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 (TEN). 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[1-Hyp,1,3-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.1,2 The reduction in cardiac output initiates a series of complex compensatory responses that increase vascular resistance and sodium retention and lead to edema formation.1,3 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.1,2,4-8 In addition, plasma levels of atrial natriuretic factor (ANF), a hormone with diuretic, natriuretic, and vasorelaxant properties, are elevated in CHF.9-12 Previously, we 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.11,13 ANF infusion induced marked natriuresis in compensated animals but not in sodium-retaining rats.11,13,14 Similar patterns of sodium excretion are found in patients with CHF2,15; 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 I2 and E2, bradykinin, and endothelium-derived relaxing factor).2,7 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.2,16

The present study was designed to assess whether bradykinin, a nine-amino acid peptide with natriuretic and vasorelaxant properties,16 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 aorto-
caval fistula was created between the abdominal aorta and the inferior vena cava according to the method 
originally described by Stumpe et al.17 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 mini-
mize contamination of urine with food) for daily mon-
itoring of their urine output and sodium excretion. Six 
to 7 days after the operation, rats with aortocaval fistula 
were divided into two subgroups according to their daily 
urinary excretion of sodium (U_{NaV}; rats with U_{NaV} <200 \mu\text{Eq per 24 hours} (decompensated) and rats with 
U_{NaV} >1,400 \mu\text{Eq per 24 hours} (compensated). The 
decompensated animals developed additional signs of 
CHF, i.e., severe dyspnea, ascites, edema, pleural effu-
sion, and hypertrophy of the heart, whereas compens-
sated 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 con-
taining [methoxy-\textsuperscript{3}H]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\textsubscript{8,33} (Peninsula Laboratories, 
Inc., Belmont, Calif.). Animals were initially given a low 
dose of ANF\textsubscript{8,33}, i.e., a bolus of 10 \mu\text{g/kg} followed by a 
continuous infusion at a rate of 10 \mu\text{g/kg per hour} for 1 
hour, and then a high dose, i.e., a bolus of 50 \mu\text{g/kg} 
followed by a continuous infusion of 50 \mu\text{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. Urine 
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 in-
travenous bolus of 100 nmol/kg of the bradykinin an-
tagont HOE-140 (\textsuperscript{D}-Arg\textsuperscript{1},\textsuperscript{Hyp}\textsuperscript{5},\textsuperscript{Thi}\textsuperscript{6},\textsuperscript{D}-Tic\textsuperscript{7},\textsuperscript{Oic}\textsuperscript{8}-
bradykinin) (Hoechst-Russel Pharmaceuticals Inc., 
Somerville, N.J.)\textsuperscript{18} 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\text{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 exper-
iment, 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-\textsuperscript{3}H]inulin in 10-\mu\text{L} samples of appropriately 
diluted urine as measured by liquid scintillation count-
ing (Beckman model LS 9000) using Hydrofluor (Na-
tional 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 \textit{t} 
test was used for comparison of each period between 
these groups. A value of \(p<0.05\) was considered statis-
tically significant.

**Results**

**Protocol 1**

The effects of low and high doses of ANF\textsubscript{8,33} on renal 
function and blood pressure are summarized in Table 1. 
Infusion of the lower dose of ANF\textsubscript{8,33} into sham-
operated controls increased urine volume 14-fold, U_{NaV}  
26-fold, and fractional sodium excretion (FE_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\textsubscript{8,33} increased urine 
volume 16-fold, U_{NaV} 30-fold, and FE_Na 36-fold and 
caus ed a further reduction in MAP to 91±3 mm Hg 
(\(p<0.05\)). Baseline values in sham-operated control rats 
were GFR, 2.3±0.1 mL/min; U_{NaV}, 0.7±0.1 \mu\text{Eq/min}; 
FE_Na, 0.23±0.05%; and MAP, 149±4 mm Hg. The same 
parameters were significantly decreased in rats with 
compensated CHF (GFR, 1.7±0.2 mL/min; U_{NaV}, 
0.3±0.1 \mu\text{Eq/min}; FE_Na, 0.11±0.03%; and MAP, 142±4 
mm Hg) and decreased even further in rats with decomp-
ensated CHF (GFR, 0.9±0.1 mL/min; U_{NaV}, 
0.06±0.01 \mu\text{Eq/min}; FE_Na, 0.05±0.01%; and MAP, 
115±7 mm Hg). Although the absolute natriuretic and 
diuretic responses to both doses of ANF\textsubscript{8,33} were 
decreased on an average of 34% in decompensated rats 
with aortocaval fistula, they were decreased an average 
of 97% in decompensated animals. Although FE_Na in
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>Group 2. Rats with ACF (compensated)</th>
<th>Group 3. Rats with ACF (decompensated)</th>
<th>Group 4. Rats with ACF (compensated)+HOE-140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td>ANF (10 μg/kg per hour)</td>
<td>ANF (10 μg/kg per hour)</td>
<td>ANF (10 μg/kg per hour)</td>
<td>ANF (10 μg/kg per hour)</td>
</tr>
<tr>
<td>ANF (50 μg/kg per hour)</td>
<td>ANF (50 μg/kg per hour)</td>
<td>ANF (50 μg/kg per hour)</td>
<td>ANF (50 μg/kg per hour)</td>
</tr>
</tbody>
</table>

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>U₉vV (μEq/min)</th>
<th>FE₉v (%)</th>
<th>U₉V (μEq/min)</th>
<th>FEₓ (%)</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±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±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±3.4</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±3.0</td>
<td>133±3</td>
</tr>
</tbody>
</table>

UV, urine flow rate; GFR, glomerular filtration rate; U₉vV, absolute sodium excretion; FE₉v, fractional excretion of potassium; U₉v, absolute potassium excretion; FEₓ, 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.

Discussion

The present study demonstrated that 1) rats with an aortocaval fistula, an experimental model of CHF, displayed 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 markedly blunted natriuretic responses 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.
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 vasconstrictors 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 hypotensive 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-Arg-Hyp-Thi-D-Phe. Thi-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 aprotenin, 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., aprotenin, is not specific and inhibits the generation or degradation of a wide range of proteases besides kallikrein, the enzyme that cleaves bradykinin from kinogen. 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.

The present study focuses mainly on the possible role of bradykinin in the acute compensatory response to CHF because the experiments are short term. 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

Bradykinin does not modulate the natriuretic response to atrial natriuretic factor in rats with aortocaval fistula.

Z A Abassi, H Klein, J Cox and H R Keiser

_Hypertension_. 1993;21:966-970
doi: 10.1161/01.HYP.21.6.966

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/21/6_Pt_2/966

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org/subscriptions/