Accelerated Secretion of Brain Natriuretic Peptide From the Hypertrophied Ventricles in Experimental Malignant Hypertension

Masakazu Kohno, Takeshi Horio, Minoru Yoshiyama, and Tadanoa Takeda

Plasma concentrations of immunoreactive (ir) atrial (ANP) and brain (BNP) natriuretic peptides were measured in the prehypertensive and hypertensive phases in spontaneously hypertensive rats (SHR) and in the malignant phase of hypertension caused by deoxycorticosterone acetate (DOCA)–salt in SHR. The secretory rate of ANP and BNP were examined in the perfusion of isolated beating heart before and after atrial removal. Plasma irANP and irBNP in mature SHR were higher than those of control Wistar-Kyoto (WKY) rats, whereas ANP and BNP values in young SHR did not differ from those of control WKY rats. DOCA-salt treatment for 8 weeks markedly increased blood pressure, ventricular weight, and plasma irANP and irBNP in SHR. ANP and BNP values were positively correlated with ventricular weight in DOCA-salt SHR. The secretory rate of ANP and BNP from the perfused whole heart were much higher in DOCA-salt SHR than other rat groups. A large amount of BNP was secreted from the hypertrophied ventricles in DOCA-salt SHR. In contrast, ANP was mainly secreted from the atrium in all rat groups. High-performance liquid chromatography profiles of extract in plasma showed that a major component of irANP and irBNP corresponded to synthetic rat ANP-(1-28) and rat BNP-45, respectively. Results suggest that both rat ANP-(1-28) and rat BNP-45 are markedly increased in plasma in DOCA-salt-induced malignant hypertension of SHR and that the major source of circulating BNP is the hypertrophied ventricles in this model. (Hypertension 1992;19:206-211)

An atrial natriuretic peptide (ANP) is a diuretic, natriuretic, and vasodilatory peptide hormone originally isolated from the mammalian heart. Brain natriuretic peptide (BNP) was first identified in the porcine brain and later isolated from the porcine heart. Porcine BNP consists of 26 amino acid residues with much homology with the sequence of ANP. BNP elicits a spectrum of diuretic, natriuretic, and hypotensive effects similar to that of ANP. Later, a low molecular weight form of rat BNP, rat BNP-45, which was identical to the amino acid sequence (51-95) of the rat BNP precursor deduced from the complementary DNA sequence, was found in the rat atrium.

Here, to assess any changes in plasma concentrations of ANP and BNP in experimental hypertension, we measured plasma concentrations of immunoreactive (ir) ANP and irBNP in young and mature spontaneously hypertensive rats (SHR) and in age-matched Wistar-Kyoto (WKY) rats. In addition, we measured plasma concentrations of irANP and irBNP in the experimental model of malignant hypertension caused by deoxycorticosterone acetate (DOCA) plus salt in SHR. Furthermore, the secretory rate of irANP and irBNP were examined in the perfusion of isolated beating heart obtained from DOCA-salt SHR, DOCA-salt WKY rats, SHR, and WKY rats by Langendorff's method before and after atrial removal.

We characterized irANP and irBNP in pooled plasma from these experimental groups and pooled perfusate from isolated hearts using high-performance liquid chromatography (HPLC) coupled with a radioimmunoassay.

Methods

Plasma Concentrations of Atrial and Brain Natriuretic Peptides in Young (6-week-old) SHR

Male 5-week-old SHR and WKY rats (Shizuoka Lab, Shizuoka, Japan) were housed in groups of five rats each in a room with controlled temperature (25°C), humidity (50–60%), and lighting (14:10-hour light/dark cycle). All rats were given tap water ad libitum and ordinary rat food containing 0.39%...
sodium and 0.98% potassium (Clea Japan, Inc., Tokyo). After 1 week of habituation, blood samples were collected from each rat by rapid decapitation.

**Plasma Concentrations of Immunoreactive Atrial and Brain Natriuretic Peptides in Mature (18-week-old) SHR and DOCA-Salt SHR**

Male 10-week-old SHR and WKY rats were divided into four groups. Groups of DOCA-salt SHR (n=7) and WKY rats (n=6) were treated with DOCA and also given 1% NaCl drinking water ad libitum as described previously. Control groups of SHR (n=6) and WKY rats (n=6) were given water ad libitum and were injected subcutaneously once a week with the vehicles for DOCA and salt. All groups received ordinary rat food containing 0.39% sodium and 0.98% potassium. At 18 weeks of age, blood samples were collected from each rat by rapid decapitation.

**Heart Perfusion Experiment**

Male 10-week-old SHR and WKY rats were divided into four groups: DOCA-salt SHR (n=7), DOCA-salt WKY rats (n=5), SHR (n=6), and WKY rats (n=5). DOCA-salt treatment was done as described above. Control groups of SHR and WKY rats were given water ad libitum and were injected subcutaneously once a week with the vehicles for DOCA and salt. At 18 weeks of age, the rats were anesthetized with intraperitoneal injection of sodium pentobarbital (35 mg/kg). Their hearts were removed and quickly placed into cold buffer solution. Perfusion of isolated rat hearts was carried out essentially as described by Yoshiyama et al. In brief, the perfusion apparatus was a Langendorff preparation with the ascending aorta cannulated and perfused from a reservoir maintained at 37°C. Hearts were then perfused at constant flow (12 ml/min) by means of a roller pump. After 10 minutes for stabilization, coronary venous effluents were collected every 1 minute. Then, after atrial removal and 10 minutes for stabilization, coronary effluent perfusates were collected again as mentioned above.

**Analytical Methods**

Systolic blood pressure was measured by the tail-cuff method with an electrophymomanometer (model RS-100, Riken Kaishatsu Co., Tokyo) under conscious conditions. Five readings were averaged for each rat.

Blood urea nitrogen (BUN) and serum creatinine were measured by a routine automatic method.

The hearts were removed after a rapid decapitation. After washing in a 0.9% saline, the atria and ventricles were carefully dissected and the ventricles (left ventricle including septum and right ventricle) were weighed.

**Measurement of Immunoreactive Atrial and Brain Natriuretic Peptides in Plasma and Perfusate**

Blood or perfusate was collected by siliconized disposable glass tubes chilled on ice and containing aprotinin (500 Kallikrein inactivator units/ml) and ethylenediaminetetraacetic acid (1 mg/ml). Plasma or perfusate was centrifuged for 10 minutes at 4°C and immediately was frozen and stored at -80°C for several days.

irANP or BNP was extracted from plasma or perfusate by a Sep-Pak C18 cartridge (Water Associates, Milford, Mass.) according to the method previously described. The recovery rate of BNP or ANP was calculated by the addition of two different amounts of cold rat ANP-(1-28) (50 or 100 pg/ml) or cold rat BNP-45 (10 or 50 pg/ml), respectively, to hormone-free plasma prepared by passage of plasma through a Sep-Pak C18 cartridge. The recovery rate of rat ANP-(1-28) and rat BNP-45 was 69% and 70%, respectively.

The radioimmunoassay for ANP was done as previously reported. The antibody used here (Peninsula Laboratories Inc., Belmont, Calif.) reacts 100% with rat ANP (1-28) and cross-reacts 100% with human ANP (1-28), 57% with human ANP (18-28), 27% with rat ANP (5-27), and 3% with rat ANP (5-25). The antibody does not cross-react with porcine BNP-26, human BNP-32, rat BNP-45, rat BNP-32, somatostatin, vasopressin, or endothelin-1.

The concentration of irBNP was measured with antibody against synthetic rat BNP-32 and iodine-125-labeled rat BNP-32 (Peninsula) essentially as reported for human BNP radioimmunoassay. This antibody reacts 100% with rat BNP-32; cross-reacts 100% with rat BNP-45; and does not cross-react with rat ANP (1-28), β-rat ANP, human ANP (1-28), porcine BNP-26, human BNP-32, angiotensin II, vasopressin, or endothelin-1.

The radioimmunoassay of BNP was performed in an assay buffer of 0.01 M sodium phosphate, pH 7.4, containing 0.05 M NaCl, 0.1% bovine serum albumin, 0.1% Nonidet NP-40, and 0.01% NaN3, as reported for human BNP radioimmunoassay.

The effective range of the standard curve was between 0.5 and 50 pg of rat BNP-32, as reported for human BNP radioimmunoassay. The interassay variations of ANP and BNP were 10.6% and 12.2% and the intra-assay variations of ANP and BNP were 6.3% and 7.6%, respectively.

**Reverse-Phase High-Performance Liquid Chromatography of Immunoreactive Atrial Natriuretic Peptide and Immunoreactive Brain Natriuretic Peptide in Extracts of Rat Plasma or Perfusate From the Isolated Hearts**

Reverse-phase HPLC was performed with an octadecysilica column (4.6×250 mm, Tosoh, Tokyo), which was eluted with a linear gradient of acetonitrile from 15% to 60% in 0.09% trifluoroacetic acid with a flow rate of 1 ml/min; 1-ml fractions were collected and assayed by radioimmunoassay. For chromatographic analysis of irANP and irBNP, 15 ml pooled plasma or 30 ml pooled perfusate was treated by reverse-phase HPLC.
Figure 1. Line graph shows standard curve of rat brain natriuretic peptide (BNP) radioimmunoassay. Mean inhibition of binding of iodine-125-labeled rat BNP-32 in the presence of 0.5-100 pg synthetic rat BNP-32 was calculated from four consecutive assays. Curves constructed from the results of serial dilutions with assay buffer of an extract of plasma from deoxycorticosterone acetate (DOCA)-salt spontaneously hypertensive rats (SHR) (a) and Wistar-Kyoto (WKY) rats (b) were parallel to the standard curve of rat BNP-32 (c) and rat BNP-45 (d). B/Bo, percent maximum iodine-125-labeled rat BNP-32 bound; ANP, atrial natriuretic peptide.

Statistical Analysis

All values are expressed as mean±SD. The statistical significance of the results was evaluated by an analysis of variance, and probability values were determined by Scheffe's test. The correlations among ANP or BNP levels and various parameters were analyzed by linear regression analysis. Values of p<0.05 were considered significant.

Results

Plasma Concentrations of Immunoreactive Atrial and Brain Natriuretic Peptides in Young SHR

Mean body weight, mean systolic blood pressure, and mean concentrations of irANP and irBNP in young (6-week-old) SHR and age-matched WKY rats are shown in Table 1. The mean body weight, the mean systolic blood pressure, and the mean plasma concentrations of irANP and irBNP were not different between SHR and WKY rats.

Plasma Concentrations of Immunoreactive Atrial and Brain Natriuretic Peptides in Mature SHR and DOCA-Salt SHR

Mean body weight, mean ventricular weight, mean systolic blood pressure, BUN, serum creatinine concentrations, and plasma concentrations of irANP and irBNP in SHR and WKY rats treated for 8 weeks with DOCA and salt and those measurements for the corresponding SHR and WKY rat controls are shown in Table 2. The mean body weight in DOCA-salt SHR was significantly lower than in the other three groups. The mean ventricular weight in DOCA-salt SHR was significantly greater than in the other three groups. The mean ventricular weight in DOCA-salt WKY rats was slightly but significantly greater than in control WKY rats. The mean systolic blood pressure in DOCA-salt SHR was much higher than in the other groups. The mean systolic blood pressure in DOCA-salt WKY rats was significantly higher than in WKY rats. BUN and serum creatinine levels in DOCA-salt SHR were significantly higher than in the other three groups. The mean plasma concentrations of irANP and irBNP in DOCA-salt SHR were markedly higher than the other groups. The mean plasma concentrations of irANP and irBNP in DOCA-salt WKY rats were significantly higher than values in control WKY rats. The mean concentrations of irANP and irBNP in SHR (18 weeks old) were significantly higher than in control WKY rats. The correlations of the plasma irANP and irBNP levels with ventricular weight in DOCA-salt SHR are shown in Figure 2. Both peptide levels, especially BNP, were closely correlated with ventricular weight. In contrast, these peptide levels were not correlated with systolic blood pressure (ANP, n=7, r=-0.45; BNP, n=7, r=0.37), BUN (ANP, n=7, r=-0.59; BNP, n=7, r=-0.03), or serum creatinine (ANP, n=7, r=0.11; BNP, n=7, r=0.51).

Secretory Rate of Immunoreactive Atrial and Brain Natriuretic Peptides from the Isolated Hearts Before and After Atrial Removal

Mean body weight, mean ventricular weight, mean systolic blood pressure, and the secretory rate of
irANP and irBNP were shown in Table 3. The mean ventricular weight in DOCA-salt SHR was significantly greater than in the other three rat groups. The mean ventricular weight in DOCA-salt WKY rats was slightly but significantly greater than in control WKY rats.

The secretory rate of irANP from the perfused whole heart was markedly higher in DOCA-salt SHR than in the other three groups. The secretory rate of ANP from the perfused whole heart was higher in SHR and DOCA-salt WKY rats than in WKY rats, but the differences were not significant. After atrial removal, the secretory rate of ANP was considerably reduced in all groups, but the secretory rate of ANP after atrial removal in DOCA-salt SHR was still higher than that of the other three groups. The secretory rate of irBNP from the perfused whole heart was also higher in DOCA-salt SHR than in the other three groups. About 70% of the secretory rate of BNP from the whole heart was maintained even after atrial removal in DOCA-salt SHR, and about 55% was maintained in WKY rats, SHR, and DOCA-salt WKY rats after atrial removal.

**Reverse-Phase High-Performance Liquid Chromatography of Immunoreactive Atrial and Brain Natriuretic Peptides in Extracts of Rat Plasma and of Perfusate From Isolated Hearts**

Reverse-phase HPLC profiles of irANP and irBNP in plasma extracts from DOCA-salt SHR, SHR, and WKY rats and in perfusate from isolated hearts obtained from DOCA-salt SHR are shown in Figures 3 and 4. A major component of irANP and irBNP was eluted in the position of synthetic rat ANP-(1-28) and rat BNP-45, respectively.

**Discussion**

In the present study, we showed first that irBNP as well as irANP was present in plasma from WKY rats, SHR, and DOCA-salt WKY rats and SHR and in the perfusate from isolated heart obtained from these rats and that a major component of irBNP and irANP in these samples corresponded to rat BNP-45 and rat ANP-(1-28) and rat BNP-45, respectively. Nakao et al have shown that the major secretory form of BNP from the rat heart is rat BNP-45. It is likely, therefore, that low molecular weight forms of ANP and BNP, rat

**Table 2. Body Weight, Ventricular Weight, Systolic Blood Pressure, Levels of Blood Urea Nitrogen and Serum Creatinine, and Plasma Immunoreactive Atrial and Brain Natriuretic Peptides in Spontaneously Hypertensive and Wistar-Kyoto Rats With or Without Deoxycorticosterone Acetate and Salt**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>WKY Control</th>
<th>WKY DOCA-salt</th>
<th>SHR Control</th>
<th>SHR DOCA-salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>350±8</td>
<td>322±8*</td>
<td>327±9*</td>
<td>302±15*†‡</td>
</tr>
<tr>
<td>Ventricular weight (g)</td>
<td>0.97±0.03</td>
<td>1.18±0.06*</td>
<td>1.10±0.06</td>
<td>1.36±0.10**†‡</td>
</tr>
<tr>
<td>Ventricular/body weight (mg/g)</td>
<td>2.77±0.05</td>
<td>3.62±0.14*</td>
<td>3.36±0.20*</td>
<td>4.53±0.52**†‡</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>134±6</td>
<td>170±7*</td>
<td>186±6*</td>
<td>275±6*†‡</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>19.0±1.3</td>
<td>22.3±2.4</td>
<td>17.7±1.4</td>
<td>55.9±6.3*†‡</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.5±0.1</td>
<td>0.6±0.1</td>
<td>0.6±0.2</td>
<td>1.2±0.2*†‡</td>
</tr>
<tr>
<td>Plasma ANP (pg/ml)</td>
<td>113±14</td>
<td>283±42*</td>
<td>251±19*</td>
<td>581±124*†‡</td>
</tr>
<tr>
<td>Plasma BNP (pg/ml)</td>
<td>3.2±0.3</td>
<td>8.5±0.6*</td>
<td>9.2±0.8*</td>
<td>23.7±5.4*†‡</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; DOCA, deoxycorticosterone acetate; BP, blood pressure; BUN, blood urea nitrogen; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

* p < 0.05 compared with control WKY rats.
†p < 0.05 compared with DOCA-salt WKY rats.
‡p < 0.05 compared with control SHR.
SHR were not different from values in age-matched WKY rats. In contrast, both peptide levels in the established hypertensive phase of SHR were significantly higher than values in age-matched WKY rats. Such an increase in plasma ANP in mature SHR was previously observed by other investigators and by us.\textsuperscript{13-16} Recently, Yokota et al\textsuperscript{17} have shown the increase in concentration of both ANP and BNP in plasma in DOCA-salt hypertensive rats. These observations suggest that the progression of hypertension is associated with the observed increase of both peptide levels. This interpretation seems to be compatible with the previous report that plasma concentration of ANP and BNP are high in patients with established hypertension.\textsuperscript{18-21}

Next, we showed that plasma concentrations of irANP and irBNP were markedly higher in DOCA-salt SHR compared with WKY rats, DOCA-salt WKY rats, and SHR. Both peptide levels were closely correlated with ventricular weight. Noresson et al\textsuperscript{22} showed that left atrial pressure in mature SHR with left ventricular hypertrophy was higher than that of age-matched WKY rats. Sesoko et al\textsuperscript{23} have shown that cardiac output in DOCA-salt SHR was markedly decreased compared with SHR and WKY rats. These observations may raise the hypothesis that atrial overload associated with the development of hypertension stimulates the release of ANP and BNP from the atrium in SHR and DOCA-salt SHR. However, the heart perfusion experiment before and after atrial removal shows that ANP is mainly (about 95%) secreted from the atrium and BNP is secreted from atrium (about 45%) and ventricle (about 55%) in WKY rats, SHR, and DOCA-salt WKY rats. On the other hand, in DOCA-salt SHR a considerable amount (70%) of BNP was secreted from the hyper-

### Table 3. Concentrations of Atrial and Brain Natriuretic Peptides of the Perfusate from Isolated Hearts Before and After Atrial Removal in Wistar-Kyoto Rats and Spontaneously Hypertensive Rats With or Without Deoxycorticosterone Acetate and Salt

<table>
<thead>
<tr>
<th>Measurements</th>
<th>WKY (g)</th>
<th>DOCA-salt (g)</th>
<th>SHR (g)</th>
<th>DOCA-salt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>363±8</td>
<td>330±15*</td>
<td>341±12</td>
<td>312±21*</td>
</tr>
<tr>
<td>Ventricular weight</td>
<td>1.02±0.06</td>
<td>1.25±0.07*</td>
<td>1.13±0.04</td>
<td>1.49±0.08* ††</td>
</tr>
<tr>
<td>Ventricular/body weight (mg/g)</td>
<td>2.80±0.12</td>
<td>3.78±0.14*</td>
<td>3.30±0.07</td>
<td>4.81±0.59* ††</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>133±6</td>
<td>168±4*</td>
<td>187±7* †</td>
<td>268±10* ††</td>
</tr>
<tr>
<td>ANP Before atrial removal (pg/min)</td>
<td>1,914±205</td>
<td>2,206±196</td>
<td>2,084±225</td>
<td>4,260±420* ††</td>
</tr>
<tr>
<td>After atrial removal (pg/min)</td>
<td>109±15</td>
<td>128±6</td>
<td>111±9</td>
<td>352±60* ††</td>
</tr>
<tr>
<td>Secretory rate from atria (%)</td>
<td>94.3±0.9</td>
<td>94.1±0.8</td>
<td>94.6±0.7</td>
<td>91.8±1.0* ††</td>
</tr>
<tr>
<td>BNP Before atrial removal (pg/min)</td>
<td>207±16</td>
<td>290±45*</td>
<td>277±26</td>
<td>603±51* ††</td>
</tr>
<tr>
<td>After atrial removal (pg/min)</td>
<td>118±15</td>
<td>154±19</td>
<td>145±11</td>
<td>422±30* ††</td>
</tr>
<tr>
<td>Secretory rate from atria (%)</td>
<td>43.3±4.1</td>
<td>46.7±1.9</td>
<td>47.0±7.3</td>
<td>29.8±4.8* ††</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; DOCA, deoxycorticosterone acetate; BP, blood pressure; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

\*p<0.05 compared with control WKY rats.

\†p<0.05 compared with DOCA-salt WKY rats.

\‡p<0.05 compared with control SHR.
trophied ventricle. Yokota et al\(^7\) have also shown that ventricular BNP increases with the accumulation of rat BNP-45 during hypersecretion in DOCA-salt hypertension. Therefore, it seems likely that the major source of increased levels of circulating BNP is the hypertrophied ventricle in DOCA-salt SHR. This interpretation seems to be compatible with the finding recently reported by us\(^1\) that plasma BNP levels were positively correlated with left ventricular mass index in patients with essential hypertension. However, since BUN and serum creatinine levels in DOCA-salt SHR were significantly higher than those of other rat groups, we cannot exclude the possibility that decreased metabolic clearance of both peptides in the kidney might influence partially the circulating levels of ANP and BNP in this model. Further studies are required to clarify whether increased levels of both peptides are associated with the pathophysiology of malignant hypertension.

Acknowledgments

We thank Machiko Johchi and Ikuko Kuno for technical assistance.

References


Key Words • atrial natriuretic peptides • brain • malignant hypertension • heart • hypertrophy • rat studies
Accelerated secretion of brain natriuretic peptide from the hypertrophied ventricles in experimental malignant hypertension.
M Kohno, T Hori, M Yoshiyama and T Takeda

Hypertension. 1992;19:206-211
doi: 10.1161/01.HYP.19.2.206

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 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/19/2/206

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