable importance in overall cardiovascular homeostasis since the blood content of the liver is large (20–25 ml/100 g liver) and represents about 10% of the animal's total blood volume (Greenway & Stark, 1971). Up to 50% of this blood can be expelled by stimulation of the hepatic

sympathetic nerves at frequencies of 1–8 Hz (Greenway, Stark & Lautt, 1969). It was of interest, therefore, to establish the effects of various vasoactive agents known to be released under a variety of physiological and pathological conditions. Although the effects of such agents on hepatic arterial and portal blood flows are well established (Greenway & Stark, 1971), quantitative data on their effects on hepatic blood volume in the anaesthetized animal are not available.

A plethysmographic technique for the measurement of regional blood volume changes and transcapillary fluid movements in skeletal muscle was described by Mellander in 1960 and the principles of the method have been summarized recently (Folkow & Neil, 1971). We adapted this technique to the liver (Greenway, Stark & Lautt, 1969 ; Greenway & Lautt, 1970) and the present study examines the hepatic blood volume changes produced by infusions of catecholamines, angiotensin, vasopressin and histamine.

Methods

Cats were anaesthetized by intraperitoneal injection of sodium pentobarbitone (Abbott Laboratories, 30 mg/kg body weight) and when reflex limb and ear movements returned, additional doses of pentobarbitone (8 mg) were given through a cannula in a forelimb vein. The trachea was cannulated and mean arterial pressure was recorded from a femoral artery.

The technique for insertion of the liver into the plethysmograph has been described previously (Greenway *et al.*, 1969) and only a brief description is given here. The abdomen was opened along the midline and right subcostal margin and the ligaments connecting the central and left lobes of the liver to the diaphragm were ligated and cut. Portal pressure was recorded from a cannula inserted through a small vein from the appendix. The hepatic nerves and lymphatics were ligated and cut (except where mentioned in the **Results**) and the gastroduodenal artery was cannulated in some experiments to allow infusions of drugs into the hepatic artery. The liver, excluding the right lateral and caudate lobes, was inserted into a perspex plethysmograph. The hepatic artery and portal vein were intact and passed through an aperture in the base of the plethysmograph which was sealed with a plasticized hydrocarbon gel (Plastibase®, Squibb). The plethysmograph was filled with Ringer–Locke solution at 37° C and connected to a float recorder operating an isotonic transducer (Harvard Apparatus Co. Model 356). The pressure in the plethysmograph was zero relative to the hilum of the liver. All recordings were made on a Beckman polygraph.

After stabilization for at least 20 min, drugs were infused for 5–10 min through a cannula in a femoral vein. On cessation of each infusion, 5 min were allowed after the measured variables had returned to the control level before the next infusion was begun. At the end of each experiment, the vessels to and from the liver were simultaneously clamped, the liver was removed and weighed, and the hepatic blood volume was determined by a haemoglobin washout technique (Greenway *et al.*, 1969).

Stock solutions (1 mg base/ml) of (−)-adrenaline HCl (Sigma Chemical Co.), (−)-noradrenaline tartrate (Winthrop Laboratories) and isoprenaline HCl (B.D.H.) were diluted as required in 0·9% w/v NaCl solution containing ascorbic acid (0·2 mg/ml). Angiotensin amide (Hypertensin, Ciba), vasopressin (Pitressin, Parke,

Davis Co.) and histamine acid phosphate (B.D.H.) were made up in 0·9% NaCl solution. All doses of catecholamines and histamine are expressed as free base.

Results

Control values and calculation of results

Twenty-two cats (mean body weight 2·5 kg) were used. The mean liver weight was 91 ± 2·3 g (mean ± S.E.). At the start of the drug infusions, mean arterial pressure was 132 ± 4·7 mmHg and mean portal pressure was 9·5 ± 0·5 mmHg (1 mmHg ≡ 1·333 mbar). The hepatic blood volume was 20 ± 1·1 ml/100 g liver. In all the experiments described in the subsequent sections, the hepatic volume became steady after a few minutes of drug infusion and no significant ($P > 0·5$; paired t test) changes occurred during the remaining period of the infusion. On cessation of the infusion, the hepatic volume returned to the preinfusion level with no consistent under- or over-shoot. The results therefore represent reversible, steady-state responses and are interpreted as changes in hepatic blood volume (see **Discussion**). For each infusion the volume change was calculated as a percentage of the hepatic blood volume determined at the end of each experiment. A representative record which shows the responses to two infusions of adrenaline is reproduced in Fig. 1.

Catecholamines

The changes in arterial pressure, portal pressure and hepatic volume in response to infusions of adrenaline and noradrenaline ((0·1–2·0 μg/min)/kg) were studied in four cats. The changes from the control values were measured and the means (± S.E.) calculated for the experiments. The results are plotted as dose-response

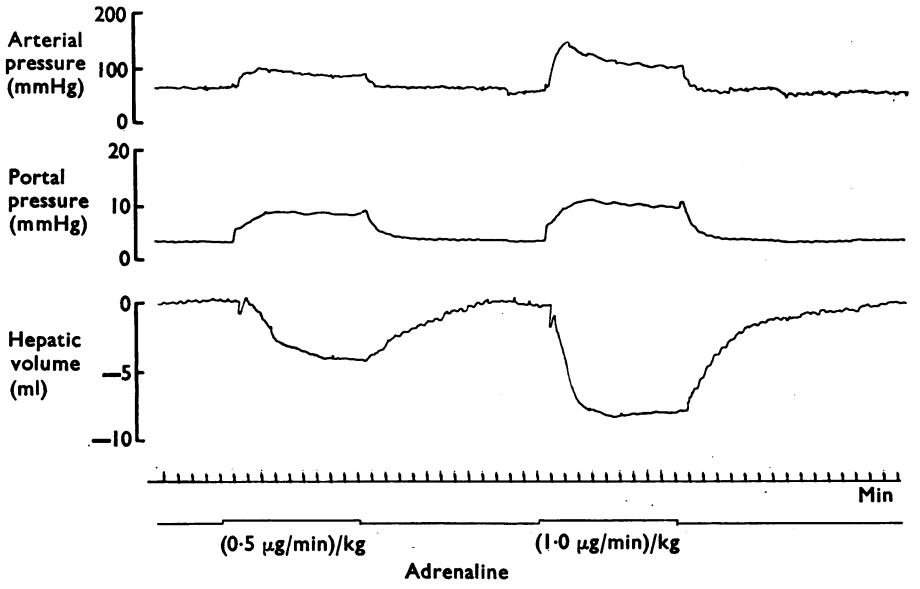

FIG. 1. Representative record of the responses to infusions of adrenaline in one cat. At the end of the experiment, the hepatic blood volume was 29·3 ml and the liver weight 112 g. Thus the change in liver volume during adrenaline infusion represents a decrease in blood volume of 15% and 27% for the first and second infusion respectively.

curves in Fig. 2. At the highest dose tested ((2 μg/min)/kg), both catecholamines expelled about 40% of the hepatic blood volume. The effects of adrenaline on hepatic blood volume were not significantly different from those of noradrenaline at any of the doses tested.

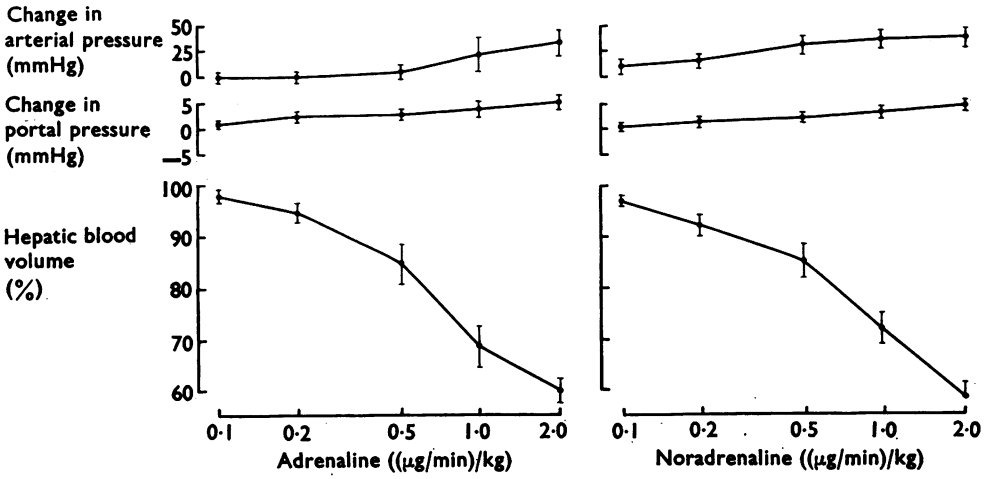

FIG. 2. Dose-response curves for adrenaline and noradrenaline. Each point represents the mean (±S.E.) for four cats.

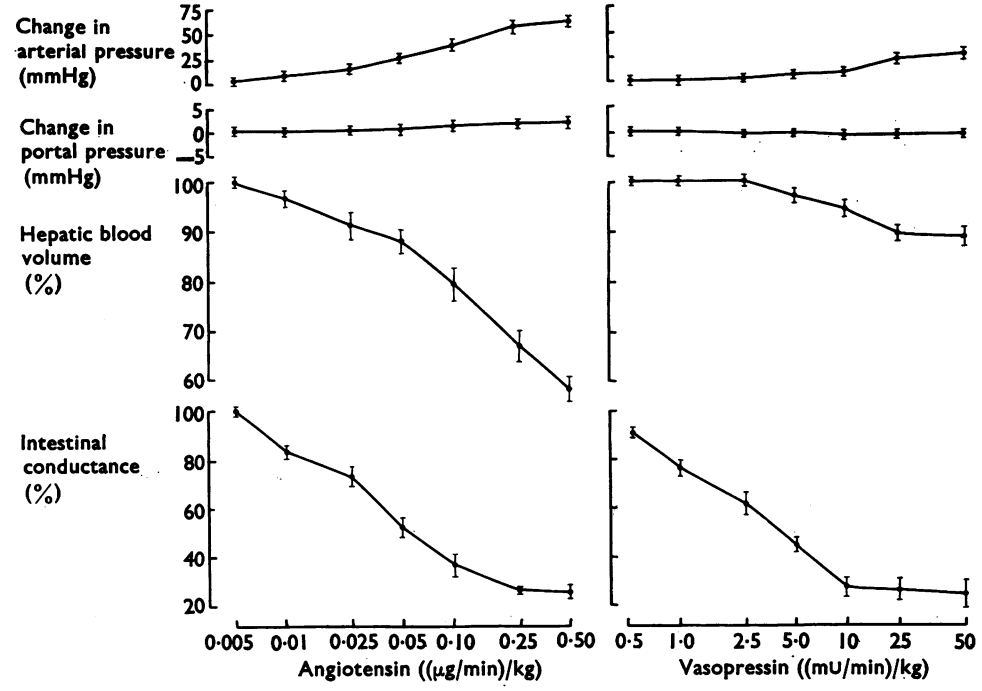

FIG. 3. Dose-response curves for angiotensin and vasopressin. The angiotensin curves represent the means (±S.E.) for eight cats and the vasopressin curves for four cats. The intestinal conductance responses are taken from a previous paper (Cohen *et al.*, 1970).

To examine whether an increased hepatic blood volume could result from stimulation of β-adrenoceptors in the capacitance vessels, isoprenaline ((0·2 μg/min)/kg) was infused into the hepatic artery on seven occasions in three cats. These doses have been previously shown to cause maximal vasodilatation of the hepatic arterial bed (Greenway & Lawson, 1969). There was no significant change in portal pressure (+0·1 ± 0·1 mmHg) or hepatic blood volume (−1·3 ± 3·7%).

Angiotensin and vasopressin

Dose-response curves were obtained to intravenous infusions of a range of doses of angiotensin ((0·005–0·50 μg/min)/kg) in four cats in which the hepatic nerves were intact and in four cats in which they were sectioned. At each dose, the responses in the two groups were not significantly different (unpaired *t* test, $P>0·1$) and the data were therefore pooled. The results are shown in Fig. 3. Dose-response curves to intravenous infusions of vasopressin ((0·5–50 mU/min)/kg) were obtained in four cats and are shown in Fig. 3. For the purposes of comparison and discussion, the dose-response curves for angiotensin and vasopressin on the arteriolar resistance vessels of the intestine, taken from our previous work (Cohen, Sitar, McNeill & Greenway, 1970), are included in Fig. 3.

Histamine

Histamine was infused on thirty occasions in three cats. Infusions were made into the hepatic artery to allow doses up to (100 μg/min)/kg to be given without gross systemic effects. In no case did histamine increase hepatic volume. Doses up to (4·0 μg/min)/kg produced no significant change in hepatic volume while higher doses produced a decrease of up to 8% of the hepatic blood volume.

Discussion

The techniques described allow quantitative measurement of hepatic volume in the anaesthetized animal without surgical interference with the hepatic artery and portal vein. The disadvantages of isolated perfusions of the liver have been stressed previously (Greenway & Stark, 1971). Our preparation avoids these disadvantages but it is more difficult to separate direct actions on the hepatic vascular bed from indirect consequences of actions elsewhere. The general state of the animals was good: corneal, ear flick and swallowing reflexes returned repeatedly as the effects of each supplementary dose of pentobarbitone wore off, the measured variables remained steady and consistent responses to drug infusions were obtained for several hours. The validity and accuracy of the measurements of hepatic volume have been discussed previously (Greenway *et al.*, 1969; Greenway & Lautt, 1970). During the drug infusions, hepatic volume changed initially but then remained steady and on cessation of the infusion the volume returned to the preinfusion level. These observations, in conjunction with our previous studies using ^{51}Cr-tagged red cells (Greenway & Lautt, 1970) suggest that the observed changes were due to changes in hepatic blood volume only and that net transsinusoidal fluid movements were insignificant. We have therefore expressed the observed changes as percentages of the hepatic blood content measured at the end of each experiment. The lack of effect of these agents on transsinusoidal fluid movements is discussed in more detail in a subsequent paper (Greenway & Lautt, 1972).

At any dose level, adrenaline and noradrenaline produced similar decreases in hepatic blood content even though their effects on arterial pressure were different and adrenaline, but not noradrenaline, caused a marked intestinal vasodilatation and hence an increase in hepatic blood flow (Greenway & Lawson, 1966, 1968). At a dose of (2 μg/min)/kg, which is probably the maximum rate of release from the adrenal medullae under normal conditions (Celander, 1954), about 40% of the hepatic blood volume was expelled. This is similar to the maximum response (50%) to hepatic nerve stimulation (Greenway *et al.*, 1969) and it suggests that circulating catecholamines could influence hepatic blood volume under physiological conditions. However, comparison of the shape of the frequency-response curve to nerve stimulation and the dose-response curve to catecholamines suggests that under conditions where the sympathetic nervous system is activated submaximally, the hepatic nerves will affect hepatic blood content to a greater extent than will circulating catecholamines.

Isoprenaline infused into the hepatic artery did not increase hepatic volume in these experiments. Propranolol did not alter and phenoxybenzamine abolished but did not reverse the decrease in hepatic volume produced by nerve stimulation (Greenway, unpublished observations). These data suggest that a relaxation of the hepatic capacitance vessels mediated by β-adrenoceptors does not occur in the anaesthetized cat. A similar situation has been reported in the splenic capsule (Davies, Robinson & Withrington, 1969 ; Greenway & Stark, 1970), and isoprenaline has at most a weak dilator effect on the capacitance vessels of skeletal muscle *in vivo* (Abboud, Eckstein & Zimmerman, 1965 ; Johnsson & Oberg, 1968 ; Mellander & Johansson, 1968). Thus it appears that β-adrenoceptor responses in capacitance vessels are not marked.

Angiotensin and vasopressin caused marked intestinal and splenic arteriolar vasoconstriction (Cohen *et al.*, 1970) and these agents play an important role in the intestinal and splenic vasoconstriction after haemorrhage (McNeill, Stark & Greenway, 1970 ; Stark, McNeill & Greenway, 1971). It was therefore of interest to determine whether they might also play a role in the venoconstriction following haemorrhage. Production rates of angiotensin in the cat after haemorrhage are not known but in the dog, amounts up to 1·5 μg/min were formed (Regoli & Vane, 1966 ; Hodge, Lowe & Vane, 1966). After administration of endotoxin to cats, angiotensin production increased up to (62 ng/min)/kg and in one cat to more than (130 ng/min)/kg (Hall & Hodge, 1971). In our experiments, infusions of (100 ng/min)/kg caused a reduction in intestinal blood flow to 38% of control but hepatic blood volume decreased only to 80% of control. Thus it is possible that endogenous angiotensin causes some reduction in hepatic blood volume but this is likely to be small. These responses to angiotensin were not mediated through actions on the central nervous system since the responses were not significantly different when the hepatic nerves were intact or sectioned. The possibility that the decreased hepatic volume was secondary to the reduction in portal flow appears to be excluded since doses of vasopressin which caused a similar reduction in hepatic flow had a much smaller effect on hepatic blood volume.

Vasopressin produced little effect on hepatic blood volume even in large doses. Endogenous secretion rates in the cat are not known but blood concentrations of 750–1,000 μU/ml have been reported after haemorrhage (Beleslin, Bisset, Haldar & Polak, 1967 ; Clark & Roche e Silva, 1967). An infusion of (10 mU/min)/kg for 5

min would produce a blood concentration of 1,000 μU/ml (blood volume 50 ml/kg; Groom, Rowlands & Thomas, 1965) if vasopressin was distributed only in the blood and was not metabolized during the 5 min period. The errors in such a calculation are enormous but it suggests that the maximum endogenous secretion rate lies somewhere in the middle of our range of doses. Thus it is unlikely that endogenously secreted vasopressin causes any decrease in hepatic blood volume even though it produces a marked arteriolar vasoconstriction in the intestine and spleen after haemorrhage (McNeill *et al.*, 1970; Stark *et al.*, 1971).

Twenty per cent of the hepatic blood volume was expelled by (0·7 μg/min)/kg adrenaline and noradrenaline, (0·1 μg/min)/kg angiotensin and approximately (0·7 μg/min)/kg vasopressin ((170 mU/min)/kg; 250 U/mg; Sawyer, 1961). Thus angiotensin is the most potent agent on the basis of molar or microgramme quantity infused intravenously.

In the dog, histamine causes outflow block and hepatic blood volume increases markedly both *in vitro* (Bauer, Dale, Poulsson & Richards, 1932) and *in vivo* (MacLean, Brackney & Visscher, 1956; Oshiro & Greenway, 1971). In the cat, histamine had little effect on hepatic volume even when massive doses were infused into the hepatic artery. This confirms previous studies on the isolated perfused cat liver (Andrews, Hecker & Maegraith, 1956; Bauer *et al.*, 1932). The hepatic venous bed in the cat either lacks the specific smooth muscle which is involved in the dog or the venous smooth muscle has no histamine receptors.

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