Atrial, B-type, and C-type natriuretic peptides cause mesenteric vasoconstriction in conscious dogs

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Woods, Robyn L., and Marcus J. M. Jones. Atrial, B-type, and C-type natriuretic peptides cause mesenteric vasoconstriction in conscious dogs. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1443–R1452, 1999.—Cardiovascular responses were compared with equimolar infusions of B-type (BNP) and C-type (CNP) with atrial natriuretic peptide (ANP) in conscious, instrumented dogs. On separate days, each natriuretic peptide or vehicle was infused (intravenously) at step-up doses of 2, 5, 10, and 20 pmol·kg⁻¹·min⁻¹ (20 min each dose) to increase circulating levels of the infused peptide from 2- to 20-fold. Like ANP, infusions of BNP caused dose-related increases (P < 0.05) in mesenteric vascular resistance, urine flow, natriuresis, and hematocrit (changes at highest doses were 60 ± 9, 334 ± 113, 313 ± 173, and 12 ± 2%, respectively). BNP also lowered (P < 0.05) plasma renin activity (–43 ± 11%) and arterial pressure (–10 ± 3%). Effects of BNP were independent of reflex sympathetic activation, since autonomic ganglion blockade did not attenuate the responses. CNP infusions had little effect except to increase (P < 0.05) mesenteric vascular resistance (27 ± 10%) and plasma ANP (41 ± 7%). Cardiovascular actions of BNP, like those of ANP, counteract the renin-ANG system and may protect the heart by lowering cardiac preload (venous return) and afterload (arterial pressure) while maintaining blood flow to extrasplanchic regions.

atrial natriuretic factor; blood pressure; atrial natriuretic peptide; brain natriuretic peptide; hemoconcentration; in vivo; kidney; plasma renin activity

METHODS

Surgical preparation. Six male greyhound dogs (31.4 ± 0.8 kg) were fed a fixed diet of raw meat and commercial kibble resulting in an average daily urinary excretion of ~135 mmol sodium and ~136 mmol potassium. Two weeks before surgery, dogs were trained to lie quietly recumbent on the padded experimental table. Each training period was extended until the dog remained on the table for a time equivalent to the duration of an experiment. Two days before surgery, each dog received metronidazole (400 mg; Flagyl; Anapharm, Queensland, Australia) twice daily and 10 ml septrin (Bactrim; Wellcome Australia, Cabarita, NSW, Australia) orally. The course of antibiotics was continued post surgery for 7 days. The dog was premedicated with 2 mg Promex-2 (acetylpromazine; Apex Laboratories, Melbourne, Australia) and 1.2 mg atropine (atropine sulfate; Apex Laboratories). Anesthesia was induced by Diprivan (6 mg/kg; propofol; ICI Australia Operations, Melbourne, Australia) and was maintained by a mixture of halothane and oxygen. Through a midline abdominal incision, a transit-time flow-probe (6 mm ID; Transonic Systems, Ithaca, New York) was placed around the cranial mesenteric artery, and two 22-gauge Barger catheters (SV 65 tubing, single lumen, 0.86 mm ID and 1.52 mm OD; Dural Plastics and Engineering, NSW, Australia) were inserted ~1 cm in the abdominal aorta distal to the flow probe. Each dog was instrumented to record blood pressure, cardiac output, mesenteric blood flow, mesenteric vascular resistance, urinary sodium and potassium excretion, plasma renin activity, and arterial pressure. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
to the renal arteries. Two vena caval catheters (SV 65 tubing, single lumen; Dural Plastics and Engineering) were inserted in the iliolumbar vein and were threaded between 23 and 40 cm downstream toward the heart. The abdominal incision was closed, and the catheters and flowprobe lead were tunnelled subcutaneously and exteriorized between the shoulders. Postoperatively, fentanyl (10 mg im; Flunixin Meglumine, Hertov Agvet, Rowville, Victoria, Australia) and 2 mg of Promex-2 were administered, and morphine (10 mg im; David Bull Laboratories, Mulgrave, Australia) was available for analgesia if required. A soft canvas coat was fitted to protect the exteriorized equipment. Before the first experiment, the dog was allowed to recover for at least 14 days during which time catheters were flushed with saline (0.9% sodium chloride), and dead space was filled with 0.5 ml heparin sodium solution (1,000 IU/ml) daily and every alternate day from then on.

Experiments. In all dogs, responses to ANP, BNP, and CNP, and vehicle infusions were determined on separate days in randomized order, with each experiment at least 7 days apart. On each of the experimental days, a bladder catheter was inserted via the urethra to collect urine. A 50-ml intravenous catheter and probe were connected and calibrated for recording data. The experimental protocol consisted of two 20-min baseline periods followed by four step-up infusions of vehicle (NaCl solution). The start of the main protocol and was continued throughout the experiment. During the 1 h of equilibration time, the catheters and probe were connected and calibrated for recording data. The experimental protocol consisted of two 20-min baseline periods followed by four step-up infusions of vehicle (NaCl solution, 100 ml/h). The experiment was repeated in the presence of an autonomic ganglionic blocker being alternated. During the equilibration period on the day with ganglion blockade, a 10-ml bolus of pentolinium tartrate (6 mg/kg; Sigma Chemical, St. Louis, MO) was given over a 10-min period followed by a continuous intravenous infusion of pentolinium (0.05 mg·kg⁻¹·min⁻¹). We previously demonstrated that this dose of pentolinium effectively blocks autonomic reflex responses in conscious dogs (40). When heart rate and arterial pressure had stabilized in response to the pentolinium, the main experimental protocol for BNP, as described above, was repeated.

Hemodynamic, hormonal, and electrolyte measurements. Cobe disposable pressure transducers measured phasic aortic blood pressure and central venous pressure (CVP) from one of the arterial and venous catheters, respectively. MBF was measured using a Transonic Flowmeter (no. T208; Transonic Systems), and heart rate was recorded from the MBF signal using a tachograph (model no. 173; BMRI, Prahran, Australia). Mean arterial pressure (MAP) was derived from a Baker Institute amplifier with a low pass filter set at 0.05 Hz. Mesenteric vascular resistance (MVR) was calculated as (MAP – CVP)/MBF. Although the preferred measure of downstream pressure to calculate MVR is portal venous pressure, the long-term patency of portal venous catheters was considered too uncertain for the current experiments, which required ±2 to 3 mo of chronic catheterization. The use of CVP does not invalidate the calculation of MVR, since mesenteric arterial resistance is much larger than that of the hepatoporal vascular resistance, with the predominant influences on MVR responses being changes in MAP and MBF. All data were continuously recorded on an eight-channel Graph-tec chart recorder (Linearcorder no. WR3310), collected at 300 Hz, digitized via an analog-to-digital conversion program in 10-s bins, and saved by personal computer (Olivetti model 280).

Arterial blood was collected at the midpoint of each 20-min period in prechilled potassium-EDTA tubes for ANP (38, 40–42) and BNP radioimmunoassays (each 5 ml), precipitin tubes (900 µl blood sample with 100 µl of dimercaprol-EDTA added) for PRA assay (40), and lithium heparin tubes (2.5 ml) for plasma sodium and potassium (by flame photometer, IL943; Instrumentation Laboratory, Milan, Italy) and osmolality (by Osmometer, Fiske One Ten Osmometer; Fiske Associates). Tubes were centrifuged for 10 min at 5,000 rpm at 4°C, and plasma was removed and stored at −70°C for radioimmunoassay or −20°C for other biochemical analyses. Blood was also collected in heparinized capillary tubes for microhematocrit determination. Urine collections were made every 20 min, and volume was measured before urinanalysis for sodium, potassium, and osmolality.

Details of the radioimmunoassay for ANP, using antibodies raised in a rabbit against α-human-ANP conjugated to thyroglobulin, have been published elsewhere (38, 40–42). There was no cross-reactivity of the ANP antiserum with BNP-32 (human), CNP-22 (human), pro-ANP-(1–67), pro-ANP-(1–30), pro-ANP-(31–67), and the fragments of human ANP-(1–11), -(13–28), and -(18–28). Recoveries of added synthetic α-human ANP to plasma were ~75%. Limit of detection was <2 fmol/ml plasma, interassay coefficient of variation (CV) was 11%, and intra-assay CV was 5%.

BNP was extracted from 1-ml aliquots of plasma with Sep-Pak C₁₈ cartridges and eluted with 80% methanol in 1% trifluoroacetic acid. Recoveries of added synthetic canine BNP-32 to plasma were ~80%. The radioimmunoassay for BNP was performed using commercial porcine-BNP-32 anti-serum (Phoenix Pharmaceuticals, San Francisco, CA) that cross-reacts 100% with canine-BNP-32, 125I-canine-BNP-32 tracer (Phoenix Pharmaceuticals), and the same canine-BNP-32 standard that was used in the experiments (Peninsula). The assay was run after 16–18 h preincubation of antigen or sample plus antibody. After a further 24 h, antibody bound from free BNP was separated with a solid-phase second antibody-coated cellulose suspension (Sac-Cell; Immunodiagnostics, Boldon, UK). Limit of detection for the assay was 4 fmol/ml, interassay CV was 14% at 30 fmol/ml (n = 15), and intra-assay CV was 11% at 5 fmol/ml (n = 8). For both ANP and BNP radioimmunoassays, all plasma samples from each dog were extracted together and measured in the same assay to reduce within-animal assay variation. Individual plasma levels have not been corrected for extraction losses.

Data and statistical analysis. Plasma hormone levels and hematocrit values were the average of duplicate (hormones) or triplicate (hematocrit) samples. Digitized hemodynamic data were averaged into 10-min blocks, and the second 10-min average from each 20-min period was used for statistical analysis and presentation in Figs. 1–5. To determine whether natriuretic peptide or vehicle infusions on each experimental day significantly influenced hemodynamic, hormonal, or urinary variables, data were analyzed by two-way ANOVA. Given the repeated nature of the data collection,
excessive correlation between measurements was corrected using the Greenhouse-Geisser epsilon to adjust the degrees of freedom for any contrast. Orthogonal partitioning of the between-column (time) sums of squares was used to determine the overall effect of treatment (4 infusion periods) versus control (2 baseline periods) and whether responses were linear or quadratic. In all cases where there was a significant effect over the four doses, responses were found to be linear \((P < 0.05)\). Where an effect of treatment was borderline, such as MBF response to CNP infusions, an additional nonorthogonal comparison between the two control values and the highest two doses was performed with a Bonferroni adjustment of the \(P\) value to account for multiple comparisons.

To determine whether there was a significant difference between hormones and vehicle infusions at a particular dose (between-days effects), two-way ANOVA was performed on the changes from baseline values at that dose. Analysis started at the 20-pmol dose, and if a significant difference between treatments (days) was found then the next dose (10 pmol) was tested and so on to minimize the number of contrasts. The Bonferroni adjustment was used to account for the number of doses (20). In the subgroup of four animals receiving BNP infusions in the presence and absence of pentolinium, the effect of pentolinium treatment alone was determined by a paired \(t\)-test on the average of the two baseline values between days.

All values depicted in Figs. 1–5 are means ± SE using between-animal estimate of variance. Significant changes were accepted at the level of \(P < 0.05\).

### RESULTS

**Hormone levels.** Plasma ANP increased progressively with ANP infusions from \(6.5 \pm 1.7\) fmol/ml (~90%) at the lowest dose to \(154 \pm 8\) fmol/ml (~13-fold) at the highest dose (Fig. 1, top left). Plasma ANP levels fell rapidly during the first 20 min after the infusion was turned off, reaching \(16.5 \pm 1.6\) fmol/ml, and recovered to baseline levels (\(10.8 \pm 0.5\) fmol/ml) during the next 20 min (the 20- to 40-min recovery period; Fig. 1, top left). During CNP infusions, endogenous ANP levels increased significantly, averaging ~25 ± 4% over all doses \((P < 0.05)\) and reaching a maximum of 43 ± 5% higher than baseline at the highest dose (Fig. 1, bottom left). During BNP and vehicle infusions, endogenous ANP levels were not altered significantly (Fig. 1, bottom left).

Plasma BNP increased progressively during BNP infusions from \(1.5 \pm 1.1\) fmol/ml (~20%) at the lowest dose to \(265 \pm 22\) fmol/ml (~30-fold) at the highest dose (Fig. 1, top right). Plasma BNP levels fell rapidly...
uring the first 20 min after the infusion was turned off (to 36.6 ± 3.8 fmol/ml) and recovered to nearly baseline levels (11.3 ± 1.2 vs. 8.6 ± 0.5 fmol/ml) during the next 20 min (the 20- to 40-min recovery period; Fig. 1, top right). There were no significant changes in endogenous BNP levels with any of the other peptide or vehicle infusions (Fig. 1, top right).

During both ANP and BNP infusions, PRA fell significantly (P < 0.05) compared with baseline values (Fig. 2). Over all doses, PRA fell by an average of 40.7 ± 6.0 and 33.6 ± 5.7%, respectively, reaching maximum falls of 63.5 ± 6.4 and 43.2 ± 11.3%, respectively, at the highest doses of each peptide. After ANP and BNP infusions were turned off, PRA levels returned to baseline at rates closely mirroring the falls during infusions (Fig. 2). During CNP and vehicle infusions, PRA levels did not change significantly, although there was a tendency for PRA to progressively rise with time in the absence of a natriuretic peptide infusion (vehicle; Fig. 2).

Hemodynamics and hematocrit. During BNP infusions there were progressive falls in MAP (P < 0.05) from 4.6 ± 2.8 mmHg at the lowest dose to 9.6 ± 2.6 mmHg at the highest dose (Fig. 3, top left). MAP returned to baseline levels during the recovery periods at a rate mirroring the fall during BNP infusions (Fig. 3, top left). There were no significant changes in MAP during ANP, CNP, or vehicle infusions (Fig. 3, top left). Heart rate did not change significantly during any of the infusion periods, although there was a tendency for PRA to progressively rise with time in the absence of a natriuretic peptide infusion (vehicle; Fig. 2).

During all infusion periods, whether vehicle or natriuretic peptide, there were small (between 1 and 2 mmHg) but significant falls in CVP (Fig. 3, top middle). The declines in CVP tended to arrest during recovery periods but still did not return to baseline levels (Fig. 3, top middle). Hematocrit rose significantly (P < 0.05) during ANP and BNP infusions but did not change significantly during CNP or vehicle infusion (Fig. 3, bottom middle). The increase with BNP [4.93 ± 0.82% red blood cells (RBC)] was significantly (P = 0.022) greater at the 20 pmol dose than with ANP (2.30 ± 0.50% RBC). Increases in hematocrit did not recover to baseline levels during the recovery periods (Fig. 3, bottom middle).

MBF fell substantially (P < 0.05) in a dose-related manner during infusions of ANP and BNP (Fig. 3, top right). During ANP infusions, MBF was −0.5 ± 7% of resting at the 2 pmol dose and −32 ± 5% at the 20 pmol dose, averaging −14.9 ± 3.6% over all doses. During BNP infusions, MBF was −13 ± 4% at the lowest dose and −42 ± 3% at the top dose, averaging 29.7 ± 2.8% over all doses. The maximum falls in MBF at the highest doses were 97 ± 19 and 132 ± 14 ml/min for ANP and BNP, respectively. There was a tendency for MBF to fall also during CNP infusions (Fig. 3, top right), but this did not reach significance (−6.0 ± 2.9% over all doses; P = 0.08). However, MBF at the top two doses of CNP were significantly lower than the baseline values (additional nonorthogonal comparison, P < 0.05). During vehicle infusions MBF did not change (Fig. 3, top right).

MVR increased (P < 0.05) in a dose-related manner during each of the natriuretic peptide infusions, including CNP, but there was no change during vehicle infusions (Fig. 3, bottom right). Increases in MVR over all doses were 22.3 ± 5.4% (ANP), 36.7 ± 4.8% (BNP), and 11.0 ± 4.3% (CNP), with maximum increases at the highest doses of each peptide of 50 ± 11% (ANP), 60 ± 9% (BNP), and 27 ± 10% (CNP). The increased MVR during CNP infusions was significantly (P < 0.05) lower than with either ANP or BNP at the 5, 10, and 20 pmol doses. There were no significant differences in MVR or MBF responses between ANP and BNP at each dose. After the peptide infusions were turned off, MBF and MVR recovered to baseline levels at a rate mirroring the falls during the infusions (Fig. 3, top right).

BNP and pentolinium. Similar to the findings from our previous study (40), pentolinium infusion to block autonomic reflexes generally had little effect on hemodynamic variables. Exceptions were heart rate, which was 52 ± 2 beats/min (n = 4) without pentolinium and 109 ± 15 beats/min in the same dogs after pentolinium (P < 0.05), and hematocrit (Fig. 4), which was 43.0 ± 1.8% RBC without and 46.3 ± 2.2% RBC with pentolinium (P < 0.05). Pentolinium treatment also significantly (P < 0.05) lowered resting PRA from 0.43 ± 0.08 to 0.13 ± 0.03 ng ANG I·ml⁻¹·h⁻¹ (Fig. 4) and increased plasma ANP levels from 11.7 ± 0.7 to 16.6 ± 0.6 fmol/ml, in line with our previous report (40). In addition, baseline plasma BNP levels were increased (P < 0.05) by autonomic blockade, from 7.7 ± 0.7 to 10.4 ± 0.5 fmol/ml.

Responses to BNP in the presence of autonomic blockade were similar to those when autonomic reflexes were intact (Fig. 4). BNP infusions, in the presence of pentolinium, resulted in significant (P < 0.05) average falls in PRA of 0.06 ± 0.01 ng ANG I·ml⁻¹·h⁻¹ (−50 ±
5%), MAP of 18.6 ± 3.0 mmHg (−17 ± 3%), and MBF of 100 ± 12 ml/min (−36 ± 4%) and increases in hematocrit of 1.7 ± 0.4% RBC (3.6 ± 0.8%) and MVR of 171 ± 37 mmHg·l⁻¹·min⁻¹ (37 ± 7%; Fig. 4).

Urinary responses. Infusions of ANP and BNP caused substantial dose-related diuresis and natriuresis (Fig. 5, top). Over all doses, the average increases (P < 0.05) in urine flow were 73 ± 16% (ANP) and 117 ± 40% (BNP), reaching maximum levels at the highest doses of 153 ± 44% (ANP) and 334 ± 113% (BNP), which were not significantly different from each other. By contrast, CNP and vehicle infusions did not increase urine flow (−2.3 ± 6.6% during CNP and 5.4 ± 5.6% during vehicle) or sodium excretion (−14 ± 6% during both CNP and vehicle; Fig. 5, top). The hormone-induced diuresis and natriuresis readily recovered to baseline levels during the recovery period, with the rates of recovery similar to the increases during infusions (Fig. 5, top).

Free water clearances were not altered by any of the peptide or vehicle infusions (Fig. 5, bottom left). Urinary potassium excretion fell significantly (P < 0.05) during CNP infusions compared with baseline by an average of 41 ± 4% over all doses (Fig. 5, bottom right) although these changes were not significantly different from those during vehicle infusions, at any dose (P > 0.33). There were no significant changes in urinary potassium excretion during ANP, BNP, or vehicle infusions. There was, however, a tendency for urinary potassium excretion to fall progressively throughout each experiment (Fig. 5, bottom right).

**DISCUSSION**

The current study reports for the first time that infusions of homologous BNP in normal conscious dogs caused dose-related, reversible mesenteric vasoconstriction. MVR increased by ~10% at the lowest dose, which raised circulating levels less than twofold, and by ~60% at the highest dose, which elevated circulating levels ~30-fold. These responses to BNP were similar to those with equimolar doses of ANP in the same dogs.
BNP infusions also resulted in modest hypotension, with MAP falling by ~5 mmHg at the lowest dose and ~10 mmHg at the highest dose. Although arterial baroreceptor activation of sympathetic reflexes may be expected to have contributed to the mesenteric vasoconstriction, we demonstrated that autonomic ganglion blockade with pentolinium did not prevent or even attenuate the BNP-induced mesenteric vasoconstriction. This suggests a direct action of the hormone on gastrointestinal vasculature, independent of the autonomic nervous system.

Equimolar infusions of CNP also increased MVR, but the vasoconstriction was much more modest than with either ANP or BNP. There was little change in MVR at the lower doses of CNP (increase of ~3% at 5 pmol·kg⁻¹·min⁻¹), but MVR increased by ~27% at the top dose. Because endogenous ANP levels rose significantly during infusions of CNP, increasing by ~27% at the top dose. Because endogenous ANP levels rose significantly during infusions of CNP, increasing by 5.6 ± 0.8 fmol/ml at the highest dose, elevated plasma ANP may account in part for the CNP-induced vasoconstriction. This contribution from ANP was probably small, however, since the lowest-dose infusion of ANP, which raised plasma ANP concentration by 7.0 ± 2.1 fmol/ml, was close to the threshold concentration for mesenteric vasoconstriction (change in MVR was <5% of baseline). Because CNP infusions did not affect the arterial blood pressure, it is unlikely that baroreflexly mediated sympathetic vasoconstriction played a major role in the response to CNP. If the remainder of CNP-induced vasoconstriction was due to a direct action from CNP, this may implicate a mechanism in common with the sister peptides ANP and BNP but with a reduced potency. This tends to rule out the natriuretic peptide subtype B receptor as a likely candidate to transduce the vasoconstrictor response, since CNP is the selective ligand for this receptor (15). The natriuretic peptide subtype A receptor is also unlikely given that this receptor, through guanylate cyclase, is linked to vasodilatation (21) and not vasoconstriction. The remaining possibilities are 1) direct vasoconstriction via the natriuretic peptide subtype C (NP-C) receptor (3), 2) an as yet unidentified NP receptor, or 3) indirect vasoconstriction through the activation of a secondary factor. Support for the third possibility derives from our recent studies in anesthetized dogs showing that hepatic vasoconstriction was more pronounced after ANP was
infused via the mesenteric artery than when the hormone was administered directly in either the hepatic artery or portal vein (39). Identity of this secondary factor remains unknown. It is highly unlikely to be circulating ANG II since PRA fell with infusions of ANP and BNP.

What impact did mesenteric vasoconstriction induced by the natriuretic peptides have on other hemodynamics? Because gastrointestinal vascular conductance comprises ~30% of the total peripheral vascular conductance in preprandial conscious dogs (42), a substantial increase in vascular resistance in this high-capacitance region may be expected to increase TPR unless there is counterbalancing extrasplanchnic vasodilatation. In earlier studies, ANP infusions increased TPR in autonomically blocked conscious dogs (31, 32, 40, 42), and, indeed, splanchnic vasoconstriction was calculated to have contributed >50% to the rise in TPR (42). In the presence of autonomic blockade, ANP infusion also caused substantial falls in cardiac output that were associated with reduction in the coronary blood flow, accounting for most of the remaining increase in TPR (31, 42). In those dogs, arterial blood pressure fell since the rise in TPR did not compensate for the falling cardiac output (31, 32, 40, 42). In the present study, arterial pressure was largely unchanged during ANP and CNP infusions but fell 5–10 mmHg during BNP infusions. Thus, with each of the peptides, the mesenteric vasoconstriction must have been counterbalanced by either extrasplanchnic vasodilatation or falling cardiac output. Because it is generally recognized that increased hematocrit responses to natriuretic peptide infusions reflect reductions in plasma volume and hence cardiac output, it is likely that cardiac output fell during each of the natriuretic peptide infusions in the present study with a potency of BNP > ANP > CNP, matching the hematocrit changes.

We propose that, even though natriuretic peptides may lower the cardiac output, this does not necessarily result in compromised tissue perfusion of vital areas, since the peptides concomitantly redirected blood flow away from the splanchnic region, with an order of potency for increasing MVR of BNP slightly > ANP >> CNP. This selective redistribution of cardiac output may help to maintain blood flow through essential organs such as brain, kidney, or heart under conditions of mildly compromised cardiac output and lowered blood pressure.

Whether the natriuretic peptide-induced redistribution of blood flow was aided by extrasplanchnic vasodi-
lalation cannot be answered from the present study. There is evidence that each of the natriuretic hormones is capable of causing vasodilatation in conscious experimental animals or normal humans, but this action occurs primarily at high doses (23, 24, 26, 27, 31, 39). Exceptionally, BNP diluted renal vasculature when infused at $4 \pm 1 \cdot 10^{-12} \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in normal humans (17), although at lower levels of $0.5 \pm 1 \cdot 10^{-12} \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ BNP was without effect on renal plasma flow (18). Thus, at the levels infused in the present study in normal animals, it is unlikely that substantial vasodilatation occurred with any of the peptides and that gastrointestinal vasoconstriction alone may be an effective means of redistributing the reduced cardiac output.

The hypotensive effect of BNP was unlikely to be accounted for by natriuresis and diuresis since 1) arterial pressure fell by $-5 \text{mmHg}$ at the lowest dose of BNP, before there was any change in sodium or volume excretion, and 2) the order of potency for the renal effects was ANP $> BNP >> CNP$, suggesting that, if volume losses through the kidneys primarily mediated blood pressure reduction, ANP should also have caused hypotension. Natriuresis and diuresis with ANP and BNP infusions are well-documented characteristics of these natriuretic peptides (e.g., see reviews in Refs. 4 and 8) and confirm the biological activity during the preparation and administration of these peptides. The present study demonstrated the dose-related and reversible nature of natriuresis and increased urine output with BNP infusion seen in other studies (11). The natriuresis and diuresis occurred in the face of falling arterial pressure but in the presence of reductions in PRA. This contrasts with the observations in conscious sheep (6), where low-dose BNP was without effect on either urine flow or sodium excretion, despite significant increases in plasma cGMP, but with concomitant hypotension and no change in PRA. These authors attributed the blunted urinary actions of BNP to the hypotension. Taken together, the results in dogs and sheep may point to the reduction in PRA as an important component in the natriuretic actions of BNP.

In our conscious dogs, CNP had a minimal effect on the sodium excretion and urine output, similar to the observations in human (13) and sheep experiments (5). By contrast, intravenous CNP was antinatriuretic in anesthetized dogs (33), although this effect appeared to be indirect, possibly related to hypotension, since intrarenal administration of CNP to anesthetized dogs did not reduce arterial pressure or sodium excretion (7).

In the present study in normal conscious dogs, there was a clear suppression of tonically released renin by both ANP and BNP in a dose-related manner, whereas CNP was without effect. If circulating ANG levels have a tonic influence on mesenteric vascular tone, PRA inhibition may have in part counterbalanced the mesenteric vasoconstrictor actions of ANP and BNP. The use of ANG-converting enzyme inhibition could result in a greater vasoconstrictor response to increased natriuretic peptide levels. In the case of BNP, the reduction in PRA occurred despite a significant fall in arterial pressure, which would normally be expected to activate renin release via the renal barostat. Moreover, even after autonomic blockade, which on its own suppressed baseline PRA, BNP inhibited PRA, further indicating a lack of involvement from the autonomic nervous system in BNP-induced inhibition of renin release. Although it is generally accepted that ANP inhibits renal renin release, this action is somewhat controversial (8, 19), depending on the species studied, whether in vitro or in vivo, and whether release of renin was activated or under tonic control. Inhibitory effects of BNP in vivo on PRA have been reported in humans (18, 22, 29) and in sheep (5), although another study using higher doses of BNP in humans failed to show any change in PRA (17). Higher-dose BNP ($50 \pm 1 \cdot 10^{-12} \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or $15 \pm 1 \cdot 10^{-12} \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) administered directly in isotonic saline-infused kidneys of anesthetized dogs caused dramatic inhibition of renin secretion rate, whereas hypertonic saline-infused kidneys responded by increasing renin secretion rate (1). Thus the sodium status may influence the effectiveness of BNP to inhibit renin release. Our findings with CNP confirm the observations by others showing a lack of effect on either plasma renin (33) or ANG II (13) levels.

Measurements of circulating levels of ANP and BNP in the present study indicated an interaction between CNP and ANP, but not BNP, which may be related to clearance mechanisms. Endogenous ANP but not BNP increased significantly during CNP infusions. In the sheep, endogenous levels of both ANP and BNP increased during CNP infusions (6), whereas, in humans, infusions of CNP increased only endogenous plasma ANP (13). These observations point to differences between sheep, on the one hand, and dogs or humans, on the other, in the degradative pathways of the two major circulating natriuretic peptides. Because infusions of ANP and BNP in our experiments did not alter the endogenous levels of each other, it would appear that CNP may have a greater affinity than BNP in vivo for the NPC (so-called "clearance") receptor, which is the preferred clearance pathway for ANP and less so for BNP, at least in humans (14). This proposal is supported by affinity binding studies for human, bovine, and rat NPC receptors that indicate a rank order of affinity of ANP $> CNP > BNP$ in all three species (37).

To date, the plasma half-life of BNP in dogs has not been reported. In humans, BNP half-life is $-22 \text{ min}$ (34), whereas the half-life of BNP in sheep is $-3 \text{ min}$ (5). The present study demonstrated a rapid return of circulating levels and of most biological activities of BNP to close to baseline levels in the first 20 min after turning off the BNP infusion (Figs. 1, 4, and 5). This observation indicates a relatively short half-life in the canine, which may be close to the $-1 \text{ min}$ half-life of ANP in this species (38). Thus, in dogs, relatively higher levels of exogenous BNP ($2 \pm 1 \cdot 10^{-12} \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) were required to increase plasma BNP (between 1.2- and 10-fold) to levels similar to those observed with low-dose infusions of homologous BNP ($0.5 \pm 1 \cdot 10^{-12} \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) used in other species (6, 12, 17).

In summary, these studies in conscious dogs have shown for the first time that low- to modest-dose...
infusions of BNP caused dose-related and reversible constriction of the gut vasculature in conscious dogs that was not due to reflex activation of the sympathetic nervous system. BNP was also strongly natriuretic and inhibited PRA despite being hypotensive, indicating that the suppressive activity of BNP was more powerful than the renal barostat to activate release of renin in response to falling arterial pressure. The mesenteric vasoconstriction and PRA inhibition, and natriuresis were similar to those effects elicited by equimolar doses of ANP. However, BNP caused greater hemococoncentration and reduction in arterial pressure than ANP. Thus the circulating ANP and BNP systems have many actions that counter those of the pressor- and volume-retaining capacity of the renin-ANG-aldosterone system. It is proposed that the circulatory effects of ANP and, particularly, BNP are to protect the heart by reducing cardiac preload (venous return) and cardiac afterload (arterial pressure) while maintaining perfusion to vital, extrasplanchnic regions. By contrast, equimolar intravenous infusions of CNP did not alter arterial pressure, hemococoncentration, urinary responses, or PRA levels but caused a modest mesenteric vasoconstrictor response, which may have been in part through the actions of elevated levels of endogenous ANP. As a local factor potentially important in conditions such as septic shock (10), CNP may contribute to changes in regional blood flow associated with such conditions. Overall, mesenteric vasoconstrictor actions of the natriuretic peptides may counterbalance other hemodynamic actions, such as plasma volume reduction, salt and water losses, and PRA inhibition, thereby preventing precipitous falls in arterial blood pressure and preferentially redistributing blood flow away from the gut.

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