Low Dose B-type Natriuretic Peptide Raises Cardiac Sympathetic Nerve Activity in Sheep

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Abstract

The reported effects of atrial natriuretic peptide (ANP) on sympathetic nerve activity (SNA) are variable, dependent on concomitant hemodynamic actions and likely to be regionally differentiated. There are few reports of the effect of B-type natriuretic peptide (BNP) on SNA and none have measured cardiac SNA (CSNA) by direct microneurography.

We measured the effects of low dose ANP and BNP (2.4pmol/kg/min infused for 120 min) on CSNA and hemodynamics in conscious sheep (n=8).

Whilst there was a trend for mean arterial pressure and cardiac output to fall with both ANP and BNP, changes were not significant compared with vehicle control. However, BNP did significantly reduce systolic arterial (97±4.2 vs 107±6.8mmHg during control; p=0.043) and pulse pressures (0.047) and increase heart rate (110±6.7 vs 96±7.3bpm; p=0.044). Trends for these hemodynamic parameters to change with ANP did not achieve statistical significance.

ANP also had no significant effect on any CSNA parameters measured. In contrast, BNP induced a rise in both CSNA burst frequency (~20 bursts/min higher than control, p=0.011) and burst area (~40% higher than control, p=0.013). BNP-induced rises in burst incidence (bursts/100beats) and burst area/100beats, however, were not significant.

In conclusion, BNP infused at low doses that only had subtle effects on hemodynamics increased CSNA burst frequency and burst area/min. This increase in CSNA may in large part be secondary to an increase in heart rate as CSNA burst incidence and burst area/100 beats were not significantly increased. This study provides no evidence for inhibition of CSNA by natriuretic peptides.

Key Words: blood pressure, catecholamines, heart rate, hormones, nervous system - sympathetic
Introduction

The sympathetic nervous system (SNS) and the cardiac natriuretic peptides are both fundamentally important in cardiovascular regulation and have been known for several decades to interact (15, 20). However, the literature describing these putatively important interactions is disparate and without an evidence-based consensus describing the effects of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) on regional sympathetic nerve activity (SNA).

Early work in various rat models showed ANP inhibition of SNA in a number of tissue beds including renal and lumbar (3, 18, 23, 29). Floras reported in humans that ANP-induced reductions in arterial and central venous pressures were not accompanied by the anticipated reflex increases in muscle SNA (MSNA) suggesting relative sympathoinhibition (15). Thus, although MSNA burst frequency and heart rate increased following ANP infusion, this was less than expected. The inhibition may have been via central or post-ganglionic pathways (16). Other workers have found that ANP inhibits MSNA in normal humans but not in patients with dilated cardiomyopathy (1). Given that SNA is regionally differentiated (22, 24) one cannot extrapolate from findings in one tissue bed to another. It is therefore important to measure SNA at each target organ/tissue of interest such as the heart. A small number of studies have measured the effect of ANP on sympathetic traffic directed to the heart. One study in anesthetized rats, whilst not showing a reduction in cardiac SNA (CSNA) in response to ANP, did show relative sympathoinhibition with no activation of CSNA in the face of a decrease in blood pressure (31). On the other hand, ANP has been reported to elicit catecholamine exocytosis in sympathetic nerves isolated from the guinea pig heart (6). Investigation in human patients with heart failure, however, showed no effect of ANP on cardiac norepinephrine (NE) spillover (2).

Although ANP and BNP are generally considered to have a similar spectrum of biological actions (8), very few studies have examined the interaction of BNP with the SNS. One human study did report that BNP decreased cardiac NE spillover in both healthy controls and patients with heart failure, whereas renal spillover decreased only in the heart failure patients (4). Given the paucity of data on BNP/SNA interaction and the lack of any studies examining effects of BNP on SNA measured by direct microneurography we have used our well-established sheep model for measuring CSNA to determine whether BNP inhibits sympathetic...
traffic to the heart and compared this to the effects of ANP. We hypothesised that, with either
no or minimal systemic hypotension, both ANP and BNP would inhibit CSNA.

Materials and Methods
These studies were approved by the Animal Ethics Committee of the University of Otago,
Christchurch. A total of eight Coopworth ewes weighing 50-69 kg (Lincoln University Farm,
Christchurch, New Zealand) were housed in an air-conditioned, light-controlled room and
received a diet of lucerne chaff and food pellets providing 75 mmol sodium and 150 mmol
potassium per day. General anesthesia was induced by 0.5 mg/kg diazepam and 4mg/kg
ketamine, and maintained with a mixture of isoflurane, nitrous oxide and oxygen. The chest
was opened via a left lateral thoracotomy in order to insert five stainless steel needle
electrodes in the thoracic cardiac nerves as previously described (19). The electrode tips were
 glued into the post-ganglionic nerve and the leads anchored to the mediastinum by means of
sutures and glue before being exteriorized dorsally through the chest wall. In addition, a
cannula (16G Angiocath; Becton Dickinson, Sandy, Utah) was placed in the carotid artery,
polyethylene catheters in the jugular vein and a Swan-Ganz thermodilution catheter (Edwards
Life Sciences, Irvine, California) in the pulmonary artery via the jugular vein for
measurements of cardiac output. Analgesia was provided by a combination of carprofen
(4mg/ml), lignocaine/marcaine (intercostal nerve block) and temgesic (0.01mg/kg). The
animals were allowed to recover for at least 4 days before study commenced.

CSNA recordings were made in fully conscious sheep from pairs of electrodes as previously
described (10, 19). Post-ganglionic efferent sympathetic activity was identified in all animals
by the following characteristics: (a) bursts were pulse (diastole) synchronised; (b) bursts
decreased during sympathetic blockade with hexamethonium infusion (2mg/kg over two
hours); and (c) an inverse relationship existed between burst area and diastolic blood pressure
during baroreflex tests undertaken on each recording day. Only recordings with a signal-to-
oise ratio of greater than two were analysed. CSNA was quantified from the integrated nerve
signal as: (a) bursts per minute (burst frequency); (b) bursts per 100 heart beats (burst
incidence); and (c) area under the integrated signal per minute (burst area/min) and burst
area/100 beats. Area under each burst was integrated individually in the first instance, thus
avoiding periods when no genuine bursts had been identified, then individual areas were
summed to give total area/minute.
As nerve recording fields are not long-lived experiments were commenced after a relatively brief post-surgical recovery period of 4 days post surgery. Each animal was studied on three occasions 1-3 days apart, receiving 2 hour infusions of ANP (Merke Sharpe & Dohme), ovine/porcineBNP-26 (Bachem, Torrance, CA) and vehicle control (haemaccel, Behring) according to a balanced random order design. ANP and BNP were infused at a dose of 2.4 pmol/kg/min which had previously been shown to raise levels within the upper range of normal physiology (8).

Arterial pressure was recorded from 30 min before start of infusion and continued for 60 min after infusion end. Heart rate and pressures were digitally integrated in five minute recording periods and data recorded at 15-30 min intervals throughout the study. Cardiac output was determined by standard thermodilution at the same time-points.

Venous blood was drawn at 30min intervals into chilled EDTA tubes, centrifuged and the plasma stored at -80°C before assay for ANP (7), BNP (25) and catecholamines (17).

Statistics

Results are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) with time as a repeated measure was used to determine time and treatment differences between control and active (ANP or BNP) arms of the study. Where significant differences were identified by ANOVA, a priori Fisher's protected least square difference (LSD) tests were used to identify individual time-points significantly different from time-matched control data. Statistical significance was assumed at p<0.05.

Results

CSNA burst area is an area under the curve measurement calculated in arbitrary units. There were significant baseline differences observed for CSNA burst area (516±101 versus 706±185 and 704±160 burst area for control, ANP and BNP days respectively, p<0.05) and CSNA burst area incidence (518±102 versus 729±208 and 684±156 burst area/100 beats for control, ANP and BNP days respectively, p<0.05). Therefore, these data are expressed as percentage change from baseline.
Infusion of ANP induced a two-fold increase in plasma ANP levels (p<0.001) with concentrations being approximately 10-15 pmol/L above baseline and control levels throughout the infusion period and returning to baseline within 30 min of cessation of infusion (Fig 1). ANP levels did not change with infusion of either control or BNP. BNP infusions induced a significant rise in circulating BNP levels with an increment of approximately 20-25 pmol/L throughout infusion (p<0.001), and with no change in plasma BNP concentrations during control or ANP infusion (Fig 1). Plasma levels of both NE and epinephrine were not significantly different between any of the experimental days (Fig 1).

Both mean arterial pressure (MAP) and cardiac output showed trends to be reduced from baseline levels following commencement of BNP infusions but the difference between control and BNP day did not achieve statistical significance (Fig 2). However, BNP did induce a significant rise in heart rate compared with control (p=0.044), with heart rate being significantly higher 60 mins into the infusion and remaining elevated for the duration of the study (Fig 2). Further analysis of the arterial pressure response revealed that systolic arterial pressure (SAP, p=0.043), but not diastolic arterial pressure (DAP, p=0.327), was significantly reduced by BNP (Fig 3). Consequently BNP, but not ANP, also induced a significant reduction in pulse pressure (p=0.047, Fig 3). There were no significant hemodynamic effects observed for ANP compared with control, although a tendency for MAP, SAP and cardiac output to decline was noted (all p values>0.2).

Compared with control, infusion of BNP induced a significant increase in CSNA burst frequency (p=0.011) and burst area/min (p=0.013) with both indices being increased from 15-30 min into the infusion and remaining elevated above time-matched control for the duration of the study (Fig 4). When these indices are corrected for heart rate, whilst CSNA burst incidence and burst area/100 beats tended to be higher than control, the differences were not statistically significant. ANP had no significant effect on any of the CSNA indices measured (all p values>0.2, Fig 4).
Discussion

While ANP is generally thought to relatively inhibit SNA, published results are variable with effects dependent on how SNA is measured, concomitant hemodynamic actions and which vascular bed is studied. There are few reports of the effect of BNP on SNA and none have measured CSNA by direct microneurography. This study shows that low dose infusions of BNP which produce only subtle changes in hemodynamics (significantly reduced SAP and pulse pressure with non-significant trends for MAP and DAP) showed no apparent inhibitory effect on sympathetic drive directed to the heart. Rather, CSNA burst frequency and burst area/min were increased, likely secondary to baroreflex unloading associated with a modest fall in arterial pressure and an increase in heart rate. Low dose infusions of ANP, which in this study showed no significant effects on hemodynamics, showed no significant effect on CSNA.

The dose of ANP and BNP employed in this study was the same as previously used in a number of sheep studies (7, 8) and as expected raised circulating levels of ANP and BNP by 12-20pmol/L to give achieved plasma concentrations of 25-30pmol/L. These levels are at the upper limit of the physiological range in sheep and similar to those seen with acute dextran loading in normal sheep (12). The achieved plasma levels of ANP and BNP are also well within pathophysiological levels observed in heart failure in both a sheep model (26) and man (27).

In addition to these doses being physiologically relevant, they were intentionally chosen to aim for minimal hemodynamic effect. When these doses have been infused into sheep for longer periods they do result in significant reductions in arterial pressure but only after ~3 hours of continuous administrations (7). Thus, the study design intentionally elected both dose and duration of peptide infusion that would be right at the threshold of inducing significant vasodilation and perturbation of systemic pressures so as not to confound or mask observing inhibition of CSNA in the setting of significant baroreflex drive to increase sympathetic activity. As it turned out, at the dose and duration utilised in the current study, ANP and BNP were actually on either side of the threshold for inducing identifiable vasodilation. Therefore, the BNP arm of the study actually assessed potential of BNP to inhibit CSNA during minimal concurrent vasodilation with statistical significance achieved for some hemodynamic parameters, namely SAP, pulse pressure and heart rate, but not others such as DAP and MAP. Of note, natriuretic peptides have been consistently noted to lower SAP more than DAP (7,8).
Meanwhile, at this dose and duration, we assessed potential sympathoinhibitory effects of ANP in the absence of statistically significant discernable changes in systemic pressures.

Other hemodynamic actions observed with longer infusions of this dose of ANP and BNP include falls in cardiac output and a tendency for increase in heart rate (7, 8). Although there was a trend for cardiac output to fall in the present study, these changes were not significant. There was however a significant increase in heart rate with BNP infusion, but not ANP.

In this setting of significant 12-20pmol/L increments in plasma ANP and BNP with either subtle falls in systemic pressure (systolic but not diastolic) for BNP or no significant falls in the case of ANP, the only indices of CSNA to change were burst frequency and burst area/min with BNP (but not ANP) infusion. BNP induced an increase in both measurements compared with control data. These increments in CSNA indices clearly mirrored the increment and time-course of the increase in heart rate and falls in SAP and pulse pressure. However, when CSNA was corrected for heart rate changes by expressing number of bursts and area indexed to 100 beats, burst incidence and burst area/100 beats were no longer significant. It is well established that CSNA bursts are entrained to heart rate (21) therefore it is expected that burst frequency and burst area/min would increase under conditions that increase heart rate. Thus, it is not clear from the present study whether the primary effect of BNP was to directly stimulate CSNA (via a central or post-ganglionic mechanism) resulting in an increase in heart rate; or to increase heart rate via baroreflex unloading and vagal withdrawal secondary to the non-significant fall in arterial pressure. The findings from a recent study by Chan et al (6) showing that BNP increases NE release in the guinea pig heart \textit{ex vivo}, as well as catecholamine exocytosis in sympathetic nerves isolated from the guinea pig heart, might suggest the former. Regardless, there was definitely no evidence of sympathoinhibition at the level of the cardiac efferent nerves in the present study either before the systemic pressures had fallen or despite the modest baroreflex stimulation as had been suggested by previous work (4). With the doses of ANP and BNP falling on either side of the threshold of inducing discernable modest but statistically significant falls in systemic pressures it is clear that either in the presence or absence of significant falls in arterial pressure, the natriuretic peptides do not inhibit sympathetic drive directed to the heart, at least in sheep at these doses.

Results in the current study are not consistent with the only other published study examining the effect of BNP on cardiac sympathetic activity (4). Those authors used NE spillover...
technique to show that low dose infusions of BNP (similar range but not identical dose or
duration to that used in the current study), that did not induce significant falls in blood
pressure or whole body NE spillover, did reduce cardiac NE spillover in both healthy control
subjects and in patients with chronic heart failure. Even with a higher dose of BNP which did
induce falls in systemic pressure, there was still evidence of relative inhibition of cardiac SNA
as even though cardiac NE spillover returned to baseline levels they were not raised above
baseline as would be expected with normal baroreflex activation of the SNS. The reasons for
the discrepant findings between the Brunner-La Rocca study (4) and the present work are not
clear, but may reflect use of different recording methods (NE spillover in man versus direct
CSNA microneurography is sheep), variations in dose and duration of BNP administration, or
may reflect species differences. The spillover technique relied on a single measurement
immediately after the 25min infusion of BNP, where as CSNA was a continuous
measurement over a much longer timeframe (120 min). It should be remembered that only
20% of the secreted NE spills into the blood stream and this may be affected by NE synthesis
and reuptake at the nerve terminal as well as nerve activity (28). It is clear that neuronal
reuptake is particularly important in the heart with regard to adrenergic effects but so far as
we are aware there is no current evidence that BNP has any effect on NE reuptake (13, 14).
Human studies measuring MSNA responses to ANP did show an immediate increase in burst
frequency over a 3-minute recording interval after the 20 minute infusion, though less than
what was seen after nitroprusside (15, 16). Furthermore immediate MSNA and CSNA
responses to some central stimuli have been shown to correlate (30). An increase in CSNA
following BNP infusion (as we observed in sheep) might explain why the human studies
using nesiritide in acute heart failure have been disappointing (5).

Plasma NE levels did not change significantly in the current study. However, circulating NE
levels are a crude index of sympathetic activity and are often not seen to change in parallel to
more direct indices of SNA, particularly in sheep studies (9, 10, 11). Thus, changes in CSNA
without parallel changes in circulating NE should not be considered discrepant findings but
emphasize the need to study regional SNA at the organ/tissue of interest as it is well
established that there is selectivity of SNA to different tissue beds (22, 24).

In conclusion, this is the first report of the effect of BNP on directly measured
(microneurography) CSNA and provides no evidence of sympathoinhibition. Rather, a
modest increase in CSNA burst frequency was accompanied by a parallel increase in heart
rate, with very subtle decreases in systemic pressure. In the absence of any evidence of systemic vasodilation with similar doses of ANP there were no effects on CSNA. Thus, there is no evidence of sympathoinhibition of CSNA by ANP or BNP but rather CSNA is very sensitive to the presence or absence of subtle baroreceptor unloading by natriuretic peptides.

**Perspectives and Significance**

For several decades it has generally been accepted that ANP and BNP inhibit the SNS but, especially in the case of BNP, there is little direct evidence to support this belief. Any agent that lowers systemic pressures is likely to increase SNA by normal baroreceptor mechanisms, unless there is some specific sympathoinhibitory action. The present study was designed to administer ANP and BNP at doses that had minimal effect on systemic pressures, thereby enhancing our ability to observe inhibition of sympathetic drive to the heart. Results show that, in this experimental setting, there is no evidence of sympathoinhibition of CSNA by ANP or BNP but rather that CSNA is very sensitive to the presence or absence of subtle baroreceptor unloading by natriuretic peptides. However, it should be noted that only one dose was utilised in the current study. Thus, it may be that levels of ANP and BNP were too low to exhibit a physiological effect on CSNA. Nonetheless, the sensitivity of CSNA activation in response to very subtle baroreceptor stimulus has obvious therapeutic implications. Although BNP (nesiritide) has been registered in the US for administration in acute heart failure for over a decade, its ongoing use has been brought into question on a number of fronts including deleterious effects on renal function and minimal or no effect on mortality observed in a number of recent meta-analyses and the recently reported ASCEND-HF trial (5). A number of commentators suggest there may still be a role for lower doses of nesiritide, which induce much less vasodilation and hypotension. However, results of the current study show that even doses of BNP that cause very subtle hypotension may have deleterious actions on the heart via cardiac sympathetic activation.
Acknowledgments

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Disclosures

There are no conflicts of interest to declare.


Legends for Figures

Figure 1: Plasma atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), norepinephrine and epinephrine responses to intravenous infusions of vehicle control [○], ANP [●] and BNP [■] at doses of 2.4pmol/kg/min. Values shown are mean ± SEM for 8 sheep. ANP induced a significant rise in plasma ANP levels (p<0.001) and BNP a significant rise in plasma BNP levels (p<0.001). Individual time points significantly different from time-matched control (Fisher’s protected LSD from two way ANOVA) are indicated by ‡ p<0.001.

Figure 2: Mean arterial pressure, heart rate and cardiac output responses to intravenous infusions of vehicle control [○], ANP [●] and BNP [■] at doses of 2.4pmol/kg/min. Values shown are mean ± SEM for 8 sheep. BNP induced a significant rise in heart rate compared with control (p=0.044). Individual time points significantly different from time-matched control (Fisher’s protected LSD from two way ANOVA) are indicated by *p<0.05 and ‡ p<0.001.

Figure 3: Systolic and diastolic arterial pressure and pulse pressure responses to intravenous infusions of vehicle control [○], ANP [●] and BNP [■] at doses of 2.4pmol/kg/min. Values shown are mean ± SEM for 8 sheep. BNP induced a significant reduction in both systolic arterial pressure (p=0.043) and pulse pressure (p=0.047) compared with control. Individual time points significantly different from time-matched control (Fisher’s protected LSD from two way ANOVA) are indicated by †p<0.01 and ‡ p<0.001.

Figure 4: Cardiac sympathetic nerve activity [CSNA] burst frequency [bursts/min], burst area [BA/min – percentage change from baseline], burst incidence [bursts/100 beats] and burst area incidence [BA/100 beats – percentage change from baseline] responses to intravenous infusions of vehicle control [○], ANP [●] and BNP [■] at doses of 2.4pmol/kg/min. Values shown are mean ± SEM for 8 sheep. BNP induced a significant rise in burst frequency (p=0.011) and burst area/min (p=0.013) compared with control. Individual time points significantly different from time-matched control (Fisher’s protected LSD from two way ANOVA) are indicated by *p<0.05, †p<0.01 and ‡ p<0.001.
Fig 1

Plasma ANP (pmol/L)

Plasma BNP (pmol/L)

Plasma norepinephrine (pmol/L)

Plasma epinephrine (pmol/L)

Control / ANP / BNP

-30 0 30 60 90 120 150 180

Time (min)
Fig 2

Control / ANP / BNP

Mean Arterial Pressure (mmHg)

Heart Rate (bpm)

Cardiac Output (L/min)

Time (min)

-30 0 30 60 90 120 150 180

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Fig 3

Systolic Arterial Pressure (mmHg)

Diastolic Arterial Pressure (mmHg)

Pulse Pressure (mmHg)

Control / ANP / BNP

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Fig 4

CSNA Burst Frequency
(Bursts/min)

CSNA Burst Incidence
(Bursts/100 beats)

CSNA BA/100 beats
(% change from baseline)

CSNA BA/min
(% change from baseline)

Time (min)

Control / ANP / BNP

* †‡

* †‡ ‡‡ ‡

* †