Effects of natriuretic peptides on load and myocardial function in normal and heart failure dogs

JOHN G. LAINCCHBURY, JOHN C. BURNETT, J R., DONNA MEYER, AND MARGARET M. REDFIELD
Division of Cardiovascular Diseases and Internal Medicine, Mayo Clinic and Foundation, Rochester, Minnesota 55902

Lainchbury, John G., John C. Burnett, J r., Donna Meyer, and Margaret M. Redfield. Effects of natriuretic peptides on load and myocardial function in normal and heart failure dogs. Am. J. Physiol. Heart Circ. Physiol. 278: H33–H40, 2000.—The effects on myocardial function and loading conditions of clinically relevant doses of the natriuretic peptides (NP) have not been established. The actions of single doses (100 ng·kg⁻¹·min⁻¹ iv over 30 min) of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) were studied in conscious normal dogs and in dogs with pacing-induced heart failure. All three NP reduced end-diastolic pressure in normal dogs, and ANP and BNP reduced end-diastolic volume. In heart failure ANP and BNP reduced EDP, and ANP reduced EDV. Arterial elastance was unchanged in normal dogs and in dogs with heart failure. ANP increased end-systolic elastance (Eₛₑ) in normal dogs, whereas BNP tended to increase Eₛₑ (P = 0.06). In dogs with heart failure, no inotropic effect was seen. In normal dogs, all NP reduced the time constant of isovolumic relaxation (τᵣ), and ANP and BNP reduced τᵣ in dogs with heart failure. Increases in plasma cGMP in dogs with heart failure were blunted. The NP reduced preload and enhanced systolic and diastolic function in normal dogs. Effects of ANP and BNP on preload and diastolic function were maintained in heart failure. Lack of negative inotropic effects in heart failure supports the validity of the NP as therapeutic agents.

The natriuretic peptide (NP) family consists of a group of structurally similar but genetically distinct peptides and includes atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) of myocardial origin and C-type natriuretic peptide (CNP) of endothelial origin (11, 25, 36). Whereas all these peptides stimulate production of cGMP via particulate guanylate cyclase-linked receptors, differences in the potency of their biological actions may exist because of potential differences in structure, receptor distribution and affinity, and susceptibility to degradation by endopeptidases or the clearance receptor (12, 14, 27, 30). For example, ANP and BNP are known to bind preferentially to the natriuretic peptide A receptor (NPR-A), whereas CNP binds preferentially to the NPR-B receptor, and BNP is the least susceptible to degradation by neutral endopeptidase and removal by the clearance receptor (NPR-C).

Both the NPR-A and NPR-B receptors are present on ventricular myocytes (17, 29). In vitro studies suggest a biphasic concentration-dependent effect of the NP second messenger cGMP on systolic myocardial function (13, 24, 28). Low and moderate concentrations of cGMP had a positive inotropic effect, whereas extremely high cGMP concentrations had a negative inotropic effect. In vitro studies have reported a monophasic effect of cGMP on diastolic function with low and high concentrations producing an acceleration of relaxation (22, 28, 32). We have previously demonstrated in vivo that intracoronary infusion of BNP is lusitropic and that antagonism of the intracardiac NP receptors slows relaxation in experimental heart failure (40).

The NP possess venodilating properties, and in vitro studies have suggested that CNP may be a more potent venodilator than the other natriuretic peptides (39). Effects on arterial tone have not been consistent with some in vivo studies suggesting a reflex-mediated increase in systemic vascular resistance and others, particularly in heart failure, demonstrating a decrease in systemic vascular resistance (16, 20).

Because of their potential for complex effects on myocardial systolic and diastolic function, afterload, and preload, the hemodynamic effects of exogenous infusion of the NP remain difficult to interpret. Because the use of NP as a therapy for heart failure is increasingly advocated, a firm understanding of the myocardial and load-altering effects of the NP is needed. Indeed, recent studies have suggested the efficacy of exogenous BNP in the treatment of heart failure, only limited data exist to establish the superiority of BNP over the other NP (23).

The importance of the NP in modulating neurohumoral and renal function in heart failure are well established; however, the effects of exogenous administration of the NP on renal production of the NP second messenger cGMP and on sodium excretion are blunted in heart failure (34). It remains unclear whether peripheral cGMP-generating capacity or myocardial and load-reducing effects of exogenous NP are qualitatively or quantitatively altered in heart failure.

From these previous in vitro and in vivo observations, we hypothesized that the NP may have a positive inotropic effect in the normal state but may exert a...
negative inotropic effect in heart failure where myocardial concentrations of the NP are already higher. Furthermore, we postulated that the predominant effect on loading conditions would be to reduce preload and that CNP may be the most potent NP in regard to preload-reducing effects. Thus the objective of the current study was to compare the effects of ANP and BNP, specific for the NPR-A receptor, and CNP, specific for the NPR-B receptor, on loading conditions and left ventricular systolic and diastolic function in vivo in conscious dogs in the absence and presence of heart failure.

METHODS

Experiments were performed in male mongrel dogs. Dogs weighed between 18 and 24 kg and were fed standard dog chow (Lab Canine Diet 5006, Purina Mills, St. Louis, MO) with free access to drinking water. The effects of a single dose (100 ng·kg⁻¹·min⁻¹ dissolved in normal saline given intravenously over 30 min at 1 ml/min) of the NP ANP, BNP, and CNP were studied in random order on separate days in eight normal conscious dogs chronically instrumented for the assessment of left ventricular (LV) pressure and volume in the presence of β-adrenergic blockade. Eight dogs were then studied after the development of pacing-induced heart failure with the same dose of the three NP again administered in random order. The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic and was conducted in accordance with the Animal Welfare Act.

Instrumentation

Dogs were anesthetized with Pentothal Sodium (20 mg/kg) and isoflurane (0.5–2.5%) and ventilated with supplemental oxygen. A left lateral thoracotomy was performed and the pericardium widely opened. A screw-in epicardial pacing lead was placed on the right ventricular free wall, and a programmable cardiac pacemaker (model 8329 or 5985, Medtronic, Minneapolis, MN) was implanted subcutaneously for chronic right ventricular pacing. A solid-state micromanometer pressure transducer (Konigsberg Instruments, Pasadena, CA) and a silicon fluid-filled catheter for transducer calibration were inserted through the LV apex. Piezoelectric ultrasound dimension crystals (Triton Technology, San Diego, CA) were implanted on opposing anterior and posterior endocardial surfaces of the left ventricle to measure the internal short-axis dimension and at the basal epicardial and apical endocardial surfaces to measure the LV long-axis dimension. Hydraulic occluders were placed on the proximal superior and inferior vena cavae (In Vivo Metrics, Heladburg, CA). A pacing wire was sutured to the left atrial free wall to control heart rate throughout the experimental protocol. All wires, leads, and catheters were exteriorized to the dorsal neck. Animals received prophylactic antibiotics postoperatively for 2 wk.

Data Collection

Studies were performed after full recovery from the thoracotomy (10–14 days) with the animals awake and standing quietly in a sling. The LV fluid-filled catheter was connected to a pressure transducer calibrated with a mercury manometer, and the signal from the micromanometer was adjusted to match that of the fluid-filled catheter. LV dimensions were measured using the implanted ultrasonic crystals (3 MHz) and a sonomicrometer (Triton Technology). The analog signals of pressure and dimension were processed with an on-line analog-to-digital converter at 250 Hz (Data Translation) and recorded continuously on a computerized data collection and analysis system, which allowed on-line display of all parameters (CA Recorder version 1.1, Data Integrated Scientific Systems, Pinckney, MI).

Experimental Protocol

Normal dogs. Eight normal dogs were studied. Dogs were given propranolol (2 mg/kg iv) and paced via the atrial pacemaker lead at ~20 beats/min above their intrinsic heart rate to block effects of sympathetic activation and control heart rate throughout the experimental protocol.

Fifteen minutes after the administration of propranolol and commencement of atrial pacing, baseline recordings were made. Three steady-state recordings, each of 20-s duration to account for respiratory variation, were made over ~5 min. After the steady-state recordings were completed, at least three sets of variably loaded pressure-volume loop were generated by transient occlusion of the cavae.

Hemodynamic variables were allowed to return to baseline between each caval occlusion. After collection of the baseline data, ANP, BNP, and CNP were infused intravenously for 30 min at 100 ng·kg⁻¹·min⁻¹ on nonconsecutive separate days. At the end of each 30-min dose, steady-state and variably loaded pressure-volume loop recordings were repeated as described above.

Venous blood samples were collected for measurement of plasma NP concentrations and cGMP at baseline and at the end of infusion. To ensure that any effects observed with NP infusion were not caused by attenuation of the effect of propranolol during the infusions, five additional normal dogs were studied in identical fashion but without peptide infusion.

Pacing-induced heart failure. Because three dogs were unable to be restudied after induction of pacing-induced heart failure for technical reasons, three additional dogs were instrumented so that a total of eight dogs with heart failure were studied.

Right ventricular pacing was initiated in the following order: 180 beats/min for 10 days, 200 beats/min for 7 days, 210 beats/min for 7 days, 220 beats/min for 7 days, and finally 240 beats/min for 7 days as previously described (15). At the completion of the pacing protocol, dogs were studied, after ventricular pacing was discontinued, with infusion of ANP, BNP, and CNP on nonconsecutive separate days at 100 ng·kg⁻¹·min⁻¹ for 30 min. Heart rate was controlled by atrial pacing during baseline measurements and throughout the NP infusions. The protocol followed was the same as in the normal dogs except that β-adrenergic blockade was not administered.

Data Analysis

Data were analyzed using the SPECTRUM software program (Wake Forest University School of Medicine). Steady-state recordings were averaged over the 20-s recording period to account for respiratory variation. LV volume was calculated as a modified ellipsoid model using the equation \( V_{LV} = \frac{(IW) \times (LA - SA)}{6} \), where \( V_{LV} \) is volume of LV, SA is short-axis LV dimension, and LA is long-axis LV dimension. This method of volume calculation gives consistent volumes of LV with or without changes in loading conditions and inotropic state (5). Stroke volume was calculated as LV EDV minus LV systolic volume. Ejection fraction (%) was calculated as stroke volume divided by EDV multiplied by 100.

Calculated rate of increase of LV pressure over time (dP/dt) was derived from LV pressure by the five-point Lagrangian fit (19). The rate of LV relaxation was analyzed by determining the time constant of the isovolumic fall of LV pressure (t.). The

Downloaded from http://ajpheart.physiology.org/ by 10.220.33.1 on October 30, 2017
dp/dt to 5 mmHg above LV end-diastolic pressure (EDP) was used to calculate $\tau$. Because the NP resulted in changes in LV EDP, the less load-sensitive method of Raff and Glantz (31) was used to calculate $\tau$. This method calculates $\tau$ as the negative inverse of the slope of dp/dt versus pressure.

Only caval occlusions that produced a fall in end-systolic pressure (ESP) of at least 30 mmHg were analyzed. Premature beats and two subsequent beats were excluded from the analysis. The LV ESP and volume data during the fall in LV pressure, caused by each caval occlusion, were fit using the least-squares technique to the equation ESP = $E_{es}(V_{es} - V_0)$, where $E_{es}$ is slope of the linear ESP volume relationship, representing the LV end-systolic elastance; $V_{es}$ is volume at end systole; and $V_0$ is intercept with the volume axis. The $E_{es}$ is sensitive to changes in contractile state but relatively insensitive to changes in loading conditions.

Arterial elastance was calculated as ESP divided by stroke volume, and LV operant stiffness was calculated as (EDP – $P_{min}$)/(EDV – ESV), where $P_{min}$ is LV minimum pressure, and EDV is LV end-diastolic volume, and ESV is LV end-systolic volume (37).

Plasma Assays

Venous blood samples were collected into EDTA and immediately placed on ice. After centrifugation at 2,500 rpm for 10 min, the plasma was decanted and stored at −20°C until analysis.

Plasma ANP, BNP, CNP, and cGMP were measured by specific radioimmunoassay as previously described (1, 2, 33, 35).

Statistics

All data are presented as means ± SE. The effect of NP infusions on the parameters measured was assessed using paired t-tests, whereas comparisons between groups were made with unpaired t-tests. Statistical significance was accepted as P < 0.05.

RESULTS

None of the measured variables were altered from baseline to the end of the study phase in normal dogs studied after β-blockade and vehicle infusion (time control, n = 5, data not shown).

Effects on Afterload

The effect of NP infusions on indices of LV afterload, ESP and ESV, and arterial elastance in the normal dogs are given in Table 1. All the NP decreased ESP and ESV (P < 0.05 for all) but arterial elastance was unchanged.

The effect of NP infusions on indices of LV afterload, ESP and ESV, and arterial elastance in the heart failure dogs are given in Table 2. Only BNP significantly decreased ESP (P < 0.05). ANP and BNP decreased ESV (P < 0.05 for both). In dogs with heart failure, CNP had no effect on ESP or ESV. None of the NP decreased arterial elastance.

Effects on Preload

The effects of NP infusions on indices of LV preload, EDV and EDP in the normal dogs are given in Table 1. The three NP had similar directional effects on LV EDV and EDP. ANP and BNP significantly decreased EDV (P < 0.05 for both). All NP significantly decreased EDP (P < 0.05 for all).

In dogs with heart failure (Table 2), ANP significantly decreased EDV (P < 0.05). ANP and BNP significantly decreased EDP (P < 0.05 for both). Again CNP was without effect in heart failure.

Effects on Systolic Function

The effect of the NP infusions on the load-insensitive index of systolic function, $E_{es}$, in the normal dogs is given in Table 1. The three NP had similar directional effects on $E_{es}$. ANP significantly increased $E_{es}$ (P < 0.05), and BNP tended to increase this variable (P = 0.06). $E_{es}$ increased with CNP in each dog, but the change was not significant due to the variability in the magnitude of this effect. $E_{es}$ was unchanged in the time control dogs (baseline vs. 30-min recordings: 6.2 ± 0.9 mmHg/ml, P > 0.05).

The effect of the NP infusions on $E_{es}$ in the dogs with congestive heart failure (CHF) is given in Table 2. The three NP had no significant effect on $E_{es}$ in CHF.

The absolute changes in $E_{es}$ with each peptide in the normal and heart failure state are shown in Fig. 1. The change in $E_{es}$ was significantly blunted in the heart failure state with BNP (P < 0.05) and tended to be with ANP (P = 0.08). The change in $E_{es}$ with CNP in the normal state was not significantly different from that observed in heart failure because of the variation in the degree of increase in $E_{es}$ with CNP in the normal state.

### Table 1. Hemodynamic response to natriuretic peptide infusion in normal dogs

<table>
<thead>
<tr>
<th></th>
<th>LVEDP, mmHg</th>
<th>LVEDV, ml</th>
<th>$E_{es}$, mmHg/ml</th>
<th>LVEDP, mmHg</th>
<th>$E_{es}$, mmHg/ml</th>
<th>$\tau$, ms</th>
<th>OS, mmHg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>162 ± 8</td>
<td>30 ± 5</td>
<td>15.5 ± 2.1</td>
<td>40 ± 5</td>
<td>24 ± 2</td>
<td>7.8 ± 1.4</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>End of infusion</td>
<td>150 ± 7*</td>
<td>27 ± 4*</td>
<td>14.2 ± 2.2</td>
<td>38 ± 6*</td>
<td>19 ± 2*</td>
<td>9.5 ± 2.1*</td>
<td>31 ± 1*</td>
</tr>
<tr>
<td>BNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>139 ± 6</td>
<td>27 ± 4</td>
<td>12.3 ± 1.5</td>
<td>37 ± 5</td>
<td>19 ± 2</td>
<td>7.3 ± 1.1</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>End of infusion</td>
<td>120 ± 5*</td>
<td>24 ± 4*</td>
<td>11.6 ± 1.6</td>
<td>34 ± 4*</td>
<td>14 ± 3*</td>
<td>8.3 ± 1.4*</td>
<td>28 ± 1*</td>
</tr>
<tr>
<td>CNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>150 ± 5</td>
<td>28 ± 5</td>
<td>14.7 ± 2.4</td>
<td>39 ± 7</td>
<td>23 ± 3</td>
<td>8.0 ± 1.8</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>End of infusion</td>
<td>142 ± 7*</td>
<td>26 ± 5*</td>
<td>13.1 ± 1.8</td>
<td>37 ± 6</td>
<td>20 ± 2*</td>
<td>10.6 ± 2.7</td>
<td>33 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CNP, C-type natriuretic peptide; LVEDP, left ventricular (LV) end-diastolic pressure; LVEDV, LV end-diastolic volume; $E_{es}$, arterial elastance; LVEDV, LV end-diastolic volume; LVEDP, LV end-diastolic pressure; $E_{es}$, end-systolic elastance; $\tau$, time constant of isovolumic pressure fall calculated assuming a nonzero pressure; OS, LV operant stiffness. *P < 0.05 vs. baseline, †P = 0.06 vs. baseline.
An example of pressure-volume loops from a single dog studied before and after the administration of ANP in the normal and heart failure states is given in Fig. 2.

Effect on Diastolic function

The effect of NP infusions on indices of diastolic function, \( t \) and operant LV stiffness in the normal dogs are given in Table 1. All NP decreased \( t \) (\( P < 0.05 \) for all). Only ANP caused a significant decrease in operant LV stiffness (\( P < 0.05 \)).

The effect of NP infusions on indices of diastolic function, \( t \) and operant LV stiffness in the CHF dogs are given in Table 2. Whereas operant stiffness decreased with all peptides, the change was only statistically significant with BNP. Both ANP and BNP significantly reduced \( t \) (\( P < 0.05 \) for both).

The absolute changes in \( t \) and operant stiffness with each peptide in the normal and heart failure state are shown in Fig. 3. The change in \( t \) with BNP was greater in heart failure (\( P < 0.05 \)). The change in \( t \) with ANP and CNP was not significantly different in heart failure. Although operant stiffness tended to decrease more with all NP infusion in CHF, the difference in the change was not statistically significant.

Effects on NP Levels and cGMP Generation

The effect of the NP infusions on the respective levels of the infused NP and on the circulating levels of cGMP in the normal dogs is given in Table 3. All NP resulted in a decrease in NP levels with ANP and BNP infusions, and an increase in cGMP levels with BNP infusion.

Table 2. Hemodynamic response to natriuretic peptide infusion in heart failure dogs

<table>
<thead>
<tr>
<th></th>
<th>LVEESP, mmHg</th>
<th>LVESV, ml</th>
<th>( E_s ), mmHg/ml</th>
<th>LVEDV, ml</th>
<th>LVEDP, mmHg</th>
<th>( E_w ), mmHg/ml</th>
<th>( t ), ms</th>
<th>OS, ml/mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>93 ± 3</td>
<td>45 ± 7</td>
<td>9.3 ± 1.4</td>
<td>56 ± 7</td>
<td>34 ± 3</td>
<td>5.0 ± 0.8</td>
<td>45 ± 5</td>
<td>2.79 ± 0.38</td>
</tr>
<tr>
<td>End of infusion</td>
<td>86 ± 5</td>
<td>44 ± 7*</td>
<td>9.7 ± 1.8</td>
<td>55 ± 8‡</td>
<td>24 ± 4‡</td>
<td>4.4 ± 0.4</td>
<td>40 ± 5‡</td>
<td>2.42 ± 0.59</td>
</tr>
<tr>
<td><strong>BNP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>91 ± 4</td>
<td>42 ± 7</td>
<td>10.4 ± 2.1</td>
<td>53 ± 8</td>
<td>31 ± 3</td>
<td>4.4 ± 0.8</td>
<td>40 ± 4</td>
<td>2.74 ± 0.59</td>
</tr>
<tr>
<td>End of infusion</td>
<td>82 ± 3*</td>
<td>40 ± 6*‡</td>
<td>9.4 ± 2.1</td>
<td>52 ± 7</td>
<td>25 ± 4*</td>
<td>4.0 ± 0.5</td>
<td>32 ± 3*‡</td>
<td>2.31 ± 0.59*</td>
</tr>
<tr>
<td><strong>CNP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>94 ± 6</td>
<td>43 ± 6</td>
<td>10.8 ± 2.3</td>
<td>55 ± 8</td>
<td>38 ± 2</td>
<td>4.6 ± 1.1</td>
<td>39 ± 4</td>
<td>3.49 ± 0.76</td>
</tr>
<tr>
<td>End of infusion</td>
<td>93 ± 6</td>
<td>43 ± 6</td>
<td>10.6 ± 2.4</td>
<td>54 ± 7</td>
<td>36 ± 3</td>
<td>3.9 ± 0.4</td>
<td>38 ± 3</td>
<td>3.07 ± 0.78‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *\( P < 0.05 \) vs. baseline, †\( P = 0.06 \) vs. baseline, ‡\( P < 0.05 \) for change from baseline vs. change from baseline with CNP infusion.
The effect of the NP infusions on the respective levels of the infused NP and on the circulating levels of cGMP in the CHF dogs is given in Table 3. All NP resulted in increases in the level of the infused peptide (\(P < 0.05\) for all). ANP and BNP increased cGMP (\(P < 0.05\) for both), but CNP did not significantly increase circulating cGMP.

The absolute changes in cGMP with infusion of each peptide in the normal and heart failure state are shown in Fig. 4. The change in cGMP with NP infusion was significantly less in the heart failure state for ANP and CNP (\(P < 0.05\) for both) and tended to be less for BNP (\(P = 0.06\)).

Comparison Between Peptides

In the normal dogs there were no significant differences in the magnitude of the hemodynamic effects between the peptides. In heart failure the decreases in LV ESV and \(\tau\) were significantly greater with BNP infusion than CNP, and the decreases in LV EDV and EDP were significantly greater with ANP infusion than with CNP (\(P < 0.05\) for all, Table 2).

The increase in plasma ANP concentration with ANP infusion was significantly greater than the increase in plasma CNP with CNP infusion in both normal and heart failure dogs (\(P < 0.05\) for both, Table 3). In heart failure the increase in plasma cGMP concentration was significantly greater with ANP and BNP than with CNP infusion (\(P < 0.05\) for both, Table 3).

**DISCUSSION**

The current study compares the effects of infusion of the three NP on load and ventricular function in vivo.

**Table 3. Plasma natriuretic peptide and cGMP concentrations with natriuretic peptide infusion in normal and heart failure dogs**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Heart Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP, pg/ml</td>
<td>cGMP, pmol/ml</td>
</tr>
<tr>
<td>ANP infusion</td>
<td>24.1±3.3</td>
<td>8.7±1.8</td>
</tr>
<tr>
<td></td>
<td>683±180*‡</td>
<td>45.7±7.9*</td>
</tr>
<tr>
<td>BNP infusion</td>
<td>6.8±2.1</td>
<td>7.1±1.2</td>
</tr>
<tr>
<td></td>
<td>631±285*</td>
<td>69.4±11.4*</td>
</tr>
<tr>
<td>CNP infusion</td>
<td>10.2±1.5</td>
<td>8.7±2.5</td>
</tr>
<tr>
<td></td>
<td>148±45*</td>
<td>36.7±9.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *\(P < 0.05\) vs. baseline, ‡\(P < 0.05\) for change from baseline vs. change from baseline with CNP infusion.
normal β-blocked dogs and in dogs with heart failure. The effects of the three NP were qualitatively similar, although CNP tended to be less active in heart failure. In both the absence or presence of heart failure, the hemodynamic effects of the NP were unassociated with significant changes in arterial elastance, a composite indicator of peripheral arterial compliance, impedance, and resistance. The NP reduced preload, and this effect was maintained in heart failure for ANP and BNP. In the normal state, NP infusion produced a mild increase in contractility that was not observed in heart failure.

Effects on Afterload

In vitro studies have shown that ANP and BNP are arterial dilators, whereas CNP, which acts preferentially through a different NP receptor, is predominantly a venodilator (3, 8, 9, 39, 41). Measurements of forearm blood flow have demonstrated that the vasodilating effects of the natriuretic peptides are attenuated in heart failure; however, systemic infusion of NP in both humans with heart failure and experimental heart failure resulted in a decrease in systemic vascular resistance (9, 26, 41). In the current study all three NP reduced ESP and ESV in the normal animals, whereas in heart failure only BNP reduced both ESP and ESV, and ANP reduced ESV. However arterial elastance was unchanged by NP infusion in either the normal or heart failure dogs, suggesting a lack of a direct effect on afterload. The presence of β-blockade in the normal dogs allowing unopposed α-adrenergic action may have contributed to the lack of change in arterial elastance. In addition in both the normal and heart failure groups, the duration of infusion may have been too brief to allow development of the full vasodilating effect (15).

Effects on Preload

Reductions in EDP were more prominent than reductions in EDV in the normal dogs, reflecting the curvilinear nature of the end-diastolic pressure-volume relationship. These reductions in EDP were maintained in heart failure for ANP and BNP and again were more prominent than changes in EDV. This may be therapeutically useful because it would tend to reduce congestion while maintaining cardiac output.

Of note, CNP had no effect on EDV in the normal dogs and no effect on EDP or volume in the heart failure dogs despite in vitro evidence suggesting it may be a more specific venodilator than the other natriuretic peptides (39).

It should be acknowledged that it is difficult to compare the changes in diastolic pressure and volume between the normal and heart failure dogs because baseline pressure and volume differ significantly between the two groups.

Effects on Systolic Function

The data in the current study are consistent with previously reported in vitro data, suggesting that cGMP has positive inotropic effects (13, 24, 28). In the normal dogs where tissue cGMP levels would be expected to be low, there is a positive inotropic response to ANP, and this is lost in heart failure where the tissue levels of cGMP would be expected to be higher. We do not however have dose-response relationships in the current study. In addition rather than reflecting alterations in tissue cGMP concentrations, the difference in inotropic response between normal and heart failure may reflect other factors such as alterations in phosphodiesterase activity in heart failure.

In terms of therapeutic use of the NP, the lack of a negative inotropic response in heart failure is reassuring.

Effects on Diastolic Function

We used a relatively load-insensitive measure of τ, which is unaffected by changes in preload (31). However, ESV and/or pressure decreased with ANP and BNP, and this would be expected to decrease wall stress so that part of the reductions in τ could be load dependent. Whereas we cannot establish the relative contribution of load changes and a direct myocardial effect to the observed improvement in LV relaxation, direct myocardial effects are suggested by previous in vitro studies (6, 22, 28, 32).

The decrease in τ with ANP and BNP was maintained in heart failure, whereas the effect of CNP was lost. An increase in the rate of LV relaxation may improve LV filling and maintain cardiac output, effects that would be beneficial in heart failure (5). The observed changes in operant stiffness are explained by the reduction in preload. Myocardial stiffness was not measured directly, though in vitro data suggests that cGMP increases resting diastolic length (28).

Effects on cGMP Production

In heart failure the generation of cGMP in response to natriuretic peptide infusion was blunted. In particular, no increase in plasma cGMP was noted with CNP infusion in heart failure. This is consistent with the blunted renal cGMP-generating effect seen in heart failure but (at least for ANP and BNP) was not associated with a blunted effect on preload and diastolic function (21). The reason for this reduced ability to generate cGMP in heart failure may reflect a number of factors, including receptor downregulation, alterations of receptor coupling to cGMP production, and upregulation of phosphodiesterases or the clearance receptor (38).

Relative Potency of NP

Effects of the NP are directionally similar (as expected), and no significant differences in the degree of changes in normal dogs were noted, although numbers of dogs are quite small. In the heart failure dogs, CNP infusion appears less active, both in terms of generation of cGMP and hemodynamic effects.

Of note, increments in plasma CNP were less than those of the other peptides, although achieved plasma peptide concentrations were quite variable with each peptide. Directionally similar results have been noted.
in other studies in dogs comparing CNP with ANP infusion at similar doses to those in the current study and may reflect species differences as well as the greater susceptibility of CNP to metabolism by both the clearance receptor and neutral endopeptidase (4, 7). This may explain some of the lack of effect of CNP infusion in heart failure but cannot be the full explanation because plasma levels of CNP were still increased to well beyond the pathophysiological range.

As mentioned, in vitro data have suggested that CNP, which binds preferentially to the β-type natriuretic peptide receptor that is preferentially expressed in veins, may be a more active venodilator than the other NP (39). The lack of a more dramatic response in preload with CNP in this study emphasizes that the actions of the NP in vivo are not just a function of dose of the NP but reflect a number of factors, including NP receptor type and distribution, ability to generate second messenger cGMP, and susceptibility to degradation.

Previous studies with NP infusion have shown that infusion of one peptide can result in increments in the plasma concentrations of the other natriuretic peptides, presumably because of competition for clearance mechanisms (3, 10). These potential interactions between the natriuretic peptides need to be considered in interpreting the results in the current study.

In conclusion, this study provides a comprehensive assessment of the effects of exogenous NP infusion on loading conditions and myocardial function in the absence and presence of heart failure. The myocardial peptides, ANP and BNP, specific for the NPR-A receptor, produced predominant effects on preload in the normal state, and these effects were maintained in the presence of heart failure despite a blunted ability to generate the NP second messenger cGMP. The operating diastolic profile was improved by the NP due to both effects on preload and potentially, direct effects on myocardial diastolic function. The endothelial peptide CNP specific for the NPR-B receptor had similar effects to ANP and BNP but was less active in heart failure. All peptides produced a mild positive inotropic effect in the normal state (significant for ANP), which was not observed in heart failure. Importantly, no significant negative inotropic effect was seen with NP infusion in heart failure. These data provide additional insight into the biological and potential therapeutic actions of the natriuretic peptide system.

This study was supported in part by grants from the Joseph P. and J. E. Bailey, S. M. Sandberg, D. M. Heublein, and J. C. Burnett. Activation of myocardial and renal natriuretic peptides during acute intravascular volume overload in dogs: functional cardioeninal responses to receptor antagonism. Am. J. Physiol. 269: R1324–R1331, 1995.


