The actions of human atrial natriuretic factor on hepatic arterial and portal vascular beds of the anaesthetized dog

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1   The vascular actions of atrial natriuretic factor (ANF) have been assessed with other vasoactive agents on the hepatic arterial and portal vascular beds of the anaesthetized dog.

2   Intra-arterial bolus injections of ANF (0.1–50 nmol) caused graded increases in hepatic arterial blood flow representing a vasodilatation of relatively short duration. Vasoconstriction was never observed.

3   The maximum increase in hepatic arterial blood was the same for ANF and isoprenaline (Iso) i.e. approximately 60–70% increase over control flow.

4   On a molar basis, ANF was less potent than Iso although over the higher dose range (10^{-9}–10^{-7} mol) its vasodilator activity exceeded that of the endogenous vasodilator adrenaline.

5   Intraportal bolus injections (1.0–50 nmol) of ANF did not alter portal inflow resistance since no changes in portal inflow pressure occurred when the portal circuit was perfused at constant inflow volume.

6   This differential action of ANF on the hepatic arterial and portal vascular beds may provide a change in total liver blood flow in favour of the arterial component.

7   ANF, by altering hepatic haemodynamics to favour formation of trans-sinusoidal fluid exchange, may provide a temporary expansion of the extravascular fluid reservoir to buffer any increased venous pressure. However, chronically elevated plasma levels of ANF would encourage the formation of ascitic fluid.

Introduction

Mammalian atrial myocytes synthesize and release the 28 amino acid residue peptide, atrial natriuretic factor (ANF). Raised venous pressure, leading to atrial distension, is one of the factors which releases ANF into the systemic circulation (Anderson et al., 1986; Gerbes et al., 1986). In addition to promoting natriuresis and diuresis the peptide causes hypotension, due principally to a fall in peripheral vascular resistance (see reviews by Genest & Cantin, 1988; Gerbes et al., 1987). However, the regional distribution of its vasodilator activity has not been fully examined.

Elevated central venous pressure also leads to raised hepatic venous pressure with profound consequences for hepatic haemodynamics and trans-sinusoidal fluid exchange (Laine et al., 1979). Enlargement of the liver with movement of fluid across the hepatic capsule into the peritoneal space may be a normal, although temporary compensating mechanism to expand the extravascular fluid space. However, chronic elevation of hepatic venous pressure leads to the persistent presence of excess peritoneal (ascitic) fluid. A significant fraction of the raised hepatic venous pressure may be transmitted through the liver to elevate portal pressure (Bennett & Rothe, 1981) causing enlargement of the spleen (Withrington et al., 1980), together with venous congestion and oedema of the gastrointestinal tract.

The aim of the present experiments was to characterize the actions of ANF on the primary intra-hepatic resistance sites in the hepatic arterial and portal vascular beds which control total liver blood inflow and, in addition, to assess its molar potency relative to other vasoactive agents. In this way some conclusions could be drawn about the role of ANF in the regulation of total liver blood flow and its distribution between the two inflow circuits both under normal conditions and in those clinical situations where the systemic circulating

levels of ANF deviate significantly from normal. A preliminary account of some of these results has been published (Dhume et al., 1988).

Methods

The experiments were performed on 10 dogs (mean weight 21.9 ± 2.0 kg, range 10.0–29.0 kg) anaesthetized with an intravenous mixture of chloralose and urethane (50 and 500 mg kg^{-1} respectively) after induction with methohexitone sodium (6 mg kg^{-1}). The trachea was cannulated although respiration was spontaneous throughout the experiment. The right femoral vein was cannulated to administer additional anaesthetic when appropriate. The right femoral artery was cannulated to provide hourly blood samples for analysis of pH, Po_2 and Pco_2 (Blood Gas Analyser, Instrumental Laboratory, Model 1302); $NaHCO_3$ was administered i.v. (1.0 mmol min^{-1}) when necessary, to maintain a normal arterial pH. The left carotid artery was cannulated and connected to a strain gauge transducer (Statham P23Gb), to provide a continuous registration of phasic systemic blood pressure from which a continuous recording of heart rate was derived electronically. Body temperature was maintained within normal limits, as indicated by a buccal thermometer, by either table heaters or overhead lamps. The animals were heparinised (500 iu kg^{-1}) once perfusion had started and half this dose was administered hourly.

The perfusion circuits and surgery required for hepatic arterial and portal perfusion have been described in detail previously (Corder & Withrington, 1988) and in all essential details the same procedure was adopted for the present series. Briefly, the hepatic artery was cannulated after ligation of major side tributaries, and perfused with arterial blood from the cannulated left femoral artery. The hepatic periarterial innervation remained intact. Incorporated into the arterial perfusion circuit was a strain gauge pressure transducer (Statham P23Gb) and a cannulating electromagnetic flow probe (Cardiovascular Instruments) to measure perfusion pressure and flow respectively. These signals were heavily

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damped electronically to provide mean values which were continuously monitored on a chart recorder (Devices M19). In addition, the signals were digitised (Data Translation DT-2801) and processed by an IBM PC-XT computer programmed to calculate and store the absolute values of hepatic arterial vascular resistance (mean pressure/mean flow) and the changes induced by any experimental procedure. A 'T' piece was included in the perfusion circuit so that vasoactive substances could be administered by bolus injection directly into the hepatic arterial circuit without necessarily entering the systemic circuit. This avoided either altering the conditions of the perfusion (i.e. constant pressure) or eliciting reflex changes in hepatic sympathetic tone.

In the portal perfusion experiments the hepatic portal vein was cleared of all attachments and principal side branches ligated. The spleen was removed and a wide bore polythene tube passed retrogradely down the splenic vein to the portal vein. The portal vein was then tied cranially just beyond the entrance of the superior mesenteric and splenic veins so that the venous drainage from the gastrointestinal tract was diverted along the splenic vein cannula to a reservoir. The hepatic end of the portal vein was then cannulated and blood from the mesenteric drainage reservoir pumped, by means of a roller pump (Watson-Marlow MHRE 1000), into the intrahepatic portal vascular bed. The pump was set to deliver the same volume flow as the mesenteric venous drainage and, once set, remained constant throughout any experiment. Incorporated into the portal perfusion circuit between the pump and liver was a low pressure strain-gauge transducer and an electromagnetic flow probe to measure continuously hepatic portal venous pressure (HPVP) and to check the pump outflow (HPVF) respectively. A long, narrow bore, polythene cannula was passed up the inferior vena cava (IVC) from the right femoral vein to a point just below the diaphragm and opposite the entry of the hepatic veins. After suitable electronic damping this provided a continuous recording of IVC pressure (IVCP). The signals from these pressure transducers were heavily damped electronically to provide mean values which were continually monitored on a pen recorder. Again the values were digitised and fed into a personal IBM computer programmed to calculate hepatic portal mean inflow resistance [(HPVP − IVCP)/HPVF] and also to store the maximum changes in portal pressure and portal inflow resistance induced by any experimental procedure. Incorporated into the portal circuit was a 'T' piece for injection of vasoactive substances directly into the portal inflow.

The flow probes were calibrated with whole blood at the end of each experiment. The liver was weighed at the end of each experiment so that hepatic arterial and portal flows and resistances could be expressed in terms of 100 g liver weight.

Drugs used and vehicles

Vasoactive substances were washed in with saline (0.9% w/v NaCl solution) to give a total injectate of 2.0 ml. ANF was obtained from three sources (Sigma, Novabiochem, Bissendorf) and made up in sterile saline which contained

human serum albumin (10 mg ml^{-1}, Elstree) and polypep (2.5 mg ml^{-1}; low viscosity; Sigma). Close arterial or intraportal injection of this vehicle did not alter either hepatic arterial or portal inflow resistance. The human serum albumin and polypep were used to reduce non-specific binding of ANF onto plastic surfaces. (±)-Isoprenaline hydrochloride (Pharmax Ltd.) and (−)-noradrenaline acid tartrate (Winthrop) were diluted immediately before injection in normal saline and the solutions maintained in ice.

Statistics

Results are presented as mean ± standard errors of the mean (s.e.mean). Tests for significance refer to Students unpaired t test.

Results

Control values

The mean liver weight was 502 ± 33 g representing 2.39 ± 0.16% of the body weight. In the 6 hepatic arterial perfusion experiments the initial mean hepatic arterial blood flow was 187 ± 12.8 ml min^{-1}. The mean hepatic arterial perfusion pressure was 151 ± 7.0 mmHg and the mean calculated hepatic arterial vascular resistance was 0.81 ± 0.05 mmHg ml^{-1} min. These values agree with those previously obtained in this laboratory.

In the four hepatic portal perfusion experiments the mean initial systemic blood pressure, once portal perfusion had started, was 115 ± 3.3 mmHg. The mean hepatic portal blood flow was 178 ± 38 ml min^{-1} or 36.7 ± 7.4 ml min^{-1} 100 g. The mean hepatic portal venous pressure was 8.50 ± 0.74 mmHg and the mean inferior vena cava pressure 0.71 ± 0.36 mmHg; giving a mean hepatic portal perfusion pressure gradient (i.e. HPVP − IVCP) of 7.82 ± 0.30 mmHg. The mean calculated hepatic portal inflow resistance was 0.23 ± 0.045 mmHg ml^{-1} min 100 g. These values are similar to those reported previously from this laboratory.

Hepatic arterial vascular responses to intra-arterial injections

Isoprenaline The non-selective β-adrenoceptor agonist isoprenaline (Iso) was administered in all the hepatic arterial preparations as a bolus injection over a range of doses (0.1–50 nmol) to construct, in each experiment, a complete dose-response curve. The only vascular response observed to i.a. Iso was an increase in hepatic arterial blood flow [HABF] which was graded with dose and of relatively short duration (Figure 1). This increase in blood flow reflects, at constant hepatic arterial perfusion pressure [HAPP], a decrease in hepatic arterial vascular resistance [HAVR] and therefore vasodilatation. Previous studies have shown this vasodilator response to be due to the activation of β_2-adrenoceptors within the hepatic arterial circuit since it is blocked by prop-
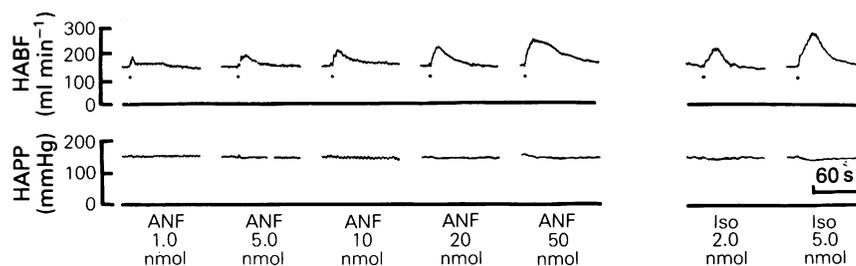

Figure 1 Experimental records obtained from a single perfusion. Liver weight 523 g. Traces are from the top: hepatic arterial mean blood flow (HABF), hepatic arterial mean perfusion pressure (HAPP). The two panels illustrate the vasodilator responses to intra-arterial bolus injections of atrial natriuretic factor (ANF; 1.0–50 nmol) and isoprenaline (Iso; 2.0 and 5.0 nmol). The black dots indicate the point of injection.
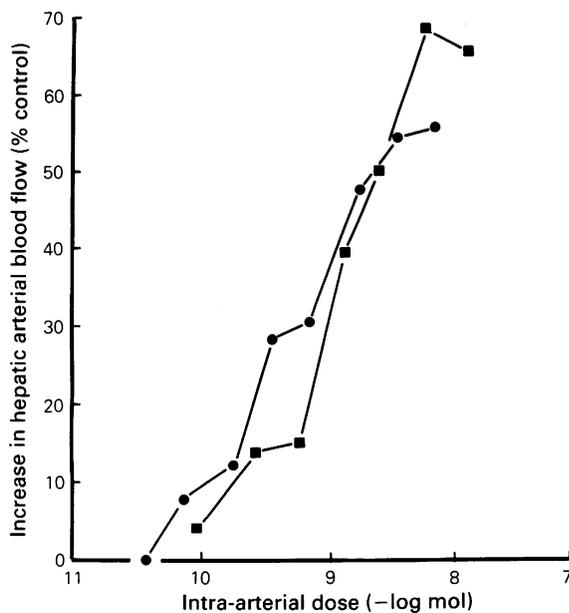
Figure 2 Dose-response curves relating the hepatic arterial vasodilator responses (expressed as the percentage increase in hepatic arterial blood flow compared to the pre-injection control) to intra-arterial bolus injections of atrial natriuretic factor (■) and isoprenaline (●). All the data were obtained in the same perfusion experiment (liver weight 294 g) under comparable conditions of vascular tone. Mean control hepatic arterial vascular resistance was 0.98 ± 0.09 mmHg ml^{-1} min.

Figure 3 Relationship between the intra-arterial molar dose of isoprenaline (●), atrial natriuretic factor (■) and adrenaline (O) and the decrease in hepatic arterial vascular resistance (hepatic arterial vasodilatation) expressed as a percentage of the control resistance prior to the injection. The points represent the means and the vertical bars the s.e. of at least 6 observations. The data for isoprenaline and atrial natriuretic factor were from the current series whilst the adrenaline results come from a previous series of similar preparations (see Richardson & Withrington, 1977; Figure 2).

ranolol (Richardson & Withrington, 1977) and the selective β_2-adrenoceptor antagonist, ICI 118,551 (Withrington, 1987). The threshold dose was usually between 0.1 and 0.5 nmol and the maximum vascular response achieved at either 10 or 20 nmol. In the current series of perfusion experiments the mean maximum increase in blood flow to Iso was $72 \pm 16\%$ of the control flow whilst the mean molar dose of Iso to decrease the hepatic arterial vascular resistance by 50% of the maximum was 0.42 ± 0.13 nmol.

Atrial natriuretic factor In the 6 arterial perfusion preparations ANF was administered as a bolus injection (on 54 occasions), in doses from 0.1–50 nmol to construct 8 dose-response curves, in most cases from the threshold value to maximum effect. The threshold dose of ANF to obtain a hepatic arterial response was usually 0.1–0.5 nmol whilst the maximum response was elicited at either 20 or 50 nmol. The only hepatic arterial vascular response to i.a. ANF was an increase in arterial blood flow, graded with dose, and of the same time course as that observed with i.a. Iso (Figure 1). These increases in arterial flow at constant perfusion pressure reflect hepatic arterial vasodilatation. A vasoconstrictor response to intra-arterial ANF was never observed. In any individual experiment (e.g. Figure 2) the dose-response curve relating the molar dose of ANF to the increase in hepatic arterial blood flow always lay to the right of that for Iso; i.e. it was less potent hepatic arterial vasodilator.

The mean maximum increase in hepatic arterial blood flow to ANF was $61 \pm 11\%$ of the control value; this is not signifi-
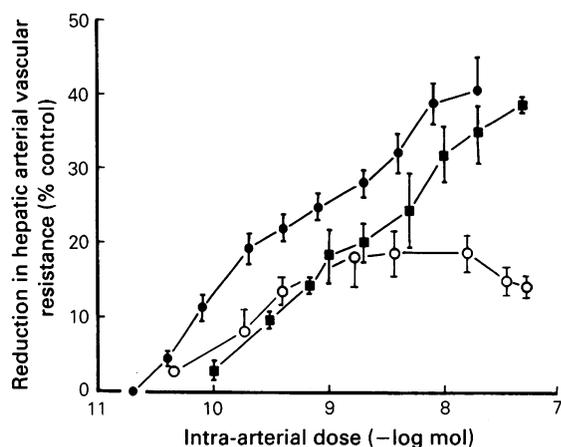
cantly different ($P > 0.5$) from the maximum vasodilator effect to i.a. Iso. However there is a difference in the position of the mean dose-response curve (Figure 3) relating the molar dose of Iso or ANF to the decrease in hepatic arterial vascular resistance. A consequence of this is that the mean molar ED_{50} to ANF i.e. the mean molar dose of ANF to reduce the vascular resistance to 50% of the maximum response, was 2.78 ± 0.87 nmol; a value significantly greater than the ED_{50} for Iso (0.42 nmol) in the same experiments ($P < 0.01$) i.e. on a molar basis ANF was less potent than Iso. In a previous series of identical experiments (Richardson & Withrington, 1977) the dose-response curve for adrenaline was constructed and this is included in Figure 3 for comparison (see Discussion).

Hepatic portal responses to intraportal injections of atrial natriuretic factor and noradrenaline

ANF was injected as a bolus into the portal circuit on 22 occasions in four perfusion preparations in doses ranging from 1.0–50 nmol. The results were unequivocal in that no change in portal inflow pressure was observed on any occasion (Figure 4). These observations clearly indicate that, at constant inflow volume, no change in portal inflow resistance was elicited with the range of doses of ANF administered in this series. In 3 of the 4 experiments the higher intraportal bolus doses of ANF were observed to cause systemic hypotension indicating passage of the peptide through the liver into the systemic circulation and peripheral vasodilatation thus confirming the biological activity of the injectate. In the same experiments noradrenaline (NA) was administered (6.0–600 nmol) intraportally to elicit graded increases in portal pressure and portal vasoconstriction confirming the reactivity of the preparation.
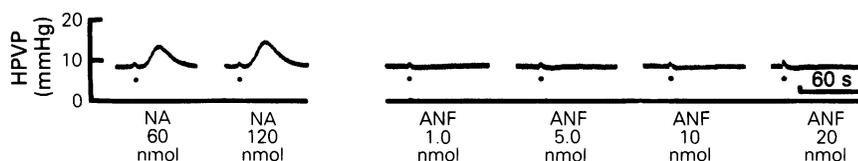

Figure 4 Records of the hepatic portal mean perfusion pressure (HPVP) when the portal circuit was perfused with mesenteric blood at constant flow (146 ml min^{-1}). The two panels illustrate the changes in portal pressure, and therefore in portal inflow resistance in response to intraportal bolus injections of noradrenaline (NA, 60 and 120 nmol) or atrial natriuretic factor (ANF 1.0, 5.0, 10 and 20 nmol). Liver weight 588 g.

Discussion

The infusion of ANF $(0.5 \mu g \min^{-1})$ into normal man causes a 20% reduction in apparent liver blood flow (Biollaz et al., 1986) as assessed by iodocyanin green clearance technique. However, such a fall in flow may arise from the reflex adjustments of the hepatic circulation to maintain systemic blood pressure. In addition such information does not provide data on any differential flow changes in the two hepatic inflow circuits. We have been concerned to establish any selective actions of ANF on those parameters of the hepatic circulation which control total liver blood flow and its distribution between the arterial and portal inflow circuits.

Histochemical studies have indicated the presence of ANF binding sites along the hepatic arterial and portal vascular territories. Using $[I^{125}]$-ANF, Bianchi et al. (1985), demonstrated labelling in the endothelium and smooth muscle of both arterial and portal inflow circuits of the rat. No binding sites were observed along the mesenteric artery.

Close-arterial injection of ANF avoids any of the reflex changes in the peripheral circulation which inevitably follow systemic administration with consequent falls in mean BP. It also mimics the normal route of access of ANF into the liver via the arterial supply. In the current experiments, ANF caused a marked hepatic arterial vasodilatation of short duration with a maximum percentage increase in blood flow not significantly different from that elicted by the non-selective β-adrenoceptor agonist, Iso. However, the molar potency of ANF, as assessed by the molar ED_{50}, was significantly less than Iso.

However, Iso is not a natural endogenous agent so that a more meaningful comparison, to assess any physiological role of ANF, is with the mixed α- and β-adrenoceptor agonist adrenaline (Ad). The hepatic arterial responses to i.a. ADR are complex (Figure 3) since they represent the overlapping excitation of both α- and β_2-adrenoceptors (Richardson & Withrington, 1977) with opposing vascular effects of vasoconstriction and vasodilatation respectively. At the lower bolus dose range (less than 10^{-9} mol) then ANF and Ad are equipotent as hepatic arterial vasodilators. As the bolus dose is increased the dilator activity of Ad declines presumably because its α-agonist vasoconstrictor properties become dominant. Therefore at doses above 10^{-9} mol, ANF is the more potent hepatic vasodilator. Since different systemic situations provoke the release of ANF and Ad, this different pattern of responses of the hepatic arterial vasculature may be appropriate. Clearly a next step in the evaluation of any potential vascular role of ANF is to examine the hepatic vascular interactions between ANF and other systemic vasoactive agents.

The effect of ANF on portal haemodynamics is more difficult to assess. In the present experiments ANF caused no change in portal inflow resistance when the bed was perfused with mesenteric venous blood at constant flow. In this series no experimental information was obtained about ANF on the

other principal determinant of portal haemodynamics, mesenteric flow. Employing chronically implanted pulsed Doppler flow probes in the conscious normotensive rat, Gardiner et al. (1988) observed a fall in mesenteric flow and vasoconstriction following bolus i.v. ANF. Garcia et al. (1984) described the relative insensitivity of isolated mesenteric resistance vessels to the relaxant properties of ANF compared to preparations from other vascular territories. Tentative conclusions from these results might explain the fall in total hepatic flow reported in man by Biollaz et al. (1986) since the change in total flow would depend upon the respective extents of arterial dilatation and the fall in portal inflow from the mesenteric circuit. ANF appears to have a unique spectrum of pharmacological activity since it causes marked hepatic arterial dilatation yet without either mesenteric or hepatic portal vasodilatation. It may therefore change total liver blood flow in favour of the arterial component. This differential activity of ANF may suggest some therapeutic potential.

It is clear, however, that in terms of its absolute molar potency, ANF is amongst the least potent vasodilator substances present in the hepatic inflow circuits. Many other vasoactive substances, particularly peptides of gastrointestinal origin (see review, Withrington & Richardson, 1989) enter the liver in the portal inflow principally during digestion. It is now established that vasoactive substances present in the portal inflow alone may nevertheless have access to hepatic arterial resistance sites by 'transhepatic routes' (Richardson & Withrington, 1978; Lautt et al., 1984). It remains an important aspect of hepatic circulatory physiology to establish any interactions, on the liver vasculature, between substances of primarily systemic origin (i.e. ANF) with those of GI origin (i.e. substance P). In this way the extent to which hepatic haemodynamics is related to, and integrated with, systemic and GI events, may be evaluated.

The physiological and pathophysiological role of ANF in liver haemodynamics remains to be fully elucidated. In the absence of other changes then hepatic arterial vasodilatation to ANF would increase the extent of fluid formation within the liver by increasing mean capillary pressure, enlarge the liver and promote movement of fluid across the liver capsule into the peritoneal space. In this way ANF may contribute towards buffering the effects of increased venous pressure by temporary occupation of an enlarged extravascular space. In the chronic condition this movement of fluid would lead to ascites and the presence of ANF in ascitic fluid (Gerbes et al., 1988) confirms the access of ANF to the hepatic microvasculature in conditions of hepatic cirrhosis and therefore its potential role as a factor contributing towards those hepatic circulatory changes characteristic of cirrhosis.

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