Comparative bioactivity of atrial and brain natriuretic peptides in an ovine model of heart failure

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1. Whereas many studies have detailed the effects of exogenous atrial natriuretic peptide (ANP) infusions in heart failure, and a limited number have examined the effects of brain natriuretic peptide (BNP), none have directly compared the bioactivity of similar doses of ANP and BNP under standard conditions of impaired cardiac function. We compared the hormonal, haemodynamic and renal effects of 3 h infusions of ANP; BNP and a vehicle control in eight sheep with pacing-induced heart failure (225 beats/min for 8–12 days).

2. Infusion of ANP and BNP increased plasma ANP (P < 0.001) (276 ± 27 versus control 142 ± 26 pmol/l) and BNP (P < 0.001) (257 ± 34 versus control 45 ± 5 pmol/l) respectively, in association with increased cyclic 3′,5′-guanosine monophosphate [control, 40 ± 6; ANP, 53 ± 6 (P < 0.05); BNP, 57 ± 7 nmol/l (P < 0.001)]. Metabolic clearance rate and half-life were similar for both peptides. Infusion of ANP and BNP similarly reduced mean arterial pressure [control, 73.0 ± 1.6; ANP, 67.6 ± 1.2 (P < 0.01); BNP, 65.7 ± 1.7 mmHg (P < 0.001)], left atrial pressure (both P < 0.05) (control, 22.0 ± 0.7; ANP, 19.9 ± 1.0; BNP, 19.8 ± 0.9 mmHg) and peripheral resistance [control, 50.3 ± 4.1 mmHg l−1 min−1; ANP, 46.0 ± 2.8 (P < 0.05); BNP, 43.8 ± 4.5 (P < 0.01)], and increased urine volume (2–3-fold, both P < 0.05), sodium excretion (>10-fold, both P < 0.01) and haematocrit levels relative to control (both P < 0.05). Infused BNP tended to raise plasma ANP levels (by 28 pmol/l), while ANP increased plasma BNP (by 18 pmol/l). Plasma aldosterone was reduced by approximately 40% by both peptides (both P < 0.05).

3. In conclusion, ANP and BNP are both powerfully natriuretic, similarly suppress aldosterone and appear equipotent in reducing preload and afterload in this model of pacing-induced heart failure.

INTRODUCTION

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are both circulating cardiac hormones. ANP is largely a product of atrial secretion and is stored as the prohormone in atrial myocyte granules, whilst BNP is predominantly secreted constitutively from the ventricular myocardium, although small amounts are co-stored with ANP in the atria [1]. ANP and BNP share functional similarities, including diuretic, natriuretic and hypotensive effects [2]. These actions appear to be mediated by the same guanylate cyclase-linked natriuretic peptide receptor (NPR)-A, resulting in the formation of the intracellular second messenger, cGMP [3]. Both natriuretic peptides are degraded and cleared by neutral endopeptidase-24.11 [4] and the non-guanylyl cyclase-linked NPR-C or clearance receptor [5,6]. Although BNP is closely related to ANP in structure (especially within the 17 amino acid ring formed between two cysteine residues), molecular forms of BNP differ considerably among species, making the actions and degradation of this peptide species-specific [4,7].

Plasma concentrations of both ANP and BNP are significantly raised in congestive heart failure [1], a disease characterized by cardiac and volume overload. This increase is due to enhanced synthesis by the failing heart, as well as reduced plasma clearance. In an animal model of early congestive heart failure, ANP attenuates sodium and water retention, renal vasoconstriction and activation of the renin–angiotensin–aldosterone system [8]. When infused in normal subjects to mimic plasma levels observed in patients with mild heart failure [9], the natriuretic, endocrine and haemodynamic effects of BNP appear to be comparable with those of ANP. Whereas many studies have detailed the effects of exogenous ANP infusions in heart failure [8, 10–12], and a limited...
number have examined the effects of BNP [13, 14], to our knowledge no studies (in either humans or experimental animals) have previously compared the bioactivity of similar doses of ANP and BNP under standard conditions of impaired cardiac function. Accordingly, we directly compared the haemodynamic, endocrine and renal effects of species-specific forms of ANP and BNP in an ovine model of heart failure.

METHODS

Surgical preparation

Eight Coopworth ewes (body weight 35–57 kg) were surgically prepared as previously described [15] via a left lateral thoracotomy. Under general anaesthesia [induced by thiopentone (17 mg/kg) and maintained with halothane and nitrous oxide], two polyvinyl chloride catheters were inserted in the left atrium for blood sampling and left atrium pressure (LAP) determination; a Konigsberg (P 6.0) high fidelity pressure-tip transducer was inserted in the aorta for measurement of mean arterial pressure (MAP); an electromagnetic flow probe was placed around the ascending aorta to measure cardiac output (CO); a 7 French Swan–Ganz catheter was inserted in the pulmonary artery for infusions and a 7 French His-bundle electrode was stitched subepicardially to the wall of the left ventricle for subsequent left ventricular pacing using an external pacemaker made in our department laboratory. All leads were externalized through individual incisions in the neck. An indwelling bladder catheter was inserted per urethra for urine collections. The animals recovered for at least 14 days before the study protocol was commenced. During the experiments, the animals were held in metabolic cages, had free access to water and ate a diet of chaff and sheep pellets (containing approximately 40 mmol/day sodium and 200 mmol/day potassium) supplemented with a further 40 mmol of sodium administered orally each morning as sodium chloride tablets using an applicator.

Study protocol

Heart failure was produced by rapid left ventricular pacing (225 beats/min) for 7 days [15]. On days eight, ten and twelve of pacing, the sheep received a 3 h intravenous infusion of vehicle (haemaccel), human ANP-28 or porcine BNP-26 in random order (purchased from Bachem, Torrance, CA, U.S.A.). Infusions were calculated at a concentration of 7.5 pmol kg⁻¹ min⁻¹ in a total volume of 60 ml and administered via the pulmonary artery catheter commencing at 10.00 hours. Partial cloning of the ovine BNP gene in our laboratory indicates that ovine BNP is identical with the C-terminal (26-residue) portion of porcine pro-BNP [16].

Similarly, the ovine ANP amino acid sequence is identical to human and porcine forms [16, 17].

Haemodynamic recordings (MAP, LAP and CO) were performed at 15 min intervals starting 1 h before infusion (baseline) until 1 h post-infusion, after which half hourly measurements were made for a further 2 h. All measurements were made with the sheep standing quietly in the metabolic cage. The left atrial catheter was connected to a Statham P50 strain-gauge transducer positioned at the level of the atria and linked to a haemodynamic monitor (M17294; Mennen-Greatbatch Ltd, Rehevot, Israel) for pressure determination relative to atmospheric pressure. The Konigsberg pressure transducer was connected to a pre-amplifier before display by the monitor. Haemodynamic measurements were determined by on-line computer-assisted analysis using methods previously described [18].

Blood was drawn from the left atrium at 30 min intervals starting 30 min pre-infusion (baseline) until 1 h post-infusion, after which hourly samples were taken for a further 2 h. The blood was taken into tubes on ice, centrifuged at 4°C, and plasma was stored at −80°C before assay of ANP [19], BNP [17], cGMP [19], plasma renin activity (PRA), aldosterone and cortisol [15]. All samples from each animal were measured in the same assay to avoid inter-assay variability. Haematocrit concentrations were measured with every blood sample taken. Upon termination of peptide infusions, additional samples were drawn at 1, 2, 3, 4, 5, 10, 15 and 20 min for determination of the disappearance rate of the infused peptide from the plasma (half-life). Plasma half-life of ANP and BNP was calculated by regressing plasma concentrations immediately post-infusion against time required for plasma levels to fall to 50% of plateau levels. The natriuretic peptide concentration of each infusate was measured to calculate the infusion rate of the peptide. Metabolic clearance rate (MCR) of the natriuretic peptides was calculated as: 

\[ \text{MCR} = \frac{\text{measured infusion rate}}{\text{plateau - baseline level}} \]

Blood samples for analysis of plasma sodium, potassium and creatinine concentrations were taken immediately before infusion (baseline), at the end of infusion, and 3 h post-infusion. Urine volume and samples for the measurement of sodium, potassium and creatinine excretion were collected hourly from 1 h pre-infusion (baseline) to 3 h post-infusion.

The study protocol was approved by the Animal Ethics Committee of the Christchurch School of Medicine.

Statistical analysis

Results are expressed as means ± S.E.M. Baseline haemodynamic and hormone values represent the means of the four and two pre-infusion measurements respectively. Significant differences between the effects of vehicle and ANP and BNP were deter-
minded by analysis of variance. Data were analysed in two separate blocks consisting of the 3 h treatment period and the 3 h post-treatment period. The response of each variable to ANP and BNP was indexed relative to the infused concentration of each peptide (magnitude of response/infusate peptide concentration) before comparison. Urine sodium excretion data were log transformed before analysis to stabilize variances. Significance was assumed when \( P < 0.05 \). Data displayed in Figures 1–5 are represented by the single pooled within-animal S.E.M. obtained from the analysis of variance and placed on the last point of each data block. Where analysis of a data block produced a significant treatment effect, the level of significance is indicated beside the appropriate S.E.M. (*\( P < 0.05 \), **\( P < 0.01 \), †\( P < 0.001 \)). Where a significant time/treatment interaction was present, paired comparisons using Fisher’s protected least-significant difference tests were used to identify time-points significantly different from vehicle control.

RESULTS

After 7 days of rapid ventricular pacing, all sheep exhibited the haemodynamic and hormonal hallmarks of established heart failure observed in previous studies [15]. Compared with normal values for our laboratory recorded in sheep before pacing (n = 12), 7 days of rapid pacing in the present study resulted in significant reductions in MAP (normal data 89±2; paced control baseline data 73±1 mmHg, \( P < 0.001 \)) and CO (normal 3.1±0.2; paced 1.5±0.11/min, \( P < 0.001 \)), whereas LAP (normal 2.3±0.3; paced 22.5±0.7 mmHg, \( P < 0.001 \)) and plasma ANP (normal 13±6; paced 122±20 pmol/l, \( P < 0.001 \)), BNP (normal 3.8±1.9; paced 40±4 pmol/l, \( P < 0.001 \)), cGMP (normal 6.3±1.6; paced 38.8±4.2 nmol/l, \( P < 0.001 \)), PRA (normal 0.6±0.1; paced 3.1±1.3 pmol ml\(^{-1}\) h\(^{-1}\), \( P < 0.001 \)) and aldosterone (normal 244±59; paced 1871±415 pmol/l, \( P < 0.001 \)) levels were elevated.

Compared with time-matched control data, infusion of ANP and BNP significantly increased plasma levels of each respective peptide (at 3 h: ANP, 276±27 versus control 142±26 pmol/l, \( P < 0.001 \); BNP, 257±34 versus control 45±5 pmol/l, \( P < 0.001 \)) (Figure 1). Measured infusate concentrations were 80% of the calculated dose for ANP and 99% for BNP, accounting (at least in part) for the differences in increment of each peptide. The calculated MCR was 1.85±0.3 and 1.80±0.24 litres/min for ANP and BNP respectively, while half-lives of the peptides in plasma on termination of infusion were rapid and averaged 2.8±0.4 min for ANP and 3.6±0.4 min for BNP. Plasma cGMP levels were significantly raised by both ANP (\( P < 0.05 \)) and BNP (\( P < 0.001 \)) (at 3 h: control, 40±6 nmol/l; ANP, 53±6; BNP, 57±7) (Figure 1). Each pmol/l rise in ANP and BNP induced similar increases in plasma cGMP (0.133 and 0.139 nmol/l respectively). In keeping with the rapid half-life of both natriuretic peptides, plasma cGMP levels had fallen to or below time-matched control levels by 30 min post-infusion. The response of endogenous natriuretic peptide levels to infusion of the other peptide is summarized in Figure 2. Plasma ANP levels tended to rise in response to infusion of BNP (a maximum increment of 28 pmol/l above control levels), whilst infusion of ANP also increased plasma BNP levels (increment of 18 pmol/l above control) (both \( 0.1 > P > 0.05 \)).

Infusion of both ANP and BNP induced similar reductions in total peripheral resistance (TPR = MAP/CO) (at 3 h: control, 50.3±4.1 mmHg l\(^{-1}\) min\(^{-1}\); ANP, 46.0±2.8, \( P < 0.05 \); BNP, 43.8±4.5, \( P < 0.01 \)), MAP (control, 73.0±1.6 mmHg; ANP, 67.6±1.2, \( P < 0.01 \); BNP, 65.7±1.7, \( P < 0.001 \)) and LAP (control, 22.0±0.7 mmHg; ANP, 19.9±1.0, \( P < 0.05 \); BNP, 19.8±0.9, \( P < 0.05 \)) compared with control, and were still significantly reduced 3 h post-ANP and BNP infusion (Figure 3). These haemodynamic changes were associated with relative haemoconcentration, as indicated by the mainten-
Fig. 2. Means ± S.E.M. of endogenous plasma ANP and BNP response during 3 h infusion of BNP (m) and ANP (a) respectively, administered at a concentration of 7.5 pmol kg⁻¹ min⁻¹ in 8 paced sheep. Vehicle data (c) are also shown. Baseline data points represent the means of samples taken in the hour preceding treatment.

Fig. 3. Means ± S.E.M. of MAP, LAP, peripheral resistance and hematocrit response during 3 h infusions of vehicle (c), ANP (a) and BNP (m) administered at a concentration of 7.5 pmol kg⁻¹ min⁻¹ in 8 paced sheep. Baseline data points represent the means of samples taken in the hour preceding treatment. Significant differences between control and natriuretic peptide data are shown by *P<0.05, **P<0.01, ***P<0.001.

DISCUSSION

To our knowledge, no previous studies have directly compared the biological and metabolic effects of ANP and BNP in heart failure. The present vehicle-controlled study shows that the administration of equimolar doses of ANP and BNP in eight sheep with pacing-induced heart failure resulted in similar increments in plasma levels of ANP and BNP and displayed similar pharmacokinetics (calculated MCRs and plasma half-lives) and increments in the intracellular second messenger, cGMP. Infusion of both ANP and BNP induced similar reductions in MAP and LAP and increased haematocrit levels relative to control, while CO was not altered significantly by either peptide. Both ANP and BNP were diuretic and natriuretic and tended to increase creatinine clearance. Infusion of one peptide raised the plasma levels of the other. Plasma aldosterone was promptly reduced by both peptides.

We found the MCR and plasma half-life of human (h)ANP and porcine (p)BNP to be similar in sheep with pacing-induced heart failure. These
results are in agreement with observations in normal sheep, where the MCR of ANP was 5.7 ± 1.2 l/min and of BNP 7.5 ± 1.4 [20]. The higher clearance rate of both peptides observed in normal sheep is likely to be related to the lower infusion rates (ANP and BNP infused at 2.5 pmol kg\(^{-1}\) min\(^{-1}\) in normal versus 7.5 pmol kg\(^{-1}\) min\(^{-1}\) in heart-failure sheep). Consistent with these findings are recent reports indicating similar binding affinities of hANP and pBNP for the rat, human [3, 21] and ovine (E. A. Espiner, M. W. Smith, A. M. Richards, M. G. Nicholls and T. G. Yandle, unpublished work) forms of the NPR-C or clearance receptor. These results are in strong contrast with findings for hBNP-32, where the half-life of the infused peptide in man is approximately 7-fold that of ANP [9] and may be attributable to the comparatively lower affinity demonstrated by hBNP for the NPR-C [1, 3, 6], as well as for the guanylyl cyclase-linked NPR-A [22], resulting in slower clearance. It is of interest to note

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\begin{align*}
\text{Table 1. Haemodynamic, hormonal and renal responses to vehicle, ANP and BNP in heart-failed sheep. Mean±S.E.M. data before (baseline), during (hours 1–3) and following (hours 4–6) infusions of vehicle, ANP and BNP (at 7.5 pmol kg}^{-1}\text{ min}^{-1}) \text{ in 8 paced sheep. Baseline data points represent the means of samples taken in the hour preceding treatment.}
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that, whilst heart failure in sheep does not appear to alter the MCRs of either natriuretic peptide, the clearance rate of BNP was found to be less than that of ANP in human hypertension [23]. This disparity in findings between sheep and man may be related to differences in species and disease states, as well as to differences in the handling of hANP compared with hBNP and pBNP.

The present study demonstrates potentially important interactions between ANP and BNP, where administration of one peptide raised the endogenous plasma levels of the other. These interactions occurred despite reduced stimulus to natriuretic peptide secretion (as indicated by falls in LAP) [5, 24]. Previous studies have reported that administration of BNP increases plasma ANP levels in normal man [14, 25] and in patients with heart failure [14]. The effect of ANP on endogenous BNP levels, however, appears less evident, with studies in humans having shown no effect [26] as well as an increase in plasma BNP [27]. The mechanisms underlying these interactions remain unclear. Cross-reactivities of the antibodies used in the assays are well below levels that could explain the observations. A possible explanation includes competitive inter-action in the degradative pathways of the natriuretic peptides. Competition by ANP and BNP for the same NPR-C, similar to the action of ANP analogues such as cANP-(4-23) [5], has been demonstrated in vitro [6], as well as in vivo in the rat [28] where administration of cANP-(4-23) reduced the clearance of exogenous rat (r)ANP, and to a lesser extent, rBNP, from the circulation. Competition for binding sites on neutral endopeptidase-24.11 is another possibility [29], since inhibition of neutral endopeptidase results in increased plasma levels of both ANP and BNP [5, 26].

Compared with time-matched vehicle control observations, both ANP and BNP significantly activated guanylate cyclase, as judged by the increase in plasma cGMP. Consistent with the short half-life of each natriuretic peptide, plasma levels of cGMP fell rapidly (within 30 min) to control levels following cessation of infusion. In agreement with reports of similar affinity of ANP and BNP for the guanylate cyclase-linked A receptor (NPR-A) in normal sheep [20], as well as similar activity in vitro [6, 30], we found that administration of ANP and BNP produced comparable increases in cGMP (relative to the plasma increments of each peptide) in this ovine model of heart failure. These results indicate a similar (receptor) site of action and are in concordance with the quantitatively and qualitatively equivalent haemodynamic responses observed during infusion of both natriuretic peptides. ANP and BNP induced significant falls in cardiac filling pressures (LAP), as well as a reduction in peripheral resistance (due to direct vasodilatation) that was associated with a decline in MAP. The fall in preload is likely to be mediated by a decrease in circulating volume and venous return (indicated by the rise in haematocrit), possibly through redistribution of plasma volume to the extravascular space [5] and through urinary loss (as evidenced by the significant diuresis in the later 2 h of the infusions). Similar haemodynamic effects have been observed by others during administration of ANP [11, 12] and BNP [13, 14] in experimental models and in human heart failure. CO was not altered compared with a control by either ANP or BNP in the present study. Previous investigations looking at ANP infusion in heart failure have observed either no change in CO [10] or a rise [11], whilst BNP has been shown to increase CO in human heart failure [14] and induce a decrease in a model of thoracic vena caval constriction [13]. These variable responses presumably depend upon the relative changes in afterload and preload in the setting of cardiac failure.

Administration of both ANP and BNP induced significant increases in urine volume and urine sodium, potassium and creatinine excretion in the present study, despite low basal arterial pressures and further reductions during infusion of both peptides. These findings are consistent with observations following both ANP [12] and BNP [14] infusions in heart failure, and may be due to actions at both glomerular and tubular levels. ANP [11, 12] and BNP [31] have both previously been seen to increase the glomerular filtration rate (and therefore sodium and water delivery to the medullary collecting ducts), even without any increase in renal blood flow, as a result of afferent arteriolar vasodilatation and efferent arteriolar vasoconstriction [5]. We observed a tendency for the glomerular filtration rate to increase (as evidenced by the rise in endogenous creatinine clearance) during infusion of both natriuretic peptides, which presumably contributed to the observed diuresis and natriuresis. Like ANP [5], BNP has been shown to reduce sodium reabsorption by the renal tubules, particularly in the inner medullary collecting ducts [32]. In addition, BNP has been demonstrated to oppose the renin-angiotensin–aldosterone system axis to a similar extent to ANP under similar conditions [33]. In the present study, the lack of effect of ANP and BNP on PRA levels presumably reflects a balance of inhibitory actions on the juxtaglomerular apparatus (through high circulatory levels of natriuretic peptides and enhanced delivery of sodium and chloride to the macula densa) and the stimulatory effect on the afferent arteriolar baroreceptor (through the fall in renal artery perfusion pressure). Indeed, these findings are in agreement with other studies where the failure of BNP to suppress PRA was associated with a significant hypotensive response [13]. Despite the absence of PRA change during the peptide infusions, plasma aldosterone was significantly reduced by both ANP and BNP, indicating a direct inhibitory effect of the peptides on the adrenal gland. Whereas renal responses to fluctuations in circulating aldosterone levels are not normally prompt, it is nonetheless possible that the
decline in aldosterone levels contributed to the sustained natriuretic response to ANP and BNP administration seen in the present study.

In summary, the present study demonstrates that ANP and BNP are both powerfully natriuretic, similarly suppress aldosterone and are equipotent in reducing preload and afterload in this ovine model of pacing-induced heart failure. These results suggest that both natriuretic peptides may play an important role in the pathophysiology of congestive heart failure. Furthermore, treatment with neutral endopeptidase inhibitors or NPR-C blockers, which increase circulating levels of ANP and BNP, may prove therapeutically beneficial in cardiac failure. Alongside our previous findings in normal sheep of pacing-induced heart failure, these results may prove therapeutically beneficial in cardiac failure. Whether this is true in humans, remains to be determined.

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