Equimolar doses of atrial and brain natriuretic peptides and urodilatin have differential renal actions in overt experimental heart failure

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Chen, Horng H., Alessandro Cataliotti, John A. Schirger, Fernando L. Martin, and John C. Burnett, Jr. Equimolar doses of atrial and brain natriuretic peptides and urodilatin have differential renal actions in overt experimental heart failure. Am J Physiol Regul Integr Comp Physiol 288: R1093–R1097, 2005. First published December 30, 2004; doi:10.1152/ajpregu.00682.2004.—A hallmark of overt congestive heart failure (CHF) is attenuated cGMP production to the endogenous cardiac natriuretic peptide ANP with renal resistance to ANP. ANP and brain natriuretic peptides (BNP) are of myocardial origin, whereas urodilatin (Uro) is thought to be derived from kidney. All three peptides are agonists to the natriuretic peptide-A receptor. Our objective was to compare the cardiorenal and humoral actions of ANP, BNP, and Uro in experimental overt CHF. We determined cardiorenal and humoral actions of 90 min of intravenous equimolar infusion of ANP, BNP, and Uro (2 and 10 pmol·kg⁻¹·min⁻¹) in three separate groups of anesthetized dogs with rapid ventricular pacing-induced overt CHF (240 beats/min for 10 days). BNP resulted in increases in urinary sodium excretion (UNaV) (2.2 ± 0.7 to 164 ± 76 μeq/min, P < 0.05) and glomerular filtration rate (GFR) (27 ± 4 to 52 ± 11 ml/min, P < 0.05) that were greater than those with Uro (P < 0.05), whereas ANP did not result in increases in UNaV or GFR. Increases in plasma cGMP (25 ± 2 to 38 ± 2 pmol/ml, P < 0.05) and urine cGMP excretion with BNP (1,618 ± 151 to 6,124 ± 995 pmol/min, P < 0.05) were similar to those with Uro; however, there was no change with ANP. Cardiac filling pressures were reduced in all three groups. These studies also support the conclusion that in experimental overt CHF, renal resistance to natriuretic peptides in increasing rank order is BNP < Uro < ANP.

THE NATRIURETIC PEPTIDES are a family of structurally similar but genetically distinct peptides, which have diverse actions in cardiovascular, renal, and endocrine homeostasis (7). Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are of myocardial cell origin, and urodilatin (Uro) is thought to be derived from the kidney (12, 14, 22). Uro has been isolated from human urine with an amino acid (AA) sequence identical to that of ANP but with an NH₂-terminal extension of four AA residues (15). All three peptides bind to the natriuretic peptide-A receptor (NPR-A), which via guanosine 3’,5’-cyclic monophosphate (cGMP) mediates natriuresis, vasodilatation, renin inhibition, antimitogenesis, and positive lusitropism (2, 3, 18, 23). All three peptides are cleaved by the natriuretic peptide-C receptor (NPR-C) and degraded by the ectoenzyme neutral endopeptidase 24.11 (NEP) (6), which is found most abundantly in the brush border of the proximal tubules in the kidney (25). Abassi et al. (1) reported that although administration of a NEP inhibitor decreased the clearance of ANP by 65%, it did not affect the clearance of Uro, thereby suggesting that Uro is more resistant to degradation by NEP. Subsequently, Kenny et al. (17) demonstrated that the Kᵢ values for hydrolysis by NEP in increasing order are ANP < Uro < BNP, thus suggesting that BNP is more resistant to degradation by NEP compared with ANP and Uro.

Congestive heart failure (CHF) represents a pathological state in which the activation of the natriuretic peptides exceeds those of all other states (7). However, previous studies have demonstrated that in overt CHF there is attenuated cGMP production to the endogenous cardiac natriuretic peptide ANP with the development of renal resistance (9). To date, it remains undetermined whether the renal resistance to the natriuretic peptides is dependent on the structure of the NPR-A receptor ligands. Recent studies have reported that Uro is more natriuretic and diuretic than ANP in CHF (16); however, little is known about the renal actions of Uro compared with BNP or the comparison between ANP and BNP.

The objective of the current study was to define the cardiorenal and humoral actions of equimolar infusions of ANP, BNP, and Uro in experimental overt CHF, thus providing new insights into the biology of the natriuretic peptides in overt CHF. We determined the cardiorenal and humoral actions of equimolar high- and low-dose infusion of ANP, BNP, and Uro in three separate groups of dogs with rapid ventricular pacing-induced overt CHF. On the basis of the resistance to degradation of these natriuretic peptides to NEP, we hypothesized that BNP would have the greatest biological actions in cardiorenal regulation in experimental overt CHF.

METHODS

Studies were conducted in three groups of anesthetized male mongrel dogs (18–23 kg) with overt chronic heart failure produced by rapid ventricular pacing at 240 beats/min for 10 days on a fixed sodium diet. The three groups consisted of an ANP group (n = 6), a BNP group (n = 5), and a Uro group (n = 6). Studies were performed in accordance with the Animal Welfare Act and with approval of the Mayo Clinic Institutional Animal Care and Use Committee.

Model of pacing-induced overt CHF. We (8) have previously shown that this model has reduced left ventricular systolic function.

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and renal function compared with normal dogs, resembling human overt CHF. All dogs underwent implantation of a programmable cardiac pacemaker (Medtronic, Minneapolis, MN). Under pentobarbital sodium anesthesia (30 mg/kg iv) and artificial ventilation (Harvard respirator; Harvard Apparatus, Millis, MA) with 5 l/min supplemental oxygen, a left lateral thoracotomy and pericardiotomy were performed. With the heart exposed, a screw-in epicardial pacemaker lead was implanted into the right ventricle. The pacemaker generator was implanted subcutaneously into the left chest wall and connected to the pacemaker lead. Dogs received pre- and postoperative prophylactic antibiotic treatment with 225 mg of clindamycin subcutaneously and 400,000 units of procaine penicillin G plus 500 mg of dihydrostreptomycin intramuscularly (Combicid; Pfizer, New York, NY). Postoperative prophylactic antibiotic was continued through the first two postoperative days. Dogs were fed a fixed sodium diet (58 meq/day, Hill’s ID) and allowed water ad libitum. All dogs were walked daily. Appetite, activity, body temperature, and condition of surgical skin sites were documented. After a 14-day postoperative recovery period, the pacemaker was turned on at 240 beats/min.

Experimental protocol. On day 11 of rapid ventricular pacing at 240 beats/min, an experiment was carried out to determined the cardioirenal and humoral effects of 2 and 10 pmol·kg⁻¹·min⁻¹ infusion of ANP, BNP, or Uro in three separate groups of dogs. On the day before experimentation, animals were fasted and given 300 mg of lithium carbonate for assessment of renal tubular function. On the day of the experiment, dogs were anesthetized with pentobarbital sodium (15 mg/kg iv), intubated, and mechanically ventilated with supplemental oxygen (Harvard respirator) at 20 cycles/min. A flow-directed, balloon-tipped thermolodilution catheter (Ohmeda; Criticath, Madison, WI) was advanced into the pulmonary artery via the external jugular vein for cardiac hemodynamic measurement. The femoral artery was cannulated for blood pressure monitoring and blood sampling. The femoral vein also was cannulated for inulin and normal saline infusion. The left kidney was exposed via a flank incision, and the ureter was cannulated for urine collection. A calibrated electromagnetic flow probe was placed around the renal artery to measure renal blood flow (RBF).

The experiment began after a 60-min equilibration period, with a 30-min baseline urinary clearance. After the 30-min baseline urinary clearance, 2 pmol·kg⁻¹·min⁻¹ of canine ANP, canine BNP, or Uro intravenous infusion was started. After a 15-min infusion lead-in period, a 30-min urinary clearance period was performed. Subsequently, the intravenous infusion was increased to 10 pmol·kg⁻¹·min⁻¹. After a 15-min infusion lead-in period, a 30-min urinary clearance period was performed. After that, the intravenous infusion was stopped and three 30-min urinary clearance periods (washout, recovery 1, and recovery 2) were performed. Cardiovascular parameters measured during the acute experiment included mean arterial blood pressure (MAP), right atrial pressure (RAP), mean pulmonary arterial pressure (PAP), cardiac output (CO), and pulmonary capillary wedge pressure (PCWP). CO was determined by thermodilution in triplicate and averaged (Cardiac Output model 9510-A computer; American Edwards Laboratories, Irvine, CA). MAP was assessed via direct measurement from the femoral arterial catheter. Systemic vascular resistance (SVR) was calculated as (MAP – RAP)/CO. Inulin was administered intravenously at the start of the equilibration period as a calculated bolus, followed by a 1 ml/min continuous infusion to achieve plasma levels of 40–60 mg/dl. Glomerular filtration rate (GFR) was measured by inulin clearance.

Cardiovascular hemodynamics were measured at the start of each urinary clearance. Arterial blood was collected in heparin and EDTA tubes and was immediately placed on ice midway through each clearance. After centrifugation at 2,500 rpm at 4°C, plasma was decanted and stored at −20°C until analysis. Urine was collected on ice during the entire period of each clearance for assessment of urine volume, electrolytes, and inulin. Urine collected for cGMP analysis was heated to >90°C before storage.

Hormonal and electrolyte analysis. Plasma and urinary samples for ANP and BNP were measured by radioimmunoassay (RIA) using the method previously described (8). Meyer et al. (19) described a specific RIA for Uro that has minimal cross-reactivity to ANP; however, this is not commercially available. In the current study, plasma and urinary samples for Uro were measured using a Uroditarin RIA kit (RK-005-18; Phoenix Pharmaceuticals, Belmont, CA) that has 100% cross-reactivity with ANP. As such, we could not interpret the baseline plasma and urine levels of Uro; however, we were able to determine the increase in plasma and urinary Uro levels with the infusion of the exogenous peptide. Plasma and urinary samples for cGMP were measured by RIA using the method of Steiner et al. (24). Urinary and plasma inulin concentrations were measured using the anthrone method (11). Urinary and plasma lithium levels were determined using flame emission spectrophotometry (model 357; Instrumentation Laboratory, Wilmington, MA). Employing the lithium clearance (CLLi) technique, we calculated the proximal fractional reabsorption of sodium (DFRNa) according to the equation

\[ \text{DFRNa} = \frac{\text{CLLi}}{\text{GFR}} \]

where GFR = \[(\text{urine Li}\times\text{urine flow})/\text{plasma Li}\] and CLLi = \[(\text{urine Na}\times\text{urine flow})/\text{plasma Na}\].

Statistical analysis. Results are expressed as means ± SE. Comparisons within a group were made by one-way analysis of variance (ANOVA) for repeated measures followed by Dunnett’s posttest analysis. This applies to data in both Tables 1 and 2 (baseline, low dose, high dose). Post hoc analysis was performed by one-way ANOVA for repeated measures followed by Dunnett’s posttest analysis. GraphPad Prism software was used for the above calculation. Statistical significance was accepted as \( P < 0.05 \).

RESULTS

Cardiovascular hemodynamics. Table 1 reports the cardiovascular hemodynamics of the three groups at baseline and in

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Baseline (2 pmol/kg·min⁻¹)</th>
<th>Low Dose (10 pmol/kg·min⁻¹)</th>
<th>High Dose (30 pmol/kg·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP</td>
<td>MAP, mmHg</td>
<td>121 ± 4</td>
<td>119 ± 5</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>35 ± 3</td>
<td>34.3 ± 3</td>
<td>33.3 ± 3</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>29 ± 3</td>
<td>26.3 ± 3*</td>
<td>23.2 ± 2*</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>12 ± 1</td>
<td>10 ± 1*</td>
<td>9 ± 1*</td>
</tr>
<tr>
<td>SVR, mmHg</td>
<td>58 ± 3</td>
<td>58 ± 3</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>BNP</td>
<td>MAP, mmHg</td>
<td>100 ± 5</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>27 ± 2</td>
<td>22.2 ± 2*</td>
<td>20.2 ± 2*</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>23 ± 1</td>
<td>17.1 ± 1*</td>
<td>16.1 ± 1*</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>12 ± 1</td>
<td>10 ± 1*</td>
<td>9 ± 1*</td>
</tr>
<tr>
<td>SVR, mmHg</td>
<td>47 ± 3</td>
<td>44 ± 3</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>Uro</td>
<td>MAP, mmHg</td>
<td>122 ± 7</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>35 ± 2</td>
<td>33.2 ± 2*</td>
<td>30.2 ± 2*</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>28 ± 2</td>
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<td>23.2 ± 2*</td>
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<td>RAP, mmHg</td>
<td>9 ± 1</td>
<td>8 ± 1*</td>
<td>6 ± 1*</td>
</tr>
<tr>
<td>SVR, mmHg</td>
<td>51 ± 1</td>
<td>53 ± 1</td>
<td>49 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANP, atrial natriuretic peptide; MAP, mean arterial blood pressure; CO, cardiac output; PAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; RAP, right atrial pressure; SVR, systemic vascular resistance; BNP, brain natriuretic peptide; Uro, uroditarin. *P < 0.05 vs. baseline.
response to ANP, BNP, and Uro. Infusion of ANP, BNP, and Uro resulted in decreases in cardiac filling pressures (RAP, PAP, and PCWP). CO was unchanged in all three groups, whereas MAP decreased in all three groups, associated with a trend for SVR to decrease. There were no statistically significant differences in the hemodynamic responses among the three groups.

**Plasma concentration and urinary excretion of infused peptides.** The plasma concentrations and urinary excretion of the infused peptide at baseline and at 2 and 10 pmol·kg⁻¹·min⁻¹ infusion are reported in Table 2. Both plasma and urinary ANP are higher compared with BNP at baseline and during infusion of the peptides (P < 0.05, 2-way ANOVA). However, the percentage increase in BNP urinary excretion (+700%) was higher compared with the percentage increase in ANP urinary excretion (+157%) and Uro urinary excretion (+350%) with the high-dose infusion. Because the UroRIA used in this study has 100% cross-reactivity with ANP, we could not interpret baseline Uro levels (see METHODS).

**Plasma cGMP and urinary cGMP excretion.** Figure 1 shows the responses in plasma cGMP and urinary cGMP excretion (UcGMP V) to ANP, BNP, and Uro infusion. In this model of overt CHF, ANP infusion did not result in a significant increase in either plasma cGMP or UcGMP V, whereas both BNP and Uro infusions resulted in increases in plasma cGMP and UcGMP V. Moreover, there was a trend for a greater increase in UcGMP V with BNP compared with Uro (P > 0.05, not significant).

**Renal function.** Figure 2 shows the responses in urinary sodium excretion (UNaV) and urine flow (UV) to ANP, BNP, and Uro. ANP did not produce a significant increase in UNaV or UV. Uro resulted in a small but significant increase in UNaV and UV; however, there was a greater increase in UNaV and UV with BNP, which was greater compared with both ANP and Uro (P < 0.05. 2-way ANOVA). Urinary potassium excretion was similar in all three groups.

Figure 3 shows the responses in GFR and DFRNa to ANP, BNP, and Uro. There was a trend for GFR to increase with ANP but did not reach statistical significance, on the other hand, the increase in GFR with BNP was greater as compared to Uro (P < 0.05 Two-way ANOVA). Only BNP significantly decreased DFRRNa, PFRRNa, was reduced significantly with high-dose ANP (97 ± 1 to 92 ± 2%, P < 0.05), and there was a strong trend for reduction with high-dose Uro (95 ± 2 to 84 ± 4%, P = 0.05) and high-dose BNP (84 ± 6 to 65 ± 14%, P = 0.1). RBF significantly increased with the high dose for both BNP (80 ± 6 to 118 ± 13 ml/min, P < 0.003) and Uro (145 ± 18 to 174 ± 10 ml/min, P < 0.05), and there was a trend for RBF to increase with the high dose for ANP (88 ± 13 to 113 ± 13 ml/min, P = 0.1).

**DISCUSSION**

The objective of the current study was to define the cardio-renal and humoral actions of equimolar infusions of ANP, BNP, and Uro in experimental overt CHF, thus providing new insights into the biology and therapeutics of these natriuretic peptides, all of which function as agonists to the NPR-A receptor. In this model of experimental overt CHF, infusion of ANP did not result in significant increases in urinary cGMP excretion, a marker of the renal activity of the natriuretic peptides. This was associated with no increase in UNaV and a trend for GFR and RBF to increase. Uro infusion produced a significant increase in urinary cGMP that was associated with increases in UNaV and GFR. Finally, BNP infusion resulted in an increase in urinary cGMP with the greatest increase in UNaV and GFR compared with both Uro and ANP infusion. Importantly, only BNP reduced tubular reabsorption at the terminal nephron, which may explain its greater natriuretic action. This study demonstrates the superior of BNP to Uro and ANP in enhancing renal function in overt experimental CHF. These studies also support the conclusion that renal resistance to natriuretic peptides in this model of canine CHF in increasing rank order is BNP < Uro < ANP. An additional conclusion is
that the renal resistance to ANP in overt CHF may not be solely due to downregulation of NPR-A receptor, given that other agonists to this receptor possesses more potent actions.

In this model of overt CHF, infusion of ANP did not result in a significant increase of plasma cGMP or urinary cGMP excretion. This is in contrast to infusion of BNP and Uro, which resulted in a significant increase in both plasma and urinary cGMP but only at the high dose, demonstrating a dose-response effect. Because all three peptides bind to the NPR-A receptor, the lack of effect of ANP on both plasma and urinary cGMP is most likely due to the increased susceptibility to degradation by NEP as previously reported (1). There was a trend for a greater increase in urinary cGMP associated with the natriuretic actions of BNP compared with Uro. This was associated with a greater increase in GFR and a greater decrease in DFRNa with BNP compared with Uro. These findings are consistent with previous in vivo studies that demonstrated that the susceptibility to hydrolysis by NEP was greatest in ANP, followed by that in Uro and then in BNP (17). Investigators in our laboratory (5) previously demonstrated that in this model of overt CHF, combined angiotensin-converting enzyme inhibition and NEP inhibition resulted in enhanced renal function-associated increases in urinary ANP excretion. Further studies investigating the interaction between Uro and NEP inhibition as well as Uro and diuretics are warranted.

The plasma concentration of the infused peptide was the greatest with ANP compared with Uro and least with BNP infusion. The difference in plasma concentration of ANP and BNP with the infusion of the peptides most likely relates to the different baseline levels and the differential effects on GFR and the renal clearance of the peptides. Because the increase in GFR was the greatest with BNP infusion, one may speculate that this resulted in the greatest amount of BNP being filtered from the plasma and presented to renal tubules, resulting in the greatest increase in urinary cGMP excretion and natriuresis. This is supported by the fact the percent increase in BNP urinary excretion (+700%) was higher compared with the percent increases in ANP urinary excretion (+157%) and Uro urinary excretion (+350%) with the high-dose infusion. The renal effects of BNP are dependent on the tubular concentration of BNP and not on plasma levels (4, 20, 21); therefore, the increased excretion of BNP would result in increased delivery of the peptide in the tubules activating the NPR-A receptors in the terminal nephron, which might explain why the lower plasma levels did not limit the actions of BNP on the kidneys. Because the Uro RIA used in this study has 100% cross-reactivity with ANP, we could not interpret baseline Uro levels (see METHODS).

Despite the significant differences in the renal actions of ANP, BNP, and Uro in this model of overt CHF, there were no statistically significant differences in the cardiovascular hemodynamic responses among the three peptides. A possible explanation for this discrepancy may be that the NEP is found most abundantly in the proximal renal tubules, and thus the renal resistance to ANP, and to some extent Uro, is more pronounced compared with cardiovascular actions (25). MAP was significantly reduced in all three groups; this is most likely
secondary to the trend for SVR to decrease and a reduction in preload.

The findings of the current study may have clinical relevance. ANP has been approved in Japan for the management of acute decompensated CHF (7), whereas BNP has been approved for the same indication in the U.S. (10). Uro is currently in phase II clinical trials in Europe for the same clinical indication (13). Our current studies support the conclusion that in our model of overt CHF, renal resistance to natriuretic peptides in increasing rank order is BNP < Uro < ANP. These results may have clinical implications given the therapeutic efficacy of these peptides in the management of overt CHF. The current study provides a strong rationale for the use of higher doses of ANP and Uro compared with BNP in overt CHF. Further studies are required to translate our current study to human CHF and determine the efficacy of the three peptides, ANP, BNP, and Uro, in enhancing renal function in human overt CHF by using clinically relevant doses for each peptide.

In conclusion, the current study demonstrated that in this model of experimental CHF, infusion of BNP results in greater increases in UNaV and GFR compared with infusion of Uro, whereas ANP did not result in significant changes. These favorable renal effects were associated with increases in both plasma cGMP and urinary cGMP excretion. At equimolar doses, BNP is superior to Uro and ANP in enhancing renal function in severe experimental CHF. These studies support the conclusion that in this model of experimental overt CHF, BNP has superior renal enhancing properties, underscoring its clinical utility in CHF.

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