Vasoconstrictor actions of atrial natriuretic peptide in the splanchnic circulation of anesthetized dogs

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Woods, Robyn L. Vasoconstrictor actions of atrial natriuretic peptide in the splanchnic circulation of anesthetized dogs. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1822–R1832, 1998.—Intravenous atrial natriuretic peptide (ANP) usually results in splanchnic vasoconstriction in humans or experimental animals that is accompanied by falls in blood pressure and/or cardiac output. To determine direct in vivo effects, in the present study, ANP was infused (12 ng·kg⁻¹·min⁻¹) directly into the mesenteric (iMA) and hepatic (iHA) arterial beds of anesthetized dogs, thereby minimizing changes in blood pressure. Over the first 2 min of iMA infusion, rate of change in mesenteric vascular resistance was 19.6 ± 5.4 mmHg·l⁻¹·min⁻¹·min⁻¹, reaching a maximum increase in resistance of 22 ± 4% compared with baseline after ~10 min. There was no evidence of vasodilatation at any stage. The mesenteric response was similar whether ANP was infused iMA, iHA, or via the femoral vein (30 ng·kg⁻¹·min⁻¹). In contrast, hepatic vasoconstrictor response to ANP infusion iHA or into the portal vein was only evident after ~5 min, reaching a maximum increase in hepatic vascular resistance of 11 ± 6% after ~15 min iHA infusion. When preinfused through the gut vasculature (iMA), ANP increased hepatic vascular resistance earlier and reached similar levels (14 ± 3%), despite a lower arterial concentration of ANP. It is proposed that a vasoconstrictor agent from the intestinal circulation contributed to ANP-induced splanchnic vasoconstriction.

atrial natriuretic factor; blood flow; fractional extraction; hepatic artery; hepatic autoregulation; intestinal tract; in vivo; mesenteric circulation; vascular resistance

DESPITE THE GENERALLY HELD view that atrial natriuretic peptide (ANP) is primarily a vasodilator hormone, evidence from in vivo studies indicates that infused ANP lowers blood pressure by reducing cardiac output rather than total peripheral resistance (31, 32, 40, 43). Indeed, in ganglion-blocked awake dogs (31, 32, 40, 43), rats (32), monkeys (32), and rabbits (42) total peripheral resistance either remained constant or increased, indicating that the lack of vasodilatation observed in intact animals was not due to overriding reflex effects from the sympathctic nervous system. The increases in total peripheral resistance were dose related (40, 41), independent of the renin-angiotensin system or vasopressin (40), and were due predominantly to vasoconstriction through the gastrointestinal vasculature (43). The vasoconstrictor actions of ANP occurred with intravenous infusions of 20–300 ng·kg⁻¹·min⁻¹ (reaching circulating concentrations ~0.05–2 ng/ml). In contrast, Shen and colleagues (31) demonstrated that at much higher intravenous bolus doses of ANP (9 µg/kg) peripheral vasodilatation occurred in all regions that were instrumented with flow probes, namely mesenteric, renal, coronary, and iliac regions. This vasodilator effect was transient and was followed by a prolonged peripheral vasoconstriction in all beds except the renal. All of this in vivo evidence that ANP causes vasoconstriction rather than vasorelaxation has largely been ignored in the prevailing literature (e.g., for reviews, see Refs. 11, 25), presumably because of the strong in vitro evidence that ANP is a vasodilator hormone acting through natriuretic peptide A-type (NPₘ) receptors coupled to membrane-bound guanylate cyclase (6). Isolated blood vessel studies have demonstrated convincingly that ANP relaxes preconstricted aortic rings and strips of large vessels such as abdominal aorta and rabbit facial vein (37). Sensitivity to the vasodilator actions of ANP was generally ~10⁻⁹ M or ~300 ng/ml, in line with the in vivo evidence of ANP activity at high levels cited above. By contrast, resistance-sized vessels, including mesenteric artery, were not sensitive to the vasorelaxant properties of ANP (1).

The hepatic microvasculature appears to react differently. Intrahepatic arterial ANP administered at doses of 0.1–50 nmol caused dose-related, transient vasodilatation in isolated blood-perfused dog liver (38) at levels >100 ng/ml, although infusion into the portal vein of this preparation was without vasoactivity. On the other hand, at much lower doses into the portal vein of isolated, Krebs-Henseleit-perfused rat liver, ANP antagonized phenylephrine-induced vasoconstriction of the hepatic bed (3), with a half-maximal effective dose for ANP activity of ~40 pmol/l (or ~120 pg/ml), which is close to the normal circulating level of ANP. NPₘ and NP₁ receptors have been localized in rat liver (27), and mRNA for all three NP receptors (NPₘ, NP₁, NP₂) has been detected in the human liver (34), supporting a vasodilator potential for ANP in this vascular bed. However, the in vitro evidence that ANP induces hepatic vasodilatation is inconsistent with the in vivo findings. In humans, hepatic blood flow fell during ANP infusion (4), although this was attributed to circulatory adjustments to reduced cardiac output with ANP. In our earlier study using radioactive microspheres to measure whole body regional blood flows in autonomically blocked conscious dogs, ANP infusion into the right atrium to steady state (75 ng·kg⁻¹·min⁻¹) resulted in an ~20% reduction, rather than a rise, in hepatic arterial blood flow (43). It is possible that complex intracellular interactions may be responsible for ANP-induced antagonism of vasoconstriction or that the isolated organ preparation has different sensi-
tivity from the in vivo preparation. Thus one of the aims of the present study was to determine the hepatic arterial blood flow response to ANP administered directly into the hepatic artery to expose in vivo the vasodilator response that had previously only been observed in vitro.

There has been no evidence from in vitro studies that ANP directly constricts mesenteric vasculature. Because all previous in vivo studies reporting ANP-induced mesenteric vasoconstriction used the intravenous route of administration (5, 13, 24, 28, 31, 32, 40, 41, 43), which could evoke release of a secondary constrictor factor from outside the splanchnic circulation, we aimed to investigate in the present study whether direct arterial versus intravenous administration of ANP would result in differential splanchnic blood flow responses to the peptide.

The role of the splanchnic circulation in the removal of ANP from the plasma is important because a number of disease states with associated poor hepatic function are known to have elevated plasma ANP levels (e.g., for review, see Ref. 9). Although the liver has been generally viewed as a site for metabolic degradation of ANP, gastrointestinal organs have not been similarly considered. Thus, in the present study, we determined the contribution from gut and liver to extraction and uptake of ANP from the plasma under baseline conditions and during continuous infusion of ANP.

METHODS

Surgical Preparation of Dogs

Experiments were performed on 15 male greyhound dogs (body wt 22–30 kg). On the day of the experiment the dog was anesthetized with intravenous pentobarbital sodium (30 mg/kg, then with additional nonhypotensive doses as needed). The trachea was intubated followed by positive-pressure ventilation with oxygen-enriched room air (large animal respirator; Harvard Apparatus, South Natick, MA) at an end-expiratory pressure of 4 cmH2O. Ventilation was adjusted to maintain arterial CO2 tension between 35 and 40 mmHg, and arterial O2 tension ranged from 140 to 220 mmHg. Ventilation was adjusted to maintain arterial CO2 tension between 35 and 40 mmHg, and arterial O2 tension ranged from 140 to 220 mmHg. Arterial pH was maintained between 7.3 and 7.4 with intravenous bicarbonate as necessary. Isotonic fluids (0.18% saline plus 4% glucose; 0.5 liter, Baxter) were administered over the first 30 min after induction of anesthesia and then infused at ~400 ml/h until the end of the experiment. A bladder catheter was inserted, and urine was drained periodically.

The left saphenous artery and vein were cannulated with polyvinyl catheters (SV65, 1.52 mm OD; Dural Plastics & Engineering, Dural, NSW), one in the artery and two in the vein. The arterial catheter was advanced 10 cm so that the tip lay in the abdominal aorta for arterial blood pressure measurement and blood sampling. One of the venous catheters was inserted 25–30 cm with the tip lying in the upper abdominal vena cava for central venous pressure measurement. The second venous catheter was inserted 5–7 cm with the tip in the femoral vein for infusion of ANP. Through a midline abdominal incision, the spleen was removed and catheters were inserted into the larger posthepatic vein (SV65 through a 14-gauge stainless steel needle), portal vein (SV65 palpatated from a mesenteric vein so that the tip lay in the portal vein 12–20 cm from the site of entry), cranial mesenteric artery (SV31, 0.80 mm OD; Dural Plastics & Engineering; inserted by the method of Herd and Barger (20)), and hepatic artery (SV31, as for mesenteric artery). Electromagnetic flow probes were placed around the cranial mesenteric (3.5–4.5 mm ID) and hepatic (2.5–3.0 mm ID) arteries, and an occluding balloon cuff was placed distally to the probe on the mesenteric artery and proximally to the probe on the hepatic artery. Electronic zero for each blood flow measurement was checked and adjusted if necessary before the start of each infusion period by momentary inflation of the occluding cuff. The size of the flow probe was matched to the size of each artery, and the flow probes were calibrated externally with a calibration factor for each probe used to convert voltage to flow (27–77 ml·min⁻¹·100 mmH⁻¹).

Physiological Measurements, Calculations, and Assays

Pulsatile blood pressures were measured in the aorta, portal vein, and abdominal vena cava via Statham P23 Db pressure transducers, and phasic and mean pressures were recorded on a Neomedix Systems model 8002F eight-channel recorder. Heart rate was obtained from a Neotrace tachograph, triggered by the aortic blood pressure signal. Blood flows were measured by a pulsed-logic electromagnetic blood flowmeter (Biotronix Lab). Vascular resistance was calculated as mean arterial pressure minus central venous pressure divided by blood flow to the particular vascular bed. During each experimental period, physiological data were continuously digitized on an Olivetti computer with an analog-to-digital conversion program via a Metabyte data acquisition card at 300-Hz sampling rate with binning of data over 5- or 10-s averages and then further averaged over 5-min intervals for statistical analysis (except for the responses to bolus doses, where 5-s data were plotted).

Whole body metabolic clearance rate was calculated from the rate of ANP infusion divided by the difference in plasma ANP concentration between the resting level and steady-state concentration during infusion (39). Fractional extraction (FE) of ANP across the gastrointestinal tract was calculated as

\[
FE = \left(1 - \frac{\text{ART}_{\text{ANP}}}{\text{PV}_{\text{ANP}}}ight) \times 100%\]

where \(\text{ART}_{\text{ANP}}\) is arterial plasma ANP concentration, and \(\text{PV}_{\text{ANP}}\) is portal venous plasma ANP concentration. This calculation was made during baseline, during femoral vein infusion of ANP, and during hepatic arterial infusion of ANP because the arterial concentration perfusing the gut was known for these times. FE of ANP across the whole of the splanchnic bed (gut plus liver) was calculated as

\[
FE = \left(1 - \frac{\text{ART}_{\text{ANP}}}{\text{HV}_{\text{ANP}}}ight) \times 100%\]

where \(\text{HV}\) is the posthepatic vein. This calculation assumed that the hepatic venous sample was representative of venous outflow from the liver, which is a reasonable assumption because the sample was obtained from the major hepatic vein draining the left lateral, left medial, quadrate and right medial lobe of the liver. Splanchnic FE of ANP was calculated only when steady-state arterial ANP concentration was known, that is, during baseline measurements and during femoral vein infusion when there was no further contribution from direct arterial infusion of ANP into the splanchnic circulation. Regression analysis was performed between FE and arterial concentration of ANP with the second-order polynomial fitting the data to an \(r^2\) value >0.9.

Clearance of ANP by the liver and gastrointestinal tract could not be determined accurately in the present study.
because direct measurements of portal blood flow were not made. However, the values for hepatic arterial blood flow were used to estimate portal venous blood flow because data from conscious, fasted dogs showed that portal blood flow was between 65 and 75% of total liver blood flow (10). Indeed, a 70% portal venous to 30% hepatic arterial blood flow has previously been used to estimate hepatic extraction of ANP in the rat (16). Removal of ANP by the liver was calculated from the amount of ANP going in to the liver

\[(PV_{ANP} \times \text{estimated PVPF}) + (ART_{ANP} \times HAPF)\]

where PVPF is portal venous plasma flow, and HAPF is hepatic arterial plasma flow, compared with that leaving the liver

\[HV_{ANP} \times (HAPF + \text{estimated PVPF})\]

and expressed as a percentage of the difference divided by the input.

Blood (5 ml) was collected into chilled tubes containing EDTA and centrifuged at 4°C, and the plasma was frozen at −20°C for subsequent radioimmunoassay measurement of ANP. Details of the radioimmunoassay were previously published (39, 40, 42, 43). Briefly, ANP was extracted from 0.5-ml aliquots of plasma using Sep-Pak C18 cartridges and eluted with 70% methanol and 0.1% trifluoroacetic acid. The concentration of ANP in plasma was measured using an antisera against α-human ANP (α-hANP) that was produced in rabbits at the Baker Institute, a commercially available synthetic standard α-hANP (Peninsula Laboratories, Belmont, CA), and an iodinated α-hANP tracer (Amersham International, Little Chalfont, Buckinghamshire, UK). There was no crossreactivity of the ANP antisera with brain natriuretic peptide-32 (human), C-type natriuretic peptide-22 (human), prepro-ANP (1–67), pro-ANP (1–30), pro-ANP (31–67), and the fragments of human ANP (1–11), (12–28), and (18–28). Recoveries of added synthetic α-hANP to plasma were ~75%, and individual plasma levels have not been corrected for extraction losses. All plasma samples from each dog were extracted together and measured in the same assay to reduce extraction losses. All plasma samples from each dog were extracted together and measured in the same assay to reduce extraction losses.

Statistical Analysis

Plasma ANP levels were analyzed for significant differences between sampling sites by one-way ANOVA with a Bonferroni adjustment for multiple pairwise comparisons (Fig. 1). All contrasts were considered significant, and the null hypothesis was rejected when P was < 0.05.

The hemodynamic data were analyzed by two-way ANOVA with three factors (dogs × treatments × blocks). The within-animal design of the study allowed the between-dogs sums of squares (SS), as well as the between-columns (treatments, blocks) SS, to be removed from the total SS to give the residual SS. The latter was used for all subsequent contrasts and to determine the SE, indicating variation within animal. Blocks were the three infusion sites and columns were 5-min averages, either as absolute values or change from the preinfusion resting value. The SS between blocks were compared to determine significant differences between infusion sites (Figs. 4 and 5). Data that are depicted in Tables 1 and 2 and Figs. 1 and 3 are means ± SE (between-animal estimate of variance). Data in Figs. 4 and 5 are means ± SE from ANOVA (within-animal estimate of variance).

To analyze the data from the first 120 s of each infusion into each dog, a regression analysis between absolute level of either blood flow or vascular resistance and time determined the rate of change (slope). Slopes from regressions were tested for significant differences by two-way ANOVA with Bonferroni adjustment for multiple comparisons (Fig. 3). To determine whether there were any significant time-related changes over the entire experiment, all preinfusion values were tested by two-way ANOVA.

RESULTS

Plasma ANP Levels, Splanchnic Fractional Extraction, and Whole Body Metabolic Clearance of ANP

Endogenous ANP levels. In 7 of 10 dogs, baseline levels of plasma ANP were higher in the portal vein than in the aorta, resulting in an overall increase of
~23% (P = 0.129; Fig. 1). Extraction ratio across the gut, however, was 22.6 ± 8.3% (P = 0.024), indicating a net production of ANP across the gastrointestinal tract. The mean fall of 4 ± 3 pg from aorta to hepatic vein was not significant (P = 0.150; Table 1 and Fig. 1). Plasma ANP concentration was significantly lower in the hepatic vein compared with the portal vein (P = 0.003; Table 1 and Fig. 1), which may reflect extraction across the venous bed of the liver. Under these baseline conditions, the fractional extraction of ANP across the whole of the splanchnic circulation was 8.7 ± 5.5% and not significantly different from zero extraction (P = 0.343), reflecting the net effect of a small amount of ANP released into the portal vein but subsequent extraction of the peptide by the liver. The estimated amount of ANP going into the liver was 10.2 ± 2.4 ng/min and outgoing was 8.4 ± 2.5 ng/min, resulting in removal of ANP by the liver of 21.6 ± 5.5%

Table 1. Plasma ANP levels, blood pressures, heart rate, and Hct

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>FV Infusion</th>
<th>MA Infusion</th>
<th>HA Infusion</th>
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</thead>
<tbody>
<tr>
<td>Arterial [ANP], pg/ml</td>
<td>28 ± 4</td>
<td>761 ± 72</td>
<td>84 ± 8</td>
<td>270 ± 58</td>
</tr>
<tr>
<td>Portal vein [ANP], pg/ml</td>
<td>32 ± 3</td>
<td>332 ± 37</td>
<td>799 ± 83</td>
<td>143 ± 19</td>
</tr>
<tr>
<td>Hepatic vein [ANP], pg/ml</td>
<td>24 ± 4</td>
<td>271 ± 39</td>
<td>270 ± 83</td>
<td>793 ± 156</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>106 ± 5</td>
<td>93 ± 5*</td>
<td>103 ± 7</td>
<td>101 ± 6</td>
</tr>
<tr>
<td>Central venous pressure, mmHg</td>
<td>5.2 ± 0.5</td>
<td>5.0 ± 0.5</td>
<td>5.2 ± 0.6</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td>Portal venous pressure, mmHg</td>
<td>7.5 ± 0.3</td>
<td>7.3 ± 0.4</td>
<td>7.6 ± 0.5</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>106 ± 6</td>
<td>111 ± 6</td>
<td>112 ± 6</td>
<td>114 ± 6</td>
</tr>
<tr>
<td>Arterial Hct, %rbc</td>
<td>49.3 ± 0.6</td>
<td>50.3 ± 0.8</td>
<td>50.6 ± 0.6*</td>
<td>50.2 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SE from between-animal estimate of variance. Blood samples were withdrawn simultaneously from 3 sites during initial control period and during the last 5 min of each 15 min infusion into the femoral vein (FV; 30 ng·kg⁻¹·min⁻¹), mesenteric artery (MA; 12 ng·kg⁻¹·min⁻¹), or hepatic artery (HA; 12 ng·kg⁻¹·min⁻¹). [ANP], plasma atrial natriuretic peptide concentration; Hct, hematocrit; rbc, red blood cells. Values presented for mean arterial pressure, central venous pressure, and portal venous pressure are from data averaged over 5 min during baseline and during the 10 to 15-min phase of the infusion period. *Significant (P < 0.05) change from baseline.
artery or femoral vein produced similar hepatic venous ANP concentrations of ~270 pg/ml, despite substantial differences between the two sites in their effects on hepatic arterial and portal venous input concentrations of ANP. Hepatic arterial concentration of ANP was about nine times higher after femoral vein infusion than with mesenteric artery infusion, whereas portal vein concentration of ANP was about three times higher with mesenteric artery infusion than with femoral vein infusion. This may indicate a preferential removal of ANP by the liver from the arterial side of the hepatoporta lbed. With direct infusion of ANP into the hepatic artery, hepatic venous concentration was ~800 pg/ml, despite the relatively low levels of ANP in the portal vein (~150 pg/ml). This was likely due to the high input of ANP from the hepatic artery (~3,270 pg/ml), calculated by (arterial$\text{[ANP]}$ - infused$\text{[ANP]}$), where infused$\text{[ANP]}$ was estimated from hepatic plasma flow of ~100 ml/min and infusion rate of ~300 ng/min.

Estimated total amount of ANP going into the liver during femoral vein infusion was 111.9 ± 14.6 ng/min and outgoing was 74.7 ± 11.2 ng/min, resulting in an uptake of ANP across the liver of 33.2 ± 6.2%. Whole body metabolic clearance rate of ANP was 1.28 ± 0.24 l/min, which was similar to our previous measurements of 1.10 l/min during steady-state infusion of ANP to conscious dogs (39). Compared with baseline (endogenous) measures, extraction of ANP across the gut and the total splanchnic region increased during infusion of the peptide into the femoral vein to 55.3 ± 3.1 and 61.1 ± 6.1%, respectively. When extraction data from endogenous levels and infusions were pooled, there was a significant association between log arterial plasma ANP level and percentage of fractional extraction of ANP (Fig. 2) across the gastrointestinal tract ($y = -222.3 + 185.3x - 30.5x^2$; where $y = \%$fractional extraction of ANP and $x = \log \text{ANP}$; $r^2 = 0.907, P < 0.001$).

**BLOOD PRESSURES, HEMATOCRIT, AND HEMODYNAMIC STABILITY OF THE PREPARATION**

Arterial blood pressures fell significantly by ~10 mmHg during the infusion of ANP into the femoral vein (Table 1) but were unchanged during the direct infusions into the mesenteric or hepatic arteries (Table 1). Central venous and portal venous pressures and heart rate were not significantly changed during any of the ANP infusions (Table 1). There was no evidence for time-related effects on any hemodynamic variable or hematocrit (Table 2).

**Splanchnic Hemodynamic Responses to ANP Infusions**

Acute (first 2 min of infusions) effects. Mesenteric blood flow fell and mesenteric vascular resistance increased over the first 2 min of ANP infusion into the femoral vein or into the mesenteric artery (Fig. 3A). Mesenteric vascular resistance (Fig. 3A, bottom) increased at mean rates of 14.7 ± 2.9 mmHg·l⁻¹·min⁻¹ (P < 0.001) during femoral vein infusion and at 19.6 ± 5.4 mmHg·l⁻¹·min⁻¹ (P = 0.003) during mesenteric artery infusion. The mesenteric vasoconstriction was in the order of ~4% change in vascular resistance per minute for the first 2 min. There were no significant differences in mesenteric response between these two infusion sites (Fig. 3A). By contrast, infusion of ANP via the hepatic artery resulted in much less mesenteric vasoconstriction than with the other two sites (Fig. 3A) over the first 2 min. The rate of change in vascular resistance during hepatic artery infusion of ANP was 3.0 ± 1.7 mmHg·l⁻¹·min⁻¹ (P = 0.054, Fig. 3A, bottom). There was no evidence of vasodilatation in the hepatoportal bed.

**Table 2. Preinfusion (resting) values**

<table>
<thead>
<tr>
<th>Value</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>105 ± 6</td>
<td>101 ± 6</td>
<td>101 ± 6</td>
</tr>
<tr>
<td>CVP, mmHg</td>
<td>5.0 ± 0.4</td>
<td>4.9 ± 0.5</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>PVP, mmHg</td>
<td>7.1 ± 0.5</td>
<td>7.2 ± 0.5</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>108 ± 6</td>
<td>111 ± 6</td>
<td>111 ± 7</td>
</tr>
<tr>
<td>MBF, ml/min</td>
<td>323 ± 30</td>
<td>305 ± 31</td>
<td>332 ± 22</td>
</tr>
<tr>
<td>MVR, mmHg·l⁻¹·min⁻¹</td>
<td>337 ± 35</td>
<td>319 ± 20</td>
<td>307 ± 24</td>
</tr>
<tr>
<td>HFR, ml/min</td>
<td>195 ± 23</td>
<td>202 ± 22</td>
<td>212 ± 23</td>
</tr>
<tr>
<td>HVR, mmHg·l⁻¹·min⁻¹</td>
<td>571 ± 64</td>
<td>511 ± 60</td>
<td>506 ± 59</td>
</tr>
<tr>
<td>Hct, %</td>
<td>49.3 ± 0.6</td>
<td>49.7 ± 1.1</td>
<td>49.9 ± 1.0</td>
</tr>
</tbody>
</table>

Values expressed are mean ± SE from between-animal variance. Order of infusions of ANP into different sites was randomized. Hemodynamic measurements over time (consecutive periods 1, 2, and 3, respectively) are shown in this table of averaged data collected during the 5-min runin at start of each infusion period. MAP, mean aortic pressure; CVP, central venous pressure; PVP, mean portal venous pressure; MFR, cranial mesenteric arterial blood flow; MVR, mesenteric arterial vascular resistance; HBF, hepatic arterial blood flow; HVR, hepatic arterial vascular resistance.
the mesenteric vasculature with any of the infusions, even within the first 5 s of infusion (data not shown).

The acute effects of ANP infusions on the hepatic vasculature (Fig. 3B) were quite different from those on the mesenteric vasculature. There was no evidence of vasoconstriction in the hepatic arterial vasculature over the first 2 min of ANP infusions, regardless of the site of infusion (Fig. 3B). Mean rates of change in hepatic vascular resistance during this time were $-11.4 \pm 10.8, -0.3 \pm 3.2,$ and $-0.1 \pm 2.3$ mmHg $\cdot$ $1^{-1}$ $\cdot$ min $^{-1}$ during hepatic arterial, mesenteric arterial, and femoral venous infusions, respectively, with no significant differences between the sites. However, during direct hepatic arterial infusions of ANP, seven of ten dogs had significant ($P < 0.05; \gamma > 0.399$) negative rates of change in hepatic arterial vascular resistance, indicating vasodilatation. Two of the other three dogs had significant positive slopes, with the third unchanged. ANP-induced vasodilatation was not as apparent with the other infusion sites because there were only three significant negative slopes during femoral vein infusion and only one during mesenteric artery infusion.

Fifteen-minute infusions and recovery. After the first 2 min of each of the infusion periods, mesenteric blood flow fell progressively and mesenteric vascular resistance increased progressively (Fig. 4A). During mesenteric artery infusion, the maximum changes in flow and resistance were $-18 \pm 2$ and $22 \pm 4\%$, respectively, and $-25 \pm 3$ and $20 \pm 4\%$, respectively, during femoral vein infusion (Fig. 4A). After turning off the infusions there was a rapid recovery of flow and resistance toward baseline levels (Fig. 4A). When the changes in blood flow were normalized for changes in arterial pressure (Table 2), there were no significant differences among responses of mesenteric vascular resistance to the three infusion sites (Fig. 4A, bottom, comparing all data points during infusion and recovery periods). Thus, although the onset of mesenteric vasoconstriction was slower in the early phase during hepatic artery infusion of ANP (Fig. 3), mesenteric vasoconstriction prevailed regardless of the site of infusion. The delayed onset with hepatic artery infusion coincided with the markedly lower arterial plasma levels of ANP (Table 1). By $\sim 10$–15 min of infusion into the hepatic artery, the increase in mesenteric vascular resistance ($20 \pm 4\%$) was equivalent to that achieved with the other two sites of infusion (Fig. 4A).

Hepatic blood flow and hepatic arterial vascular resistance were slower to respond to ANP infusions than the mesenteric vasculature (Figs. 3 and 4). After 10 min of infusion, however, the hepatic arterial vasculature also constricted, regardless of infusion site (Fig. 4B). Between 10 and 15 min, hepatic vascular resistance increased by $12 \pm 4$ and $14 \pm 3\%$, with femoral venous and mesenteric arterial infusions, respectively. When all the data over the infusion plus recovery periods were compared, the changes in hepatic vascular resistance were significantly less when ANP was infused into the hepatic artery compared with the mesenteric artery ($P = 0.033$) (Fig. 4B). After each infusion was turned off, there was a tendency for vasoconstriction to continue unabated for the first 5 min, then vascular resistance fell to preinfusion levels (Fig. 4B).

In contrast to the mesenteric vascular response to ANP, there was no dose relationship between arterial ANP levels and hepatic arterial vasoconstriction. Indeed, the least hepatic vasoconstriction occurred when the hepatic arterial ANP level was the highest (estimated 1,800 pg/ml). At the same time, however, portal venous concentration of ANP was the lowest (Table 1). This observation lead to the possibility that, if ANP caused vasoconstriction directly, then the peptide may need to be presented preferentially from the hepatoporal rather than arterial side of the dual hepatic circulation. To test this, ANP was infused directly into the portal vein and compared with infusions into the hepatic artery and mesenteric artery in an additional group of five dogs.

Portal Vein Infusions Versus Hepatic and Mesenteric Arterial Infusions

Direct infusion of ANP ($12$ ng $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$) into the portal vein resulted in time-related increases in hepatic vascular resistance (Fig. 5). These changes were not different from the responses to intrahepatic arterial infusions of ANP at the same concentration (Fig. 5). As found in the first group of 10 dogs, the increase in
hepatic vascular resistance in response to mesenteric arterial infusion of ANP was approximately twice as great as either the hepatic arterial (P < 0.05) or portal venous (P < 0.05) infusions (Fig. 5).

Direct Bolus Injections of ANP

Bolus injections of ANP of 1, 2, 5, and 10 µg directly into the mesenteric artery or hepatic artery of two dogs resulted in transient dose-related vasodilatation in the respective vascular beds (Fig. 6, illustrating 5-s averages for each dog, 30 s before and 90 s after administration of ANP).

DISCUSSION

Major Findings

The major new findings of the present study were threefold. First, ANP caused marked vasoconstriction of both mesenteric and hepatic arterial regions in vivo, whether administered directly into the splanchnic region or via the systemic circulation. Second, the effect of ANP on liver blood flow appeared secondary to the activation of a constrictor agent released into the portal venous blood because the hepatic vasoconstriction was greatest after ANP had previously passed through the gastrointestinal bed. This factor was unlikely to be ANP itself because infusions of the peptide directly into the portal vein resulted also in attenuated hepatic vasoconstriction compared with that after ANP had previously passed through the gut vasculature. Third, the gastrointestinal region was an important site for extraction of ANP from the circulation as well as an extracardiac site for release of the peptide into plasma, detectable when circulating levels were low.

Vasoconstrictor Actions of ANP

Previous studies from our laboratory and others’ demonstrated that ANP caused splanchnic vasoconstriction in vivo when the peptide was administered intravenously to experimental animals (5, 13, 24, 26, 28, 31, 40, 41, 43) and to humans (4, 29). In those studies, ANP caused systemic hemodynamic effects, such as hypotension and/or reduced cardiac output, which could significantly influence the distribution of blood flow to the splanchnic region independently of any direct actions of the hormone. Given that a major prevailing view is that ANP is primarily a vasodilator hormone, it was expected that the present experiments using direct admin-
administration of ANP into the mesenteric and/or hepatic arteries may result in splanchnic vasodilatation. Only when we administered the peptide at very high bolus doses (µg or nmol) directly into these arteries was there transient, dose-related vasodilatation. These observations support the findings from an earlier study by Shen and colleagues (31) who demonstrated that an intravenous bolus of ANP at 9 µg/kg into conscious, ganglion-blocked dogs caused transient vasodilatation in all vascular beds studied, including the mesenteric, but a lower intravenous continuous infusion of ANP (300 ng·kg⁻¹·min⁻¹) resulted in vasoconstriction in the mesenteric and iliac beds (31). So what is the mechanism for the vasoconstrictor responses to ANP in both mesenteric and hepatic arterial beds? The natriuretic peptide receptors NPA and NPB are coupled to guanylate cyclase and activation of cGMP, which mediates vasodilatation. The NPR receptor is coupled to the inhibition of cAMP and enhanced phosphatidylinositol turnover. Until recently this receptor was thought to be merely a clearance receptor but it could cause vasoconstriction through the adenylyl cyclase and phosphatidylinositol signal transduction pathways (2). However, because there is no in vitro evidence that ANP acts directly on blood vessels to cause vasoconstriction, the present study addressed the possibility that the splanchnic vasoconstrictor action of ANP was through activated release of a secondary constrictor agent. From analysis of the time course and magnitude of the splanchnic vasoconstrictor responses to ANP with the three different sites of administration of the peptide, it is unlikely that a secondary agent from a site outside the splanchnic circulation, such as the lungs, is involved. Moreover, hepatic vasoconstriction was greatest after ANP had already perfused the gastrointestinal circulation, implicating the gut as the site for release. One such hormonal candidate is a catecholamine released from extra-adrenal abdominal chromaffin cells, because we recently demonstrated in conscious dogs that ANP-induced mesenteric vasoconstriction was largely prevented by α-adrenergic blockade (unpublished observations) but not by autonomic ganglion blockade (32, 40, 43).

Vasodilatation Versus Vasoconstriction

Whether the overall vascular responses to ANP in both mesenteric and hepatic arterial beds were vasore-

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**Fig. 5.** Hepatic vascular resistance responses to infusion of ANP (12 ng·kg⁻¹·min⁻¹) directly into the portal vein (iPV; ▼), iHA (●), or iMA (○). Baseline level of vascular resistance (before infusions) is given in parenthesis as mean of 10 min averages ± SE (between-animals estimate). Points are mean changes from preinfusion values averaged over consecutive 5-min periods during the 10-min ANP infusion and 10-min recovery period in 5 dogs. Error bars refer to SE from 2-way ANOVA (within-animal estimate of variance). *Significant (P < 0.05) differences between iHA or iPV and iMA sites of infusion (using contrasts between blocks of all data points during infusion and recovery periods from ANOVA).

**Fig. 6.** Mesenteric (A) and hepatic (B) arterial blood flow responses to bolus injections of ANP at 1, 2, 5, and 10 µg directly into the mesenteric artery (MA) or hepatic artery (HA) into 2 dogs (● and ○, respectively). Points are the average of consecutive 5-s intervals.
laxant or vasoconstrictor was dose related. Similar to the observations of Shen and colleagues (31), who administered ANP intravenously to conscious, instrumented dogs, high bolus doses of ANP given directly into the mesenteric bed elicited transient vasodilator effects (Fig. 6), whereas lower dose infusions caused only vasoconstriction. Shen and colleagues also showed a prolonged vasoconstrictor response that followed the vasodilatation after bolus administration. In the liver, intrahepatic arterial administration of high bolus doses evoked transient, dose-related vasodilatation (Fig. 6). At the lower doses, with direct infusion of ANP into the hepatic artery, there was a tendency toward vasodilatation over the first few minutes (Fig. 3), although this was not evident in all dogs. With the other sites of administration, where the dose of ANP reaching the hepatic vasculature was lower than with the direct intrahepatic route, there was no evidence for hepatic vasodilatation (Fig. 3). The subsequent vasoconstriction during infusions of ANP depended on 1) the route of administration of the hormone, that is, whether ANP had previously passed through the gut, and 2) any counteracting vasodilatation induced directly by ANP through NP receptors. Studies with a selective NP receptor antagonist are needed to address this issue further.

Hepatic Autoregulation

Hepatoportal venous and hepatic arterial blood flows vary reciprocally to regulate total liver blood flow, with the arterial system autoregulating to changes in portal venous pressure and flow (for review, see Ref. 10). In the present study, vasoconstriction in the gastrointestinal region should have reduced portal venous inflow, resulting in autoregulatory increase in hepatic arterial blood flow. The observations that ANP induced simultaneous vasoconstriction in hepatic and mesenteric arterial beds with subsequently reduced hepatopetal flow demonstrate that the vasoconstrictor capacity of the peptide can override the autoregulatory processes within the hepatic arterial bed. Furthermore, responsiveness of the hepatic arterial bed to ANP was not restricted to the peptide’s access from the arterial blood, because infusion directly into the portal vein also resulted in hepatic arterial vasoconstriction. In a study by Withrington and colleagues (38) using the isolated dog liver, ANP was without apparent effect on hepatoportal hemodynamics when infused directly into the portal vein. The present study indicated that the maximum hepatic vasoconstrictor effect, in vivo, occurred after ANP was perfused through the gut vasculature before reaching the hepatic circulation. Thus, in vitro, the absence of a constrictor agent released from the gut could explain why ANP did not cause vasoconstriction in the isolated liver (38).

Production of ANP by the Gut

ANP immunoreactivity has been found in extracts of rat (35), guinea pig (33), and human gastrointestinal tissues (15). Both the circulating form of ANP and the prohormone were detected, suggestive of synthesis of ANP within the intestine. The presence of ANP-mRNA in the adult rat (18) and human (14) gastrointestinal tract at levels of 1–10% those found in the cardiac atrium strongly supports the suggestion that ANP can be produced by gastrointestinal tissue. These observations have led to the suggestion that ANP may play a paracrine or autocrine role in salt and water balance of the gastrointestinal tract. Our findings are the first to detect spillover of ANP into the plasma from the gastrointestinal tract, although the peptide released into the hepatoportal vessels is probably confined to the splanchnic circulation because there was a significant drop in ANP concentration between portal vein and hepatic vein (Fig. 1). The overload of ANP from the gut into the portal vein was detected only at arterial concentrations of ANP < 40 pg/ml (Fig. 2). Because this endogenous contribution to plasma levels was small, fractional extraction ratio readily shifted from negative to positive when arterial levels were elevated. These two variables were positively associated, reflecting the substantial capacity of the organs draining into the portal vein to take up ANP as the arterial plasma levels rose (Fig. 2). Another study measuring extraction ratio across the gastrointestinal region in the anesthetized rat did not detect production of ANP (16), possibly due to the high aortic levels of ANP, averaging ~165 pg/ml (16). With the use of the second-order polynomial relationship described in Fig. 2, the fractional extraction of ANP across the gut at arterial ANP concentration of 165 pg/ml was 39% in the dog, very close to the measured mesenteric extraction ratio of 42% in rats (16). Because the splanchnic region makes a major contribu-
tion to whole body metabolic clearance (21, 26, 39), it is likely that the maintenance of relatively constant metabolic clearance as circulating levels of ANP rise (22) is, at least in part, due to 1) the high fractional extraction of ANP across the gut and 2) the effects of ANP to reduce mesenteric blood flow (Figs. 2, 4, and 5).

Hepatic Removal of ANP

There has been considerable interest in the role of the liver to remove ANP from the circulation in humans, because the elevated plasma levels of ANP in diseases such as cirrhosis and congestive cardiac failure may be related to hepatic dysfunction. Because it is not possible to measure directly the contribution of the liver to removal of a substance from the circulation without portal vein sampling, splanchnic extraction (arterial-hepatic vein) has been taken to reflect removal predominantly by the liver, estimated as 35-50% in humans (e.g., Refs. 7, 19). As noted above, from studies by others and the present data, the gastrointestinal bed makes a considerable contribution to removal of ANP from the arterial plasma. Nevertheless, with the use of indirect estimates of hepatic uptake of ANP, the present and other studies (16, 21) indicate a major contribution also from the liver to removal of the peptide from portal venous and arterial plasma. In the present study, hepatic removal of ANP was in the range of 20–50%, related to the level of ANP in the portal vein over the range of 30–800 pg/ml or hepatic artery (Table 1). By contrast, when the hepatic component of splanchnic extraction was measured directly during infusion of ANP at 300 ng·kg\(^{-1}\)·min\(^{-1}\) into conscious dogs, which increased circulating levels to >4,000 pg/ml, Matsushita and colleagues (26) calculated that only 1% of circulating ANP was removed by the hepatic vasculature. This calculation of hepatic removal was a cumulative estimate during 30 min of ANP infusion plus a period of 10 min after the infusion was turned off when hepatic and portal blood flows were changing considerably. Thus measurements of uptake under these non-steady-state conditions must be viewed with caution. Moreover, it is possible that the very high circulating levels in the Matsushita study approached saturation of the uptake pathways in the liver.

In conclusion, infusion of ANP directly into the hepatic artery, mesenteric artery, and portal vein to achieve local levels within the range observed through endogenous release of the peptide, resulted in vasoconstriction in both mesenteric and hepatic arterial beds. The constriction was reversible and occurred in the absence of arterial or venous pressure changes. ANP-induced hepatic arterial vasoconstriction occurred when hepatoportal blood flow was also reduced, indicating that the vasoconstrictor response to ANP was powerful enough to override autoregulation of hepatic arterial blood flow. Maximum hepatic vasoconstriction was observed only after ANP had first perfused the gastrointestinal bed, implicating a secondary factor released from the gut region contributing to the vasoconstriction in the liver. At very high doses, ANP caused transient dose-related vasodilatation in both hepatic and mesenteric beds when administered intra-arterially. At the infusion rate of 12 ng·kg\(^{-1}\)·min\(^{-1}\) directly into the hepatic artery, there was evidence of hepatic arterial vasodilatation in some animals, suggestive of the possibility that ANP-induced vasodilatation may counteract subsequent vasoconstriction in the liver induced by a secondary agent from the gut. The gut was shown to be both a source of ANP and an important region for removal of the peptide from plasma. Our data support the conclusion that both the hepatic and the gastrointestinal beds make an important contribution to the removal of ANP from the circulation.

Perspectives

This study raises the possibility of two major functions of ANP. First, the existence of a local ANP system where the hormone is produced, acts, and is degraded within the splanchnic bed. The stimulus for activation of such a system is unknown but one might speculate that salt ingestion could be a trigger. Subsequent gastrointestinal vasoconstriction may prevent rapid absorption of excess salt and water. Second, splanchnic vasoconstriction caused by ANP released from the heart may have a teleological advantage, related to its effects on cardiac output. We know that a major hemodynamic response to ANP is a reduction in atrial filling pressures and thereby cardiac output (31, 32, 40, 43). Although this cardioprotective action of ANP guards against excessive increases in preload, without a selective redistribution of the reduced cardiac output, blood flow to vital areas of the peripheral circulation could be compromised. By vasoconstriction, ANP can redirect flow away from digestive organs to other beds requiring a greater proportion of the cardiac output. The splanchnic region is an appropriate target for such a redistribution of blood flow because it has a high capacity and demands most oxygen when postprandial. The physiological role of ANP in postprandial gastrointestinal hyperemia awaits our understanding.

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REFERENCES
