Bradykinin Does Not Modulate the Natriuretic Response to Atrial Natriuretic Factor in Rats With Aortocaval Fistula

Zaid A. Abassi, Henry Klein, James Cox, and Harry R. Keiser

Rats with aortocaval fistula, an experimental model of congestive heart failure (CHF), display two distinct patterns of sodium excretion: some rats develop marked sodium retention and worsening edema with urinary excretion of sodium (UN/V) <200 µEq per 24 hours, i.e., uncompensated CHF, whereas in others sodium balance rapidly returns to normal (UN/V >1,400 µEq per 24 hours), i.e., compensated CHF. Similar patterns of sodium excretion are found in patients with CHF. The mechanisms underlying these responses are not fully understood. The present study was designed to assess whether bradykinin plays a role in the compensatory response to CHF. Infusions of either 10 or 50 µg/kg per minute of synthetic atrial natriuretic factor (ANF) into sham-operated control animals produced significant increases in urine flow and fractional excretion of sodium (FEN). Infusions of ANF at the same doses into rats with compensated CHF increased FEN from 0.11±0.03% to a maximum of 6.10±1.30%, whereas the rise in FEN in animals with uncompensated CHF was significantly reduced (0.05±0.01% to 0.59±0.18%) compared with sham-operated controls (0.23±0.05% to 8.32±1.0%) or the group with compensated CHF. Treatment of the compensated rats with the bradykinin antagonist HOE-140 (D-Arg,[Hyp,Thi',D-Tic,Oic]*-bradykinin) given at a rate of 100 nmol/kg per hour did not affect their renal response to the ANF. In addition, infusion of the bradykinin antagonist alone into compensated rats with aortocaval fistula had no significant effect on their basal urinary flow rate or sodium excretion during the infusion. These findings indicate that kinins do not modulate either the natriuretic response to ANF or basal sodium excretion in rats with compensated CHF. (Hypertension 1993;21:966-970)

KEY WORDS • atrial natriuretic factor • bradykinin • heart failure, congestive • fistula • renal function

Congestive heart failure (CHF) is characterized by a reduction in both cardiac output and arterial blood pressure. The reduction in cardiac output initiates a series of complex compensatory responses that increase vascular resistance and sodium retention and lead to edema formation. The mechanisms underlying these compensatory responses appear to be multifactorial and have been attributed to hemodynamic alterations as well as to the activation of the sodium-retaining systems, i.e., the renin-angiotensin-aldosterone system and the sympathetic nervous system. In addition, plasma levels of atrial natriuretic factor (ANF), a hormone with diuretic, natriuretic, and vasorelaxant properties, are elevated in CHF. Previous studies have shown that rats with an aortocaval fistula, an experimental model of CHF, either decompensate and develop progressive sodium retention or compensate and return to an almost normal sodium balance. ANF infusion induced marked natriuresis in compensated animals but not in sodium-retaining rats. Similar patterns of sodium excretion are found in patients with CHF; i.e., some patients compensate and maintain normal sodium balance, and others display unstable cardiac compensation and avid sodium retention. It is generally accepted that the severity of CHF, as reflected by sodium excretion, depends on the balance between opposing hormonal systems: vasoconstrictors (angiotensin II, norepinephrine, vasopressin, and endothelin) and vasodilators (ANF, prostaglandins I 2 and E2, bradykinin, and endothelium-derived relaxing factor). However, the relative contribution of each hormone or factor to the development of either normal or positive sodium balance is not known. Because bradykinin is thought to be involved in sodium and water homeostasis, a possible role of this substance in the compensation to heart failure has been speculated.

The present study was designed to assess whether bradykinin, a nine-amino acid peptide with natriuretic and vasorelaxant properties, is involved in the compensatory response to CHF. In addition, we studied whether bradykinin modulates the natriuretic response to ANF in rats with compensated CHF.

Methods

This study was performed on male Munich-Wistar rats weighing 250–300 g (Harlan Sprague Dawley, Inc., Indianapolis, Ind.). The animals were kept in individual metabolic cages at a controlled temperature of 23°C on a 6 AM to 6 PM light cycle and fed a standard rat chow diet containing 170 mEq/kg Na+ (Agway, Inc., Syracuse,
N.Y.). Tap water was provided ad libitum. An aortic-caval fistula was created between the abdominal aorta and the inferior vena cava according to the method originally described by Stumpe et al. Briefly, on the day of the surgery, the animals were anesthetized with pentobarbital sodium (40 mg/kg), and the vena cava and aorta were exposed via a midline abdominal incision. A side-to-side (1.2–1.3 mm) surgical anastomosis was placed between the two blood vessels distal to the origin of the renal arteries. Animals that underwent a sham operation served as a control group. The rats were placed in individual metabolic cages (designed to minimize contamination of urine with food) for daily monitoring of their urine output and sodium excretion. Six to 7 days after the operation, rats with aortic-caval fistula were divided into two subgroups according to their daily urinary excretion of sodium (U_{\text{NaV}}): rats with U_{\text{NaV}} <\text{200 }\mu \text{Eq per 24 hours} (\text{decompensated}) and rats with U_{\text{NaV}} >\text{1,400} \text{ }\mu \text{Eq per 24 hours} (\text{compensated}). The decompensated animals developed additional signs of CHF, i.e., severe dyspnea, ascites, edema, pleural effusion, and hypertrophy of the heart, whereas compensated rats did not show these signs.

On the day of the experiment, rats were anesthetized with 100 mg/kg i.p. Inactin (BYK-Golden, Konstanz, FRG) and prepared for clearance studies. The animals were placed on a temperature-regulated table, and a tracheostomy was performed. Polyethylene catheters (PE-50) were inserted into the right carotid artery to measure arterial blood pressure (Grass model 79D), into the jugular vein for infusions, and into the bladder for urine collections. A solution of 0.15 M NaCl containing [methoxy-\text{3H}]inulin (New England Nuclear, Boston) at a concentration of 4 \mu \text{Ci/mL} was infused at a rate equal to 1\% body weight per hour. After an equilibration period of 60 minutes, two baseline urine collections were made, each of 30 minutes duration. A blood sample was drawn halfway between these two clearance periods. After the control collections, one of the following protocols was performed.

**Protocol 1: Dose Response to Atrial Natriuretic Factor**

This series of studies was performed to assess the response of rats with compensated CHF (n=10), rats with decompensated CHF (n=5), and sham-operated controls (n=10) to ANF_{8-33} (Peninsula Laboratories, Inc., Belmont, Calif.). Animals were initially given a low dose of ANF_{8-33}, i.e., a bolus of 10 \mu g/kg followed by a continuous infusion at a rate of 10 \mu g/kg per hour for 1 hour, and then a high dose, i.e., a bolus of 50 \mu g/kg followed by a continuous infusion of 50 \mu g/kg per hour for 1 hour. Urine was collected in periods of 30 minutes beginning with each dose of ANF. Blood samples were drawn at the midpoint of each clearance period. Urinary losses were replaced with an equal volume of 0.9\% saline. All urine was collected into preweighed vials and kept on ice.

**Protocol 2: Effect of a Bradykinin Antagonist on the Natriuretic Response to Atrial Natriuretic Factor**

Rats (n=7) with compensated CHF received an intravenous bolus of 100 nmol/kg of the bradykinin antagonist HOE-140 (D-Arg,[Hyp^5, Thi^2, D-Tic^7, Oic^8]-bradykinin) (Hoechst-Russel Pharmaceuticals Inc., Somerville, N.J.) followed by a sustained infusion (100 nmol/kg per hour) throughout the experiment, i.e., 2.5 hours. Starting 30 minutes after the beginning of the HOE-140 infusion, low and high doses of ANF were infused as described in protocol 1. We have found that 100 nmol/kg per hour of HOE-140 is sufficient to block the hypotensive effect of 0.2 and 2.0 \mu g/kg per hour of bradykinin (unpublished data).

**Protocol 3: Effect of HOE-140 Alone**

Five rats with compensated CHF were given a bolus of 100 nmol/kg of HOE-140 followed by a sustained infusion of 100 nmol/kg per hour throughout the experiment, i.e., 1.5 hours. Three clearance periods of 30 minutes each were obtained.

**Analytical Methods**

Glomerular filtration rate (GFR) was determined via inulin clearance calculated from the concentration of [methoxy-\text{3H}]inulin in 10-\mu L samples of appropriately diluted urine as measured by liquid scintillation counting (Beckman model LS 9000) using Hydrofluor (National Diagnostic Inc., Manville, N.J.). Sodium and potassium concentrations in plasma and urine samples were measured by flame photometry.

**Statistical Analysis**

All data were averaged and expressed as mean±SEM. One-way analysis of variance was used for statistical evaluation of repeated measurements within groups. Two-way analysis of variance was used for comparisons between the different experimental groups; whenever a significant difference was found, unpaired Student's t test was used for comparison of each period between these groups. A value of p<0.05 was considered statistically significant.

**Results**

**Protocol 1**

The effects of low and high doses of ANF_{8-33} on renal function and blood pressure are summarized in Table 1. Infusion of the lower dose of ANF_{8-33} into sham-operated controls increased urine volume 14-fold, U_{\text{NaV}} 26-fold, and fractional sodium excretion (FE_{\text{Na}}) 28-fold. The natriuresis and diuresis were accompanied by a significant fall in mean arterial pressure (MAP) from 149±4 to 109±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged. The larger dose of ANF_{8-33} increased urine volume 16-fold, U_{\text{NaV}} 30-fold, and FE_{\text{Na}} 36-fold and caused a further reduction in MAP to 91±3 mm Hg (p<0.05). However, GFR was unchanged.
Table 1. Renal and Systemic Effects of Atrial Natriuretic Factor in Sham Controls and Compensated and Decompensated Rats With Aortocaval Fistula

<table>
<thead>
<tr>
<th>Group 1. Sham controls</th>
<th>UV (μL/min)</th>
<th>GFR (ml/min)</th>
<th>( U_{\text{Na}}V ) (μEq/min)</th>
<th>( FE_{\text{Na}} ) (%)</th>
<th>( U_{\text{K}}V ) (μEq/min)</th>
<th>( FE_{\text{K}} ) (%)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>6±1</td>
<td>2.3±0.1</td>
<td>0.7±0.1</td>
<td>0.23±0.05</td>
<td>1.5±0.2</td>
<td>15.4±1.6</td>
<td>149±4</td>
</tr>
<tr>
<td>ANF (10 μg/kg per hour)</td>
<td>83±15*</td>
<td>2.3±0.1</td>
<td>18.2±2.4*</td>
<td>6.39±1.0*</td>
<td>2.8±0.2*</td>
<td>35.7±5.3*</td>
<td>109±3*</td>
</tr>
<tr>
<td>ANF (50 μg/kg per hour)</td>
<td>98±15*</td>
<td>1.9±0.2</td>
<td>20.9±2.3*</td>
<td>8.32±1.0*</td>
<td>2.1±0.1*</td>
<td>34.1±3.2*</td>
<td>91±3*</td>
</tr>
</tbody>
</table>

Group 2. Rats with ACF (compensated)

<table>
<thead>
<tr>
<th>UV (μL/min)</th>
<th>GFR (ml/min)</th>
<th>( U_{\text{Na}}V ) (μEq/min)</th>
<th>( FE_{\text{Na}} ) (%)</th>
<th>( U_{\text{K}}V ) (μEq/min)</th>
<th>( FE_{\text{K}} ) (%)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>5±1</td>
<td>1.7±0.2†</td>
<td>0.30±0.1†</td>
<td>0.11±0.03†</td>
<td>1.1±0.1†</td>
<td>15.5±1.8</td>
</tr>
<tr>
<td>ANF (10 μg/kg per hour)</td>
<td>55±12*†</td>
<td>1.7±0.1†</td>
<td>11.3±2.7††</td>
<td>4.90±1.20††</td>
<td>2.5±0.4††</td>
<td>38.6±4.9*</td>
</tr>
<tr>
<td>ANF (50 μg/kg per hour)</td>
<td>65±16††</td>
<td>1.6±0.1†</td>
<td>14.4±3.6††</td>
<td>6.10±1.30††</td>
<td>2.3±0.2††</td>
<td>38.4±3.0*</td>
</tr>
</tbody>
</table>

Group 3. Rats with ACF (decompensated)

<table>
<thead>
<tr>
<th>UV (μL/min)</th>
<th>GFR (ml/min)</th>
<th>( U_{\text{Na}}V ) (μEq/min)</th>
<th>( FE_{\text{Na}} ) (%)</th>
<th>( U_{\text{K}}V ) (μEq/min)</th>
<th>( FE_{\text{K}} ) (%)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4±1†</td>
<td>1.6±0.1††</td>
<td>0.33±0.1††</td>
<td>0.14±0.05†</td>
<td>1.2±0.1†</td>
<td>18.5±2.9</td>
</tr>
<tr>
<td>ANF (10 μg/kg per hour)</td>
<td>56±12††</td>
<td>1.6±0.2†</td>
<td>10.9±2.8††</td>
<td>5.20±1.53††</td>
<td>2.8±0.3††</td>
<td>43.8±4.9††</td>
</tr>
<tr>
<td>ANF (50 μg/kg per hour)</td>
<td>49±11††</td>
<td>1.7±0.1†</td>
<td>11.8±2.7††</td>
<td>5.50±1.22††</td>
<td>2.2±0.2††</td>
<td>37.2±5.2*</td>
</tr>
</tbody>
</table>

UV, urine flow rate; GFR, glomerular filtration rate; \( U_{\text{Na}}V \), absolute sodium excretion; \( FE_{\text{Na}} \), fractional sodium excretion; \( U_{\text{K}}V \), absolute potassium excretion; \( FE_{\text{K}} \), fractional excretion of potassium; MAP, mean arterial pressure; ANF, atrial natriuretic factor; ACF, aortocaval fistula. Values are mean±SEM. Baseline values are the average of two baseline urine collections.

*tp<0.05 compared with baseline values with no ANF.

†p<0.05 compared with sham control.

‡p<0.05 compared with compensated rats.

compensated rats increased from a basal value of 0.11±0.03% to a maximum of 6.1±1.3% (55-fold) after the larger dose of ANF, it increased in compensated rats from 0.05±0.01% to only 0.59±0.18% (12-fold). Similar differences between these subgroups were also observed in urine volume and \( U_{\text{Na}}V \) responses to ANF.

Protocol 2

Administration of both doses of ANF to compensated rats pretreated with the bradykinin antagonist induced diuretic and natriuretic responses that were not significantly different from those obtained in compensated rats without the antagonist (Table 1). Furthermore, the fall in blood pressure observed after ANF infusion was the same in compensated rats whether or not they were treated with the bradykinin antagonist.

Protocol 3

HOE-140 infusion into compensated rats caused a slight but insignificant increase in urine volume, \( U_{\text{Na}}V \), \( FE_{\text{Na}} \), and GFR (Table 2). However, the absolute and fractional excretion of potassium increased from baseline values of 0.9±0.12 μEq/min and 13.8±1.6% to 2.1±0.2 μEq/min (p<0.05) and 27.3±3.0% (p<0.05), respectively. These effects were accompanied by a small but significant reduction in MAP (Table 2).

Discussion

The present study demonstrated that 1) rats with an aortocaval fistula, an experimental model of CHF, display two different clinical presentations: some rats decompensate and develop sodium retention, and other rats compensate and increase their daily sodium excretion to normal; 2) decompensated rats express marked natriuresis to ANF, whereas compensated rats show marked natriuresis to ANF; and 3) infusions of the bradykinin antagonist HOE-140 alone or with ANF into compensated rats have no effect on the magnitude of their renal responsiveness to either endogenous or exogenous ANF.

Table 2. Renal and Systemic Effects of Bradykinin Antagonist HOE-140 in Compensated Rats With Congestive Heart Failure

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>UV (μL/min)</th>
<th>GFR (ml/min)</th>
<th>( U_{\text{Na}}V ) (μEq/min)</th>
<th>( FE_{\text{Na}} ) (%)</th>
<th>( U_{\text{K}}V ) (μEq/min)</th>
<th>( FE_{\text{K}} ) (%)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3±0.5</td>
<td>1.63±0.2</td>
<td>0.29±0.08</td>
<td>0.13±0.03</td>
<td>0.91±0.12</td>
<td>13.8±1.6</td>
<td>147±4</td>
</tr>
<tr>
<td>30</td>
<td>6.5±1.3</td>
<td>1.88±0.2</td>
<td>0.47±0.18</td>
<td>0.17±0.06</td>
<td>1.54±0.18*</td>
<td>21.1±2.0*</td>
<td>133±3*</td>
</tr>
<tr>
<td>60</td>
<td>5.9±1.3</td>
<td>1.56±0.2</td>
<td>0.34±0.15</td>
<td>0.17±0.08</td>
<td>1.70±0.26*</td>
<td>29.3±3.4*</td>
<td>133±3*</td>
</tr>
<tr>
<td>90</td>
<td>6.7±0.7</td>
<td>1.99±0.3</td>
<td>0.40±0.13</td>
<td>0.15±0.05</td>
<td>2.10±0.20*</td>
<td>27.3±3.0*</td>
<td>133±3*</td>
</tr>
</tbody>
</table>

UV, urine flow rate; GFR, glomerular filtration rate; \( U_{\text{Na}}V \), absolute sodium excretion; \( FE_{\text{Na}} \), fractional sodium excretion; \( U_{\text{K}}V \), absolute potassium excretion; \( FE_{\text{K}} \), fractional excretion of potassium; MAP, mean arterial pressure. Values are mean±SEM.

*p<0.05 compared with first period when no HOE-140 was given.
This classification of rats with aortocaval fistula is of special interest, because it resembles the clinical presentations of CHF in humans, namely, compensated or decompensated. Moreover, patients with decompensated CHF develop avid sodium retention when placed on a normal sodium diet, whereas patients with compensated CHF maintain a normal sodium balance under the same condition. These differences between compensated and decompensated patients or animals with CHF are probably due to major differences in their hormonal status. A common feature between patients with decompensated CHF and sodium-retaining rats with aortocaval fistula is the activation of the renin-angiotensin-aldosterone system. Previously, we have shown that plasma renin activity and plasma aldosterone concentration were higher in rats with decompensated CHF when compared with rats with compensated CHF. Dzau has reported similar findings in humans; i.e., plasma renin activity and plasma aldosterone concentration were markedly increased in patients with acute decompensated CHF, and they were in the normal range in those with compensated or stable CHF. Villareal et al demonstrated in dogs with aortocaval fistula that the initial period of sodium retention was accompanied by elevation in plasma renin activity and plasma aldosterone concentration, whereas the late phase of compensation and normal sodium balance was associated with sharp falls in plasma renin activity and plasma aldosterone concentration. Taken together, these results suggest that activation of the renin-angiotensin-aldosterone system attenuates the natriuretic effect of the elevated endogenous ANF observed in CHF. Therefore, this system is likely to play a major role in the pathogenesis of the sodium retention in decompensated CHF. However, inhibition of that system by means of angiotensin converting enzyme inhibitors only partially restored the natriuretic response to ANF, suggesting that other systems, such as the sympathetic nervous system, may contribute to the avid sodium retention in CHF.

On the other hand, body homeostasis for fluids and sodium is maintained by a fine balance between vasocostrictors versus vasodilators and natriuretic versus antinatriuretic mechanisms. ANF was initially thought to be elevated in compensated CHF and therefore responsible for an increased natriuresis leading to compensation. However, ANF was found to be elevated to the same extent in both compensated and decompensated CHF. Therefore, other mechanisms such as bradykinin and prostaglandins were thought to be responsible for the development of the compensatory response to CHF. However, the present study demonstrates that inhibition of bradykinin in rats with compensated CHF, by the specific bradykinin antagonist HOE-140, did not decrease significantly sodium excretion or urine flow. The observed hypertensive and kaliuretic effects of HOE-140 could be explained by a possible partial agonistic activity of this agent. Moreover, pretreatment of these rats with a bradykinin antagonist had no effect on their renal response to exogenous ANF. It is unlikely that bradykinin plays a significant role in the renal compensatory response to experimental CHF. Our finding that bradykinin does not participate in the regulation of sodium and water balance is in agreement with recently published data by Strick et al. According to these authors, infusions of the bradykinin antagonist D-Arg⁵Hyp⁷Thr⁷D-Phe⁷-Thr³-bradykinin into the renal artery of anesthetized dogs had no effect on basal renal hemodynamic or excretory parameters. Furthermore, the antagonist had no effect on pressure-induced natriuresis. Similarly, Legault et al reported that half of the dogs with long-term stenosis of the vena cava showed a significant natriuretic response to ANF, whereas the other half failed to develop a significant natriuresis to ANF infusion. In contrast to the present study, when “responder” dogs were treated with the kallikrein inhibitor aprotinin, they developed a blunted natriuretic response to ANF. In addition, when “nonresponder” dogs were infused simultaneously with ANF and bradykinin, they showed an improved natriuresis compared with ANF infusion alone. The authors concluded that bradykinin modulates the natriuretic response to ANF in dogs with long-term stenosis of the vena cava. These differences in findings are probably due to the differences in the protocols and drugs used. These authors used low-output experimental heart failure, whereas we used a high-output model of CHF. Moreover, the kallikrein inhibitor they used, i.e., aprotinin, is not specific and inhibits the generation or degradation of a wide range of proteases besides kallikrein, the enzyme that cleaves bradykinin from kininogen. HOE-140 is a potent, long-acting specific bradykinin antagonist that blocks the biological effects of bradykinin in different organs. In addition, their finding that bradykinin improved the natriuretic response to ANF in “nonresponder” dogs may be attributed to the fact that bradykinin and ANF acted synergistically, inhibiting sodium transport in the collecting duct. Recently, Stoos et al reported that neither ANF nor bradykinin alone inhibits the transport of sodium in the M-1 cortical collecting cell line, whereas when bradykinin and ANF were added simultaneously, sodium transport decreased significantly. These results do not rule out the possibility that kinins might play a role in sodium and water homeostasis in the long term or in the very early stage of CHF. Nevertheless, our data clearly show that kinins neither modulate the natriuretic response to ANF nor regulate electrolyte and fluid balance in rats with compensated CHF.

References

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