Immunoreactive Atrial Natriuretic Peptide in Ventricles, Atria, Hypothalamus, and Plasma of Genetically Hypertensive Rats

Heikki Ruskoaho and Juhani Leppäläuto

To evaluate the role of extra-atrial atrial natriuretic peptide (ANP) in volume and blood pressure regulation, the plasma, atrial, ventricular, and hypothalamic levels of immunoreactive atrial natriuretic peptide (IR-ANP) were measured simultaneously in the spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) at the ages of 2, 6, and 12 months. Plasma IR-ANP in the 12-month-old, conscious SHR was significantly higher than that of the WKY (300 ± 18 versus 200 ± 20 pg/ml, p<0.05, n = 9), while no differences in plasma IR-ANP levels were found between the strains in younger rats. Acute volume expansion with saline (1.1 ml/100 g body wt) in hypertensive as well as in normotensive rats resulted in marked increases in right atrial pressure and plasma IR-ANP concentration. The older SHR had attenuated ANP release to volume loading as shown by the shift of the ANP versus right atrial pressure curve to the right. Right auricular IR-ANP concentration decreased, while that of left auricle increased with increasing age in both strains. No substantial differences were noted in auricular ANP concentration between SHR and WKY. However, the total atrial IR-ANP content (µg/atria) was consistently lower in SHR compared with WKY. In both ventricles, IR-ANP concentrations and contents increased with increasing age in WKY and SHR, but the ventricular levels of ANP were reduced in ventricles of the SHR heart compared with normotensive controls. The depletion of total ventricular IR-ANP was greatest in SHR with severe ventricular hypertrophy and coincided with the attenuated ANP release to acute volume load. The increase of left but not right ventricular weight occurring secondary to 6 weeks minoxidil treatment was accompanied by higher ANP concentration in both strains. In contrast to the ventricles, the hypothalamic IR-ANP concentration was significantly increased in SHR compared with that of WKY and decreased in both strains after 6 weeks' treatment with antihypertensive drugs. Thus, ventricular and hypothalamic, as well as atrial, ANP respond to increased pressure overload in genetically hypertensive rats. Our results suggest that chronic stimulation of ANP release from ventricles is associated with depleted stores of ANP from both ventricles and reduced response to acute volume load. Our findings that ventricular ANP increased with increasing weight and in response to a hypertrophic stimulus in WKY and was decreased in SHR with severe ventricular hypertrophy suggest that ANP may locally have an inhibitory effect on the development of cardiac hypertrophy. (Circulation Research 1988;62:384-394)

Atrial natriuretic peptide (ANP) is synthesized in cardiac atria, where it is processed and then stored in membrane-bound secretory granules. It is released by increased blood volume, presumably leading to augmented atrial stretch, appears to be the major signal for its release. After release into the circulation, ANP produces a variety of hemodynamic effects due to its potent natriuretic, diuretic, and vasorelaxant properties, in addition to influencing other hormonal modulators of blood volume and arterial pressure, such as renin, aldosterone, and vasopressin.

Following the description of ANP bioactivity by de Bold et al., it was thought that the synthesis and secretion of ANP were confined to the cardiac atria and were absent from ventricular or other tissues. However, recent evidence suggests that expression of the ANP gene occurs in extra-atrial tissues. Radioimmunological and immunocytochemical studies have revealed ANP to be present in the cardiac ventricles and central nervous system, where the highest levels have been found in the hypothalamus. The identification of ANP messenger RNA (mRNA) in cardiac ventricles, lung, central nervous system, and aorta further emphasizes that the capacity for ANP gene expression extends beyond atrial tissue.

Nevertheless, the importance of ANP in extra-atrial tissues is still not clear. The aim of the present study was to investigate in genetically hypertensive and age-matched normotensive control rats possible changes in immunoreactive ANP (IR-ANP) in plasma, atria, ventricles, and hypothalamus at various ages in response to a volume load and after antihypertensive therapy.

**Materials and Methods**

**Animals**

Male spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain (F58) and age-matched Wistar-Kyoto rats (WKY) from the Department of Pharma-
colony colony at the University of Oulu, Oulu, Finland, were used. Both strains were originally obtained from Mollegaard's Avslaboratorium, Skensved, Denmark. The rats were housed in plastic cages in a room with controlled 40% humidity and a temperature of 22° C. A 6 AM–6 PM light/6 PM–6 AM dark environmental cycle was maintained.

Indirect Measurement of Blood Pressure and Heart Rate

Systolic blood pressure and heart rate were measured in conscious animals by a tail-cuff method using a commercially available blood pressure recorder (model 8002e, W + W Electronics, Basel, Switzerland). The rats were maintained in a warming room at 36° C for 40 minutes prior to blood pressure measurement. Six consecutive systolic blood pressure measurements were obtained from each rat, and the arithmetic mean of these readings was taken as the systolic blood pressure. Pulse rate was obtained from signals picked up by the piezocrystal during the blood pressure recording.

Chronically Instrumented Rats

The surgical preparation and the experimental setup, which have been previously described, were slightly modified. Under chloralhydrate (30 mg/kg i.p.) anesthesia, a PE-60 catheter was placed into the abdominal aorta for measurement of blood pressure and heart rate and collection of blood samples. PE-50 catheters were inserted into the right atrium via the jugular vein for measurement of right atrial pressure and into the femoral vein for infusion of saline. All catheters were exteriorized behind the neck, filled with a heparinized saline solution, and plugged with a stainless pin. After operation, rats were housed individually in the experimental cages and had continuous access to food and water.

The day after the operation, the arterial and right atrial catheters were attached to pressure transducers (model MP-15, Micron Instruments, Los Angeles) and Grass polygraph (model 7D, Grass Instrument, Quincy, Massachusetts) for mean arterial pressure, heart rate, and right atrial pressure recording. The venous catheter was connected to a syringe for saline infusion. After an equilibration period (30 minutes), hemodynamic variables were recorded in the conscious, freely moving animals for 30 minutes. The rats were then given 1.1 ml/100 g body wt of 0.9% saline intravenously over 1 minute. Since our previous results (see Lang et al*) revealed that peak circulating levels of IR-ANP occurred about 1 minute after infusion, blood samples (0.8 ml) were taken 15 minutes before and 1 minute after the saline infusion, put into precooled EDTA tubes on ice, and immediately centrifuged, and the plasma was stored at −20° C until assayed by radioimmunoassay (RIA). Each blood sample taken was replaced by an equal volume of saline. Preliminary experiments showed that there was no difference in plasma IR-ANP concentration between two samples taken at 15-minute-intervals (first sample: 176 ± 19 pg/ml; second sample 195 ± 25 pg/ml, n = 7, NS).

Isolated Perfused Rat Heart

To eliminate the possible contribution of contaminating plasma for ventricular ANP levels, hearts were perfused according to the method of Langendorff as described previously.25 After the second blood sample, rats were decapitated, the abdominal cavity opened, the diaphragm transected, and lateral incisions made along both sides of the rib cage. The anterior chest wall was retracted, and the heart was cooled with perfusion fluid (4–10° C). The aorta was cannulated superior to the aortic valve, and retrograde perfusion was begun with a modified Krebs-Henselet bicarbonate buffer, pH 7.4, and equilibrated with 95% O2-5% CO2 at 37° C. Final concentrations of the salts in the buffer were (mmol/l): NaCl 113.8, NaHCO3 22.0, KCl 4.7, KH2PO4 1.2, MgSO4×7 H2O 1.1, CaCl2×2 H2O 2.5, glucose 11. Hearts were perfused with this solution for 4.5–5 minutes.

Organ Weights

After the perfusion of hearts, right and left auricles were removed, and the remaining atrial tissue, the aorta, and the pulmonary artery were carefully cut close to the ventricular surface. The ventricles were divided into a right ventricular free wall portion and a left ventricular-septal portion (combined right and left septa). All samples were blotted dry and weighed. To avoid the possible contamination of atrial tissues in the ventricular samples, individual ventricles were cut into the superior (about 15–20% total weight) and inferior parts, the latter being used for ventricular ANP determinations. To rule out the possibility that changes in cardiac weights were a nonspecific, both kidneys were removed and weighed.

After decapitation, the hypothalamus was removed and weighed. The anterior cut for the hypothalamic samples was at the chiasma, the lateral cuts at the hypothalamic sulci, and the posterior at the mamillary bodies. All tissue samples were stored at −70° C until assayed by RIA.

Assay of Immunoreactive Atrial Natriuretic Peptide in Plasma and Tissue

ANP was extracted from the tissues by homogenizing atrial and ventricular samples with 3 ml of 0.3 M HCl containing 10 mg/l of phenylmethylsulfonylfluoride for 30 seconds. The Ultraturrax (Janke & Kunkel, Staufen, FRG) was washed by 3 ml of the hydrochloride buffer; the homogenate and the wash were combined and centrifuged for 20 minutes at 3,000g. For the hypothalamic samples, volumes for the homogenization and wash were 1 ml. The supernatants were stored at −20° C before the assays. For the radioimmunoassay, the atrial and ventricular samples were diluted 3 × 104 and 5,000-fold, respectively. The hypothalamic samples were lyophilized and reconstituted with 0.5 ml of the radioimmunoassay buffer. Tissue ANP is expressed as both concentration per milligram wet weight and as content per organ.

Synthetic rat ANP, was added (15 µg per an atrial homogenate and 0.4 µg per a ventricle homogenate).
The recovery of the synthetic ANP from the atrial homogenate was 91.2 ± 10.0% (mean ± SD, n = 4) and from the ventricular homogenates 99.1 ± 4.6% (mean ± SD, n = 5).

Blood samples were centrifuged (2,000 g, 10 minutes, at 4 °C), and ANP was extracted from plasma according to the method of Larose et al.28 Modified in some details. Briefly, plasma sample (0.45 ml) acidified to pH 4 with 10% trifluoroacetic acid was applied into a Sep-Pak C18 cartridge (Waters Associates, Milford, Massachusetts) that had been activated previously with methanol followed by triethylamine acetate buffer (TEA, 20 mM, pH 4.0). After wash with 0.3% y-globulin, pH 6.0. The recovery of added rat ANP26 ranging from 0 to 1,250 pg/tube were 81.7 ± 3.3% (mean ± SEM, n = 6).

The recovery of the synthetic ANP from the atrial tissue extracts were diluted with the radioimmunoassay buffer and incubated in duplicates of 100 μl. At the end of treatment period, rats were decapitated, hearts prepared for RIA as described above.

Materials

Synthetic rat ANP26 was a gift from Dr. N. Ling, The Salk Institute, La Jolla, California. Minoxidil hydrochloride and α-methyldopa hydrochloride were gifts from Dr. A. Karjalainen, Farmos-Group Ltd., Oulu, Finland. Heparin was from Medica, Helsinki, Finland, and other chemicals were from Sigma Chemical, St. Louis, Missouri.

Data Analysis

The results are expressed as mean ± SEM. The data

### Table 1. Body and Organ Weights of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>2</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>BW (g)</td>
<td>236 ± 9</td>
<td>227 ± 3</td>
<td>402 ± 8</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>556 ± 21</td>
<td>598 ± 12</td>
<td>918 ± 18</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>2.36 ± 0.03</td>
<td>2.63 ± 0.04</td>
<td>2.28 ± 0.02</td>
</tr>
<tr>
<td>RVW (mg)</td>
<td>159 ± 7</td>
<td>152 ± 5</td>
<td>243 ± 19</td>
</tr>
<tr>
<td>RVW/BW (mg/g)</td>
<td>0.68 ± 0.03</td>
<td>0.67 ± 0.02</td>
<td>0.60 ± 0.04</td>
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<tr>
<td>VW (mg)</td>
<td>715 ± 28</td>
<td>750 ± 16</td>
<td>1,162 ± 33</td>
</tr>
<tr>
<td>VW/BW (mg/g)</td>
<td>3.03 ± 0.03</td>
<td>3.30 ± 0.05</td>
<td>2.88 ± 0.04</td>
</tr>
<tr>
<td>LVW/RVW (mg/mg)</td>
<td>3.50 ± 0.09</td>
<td>3.94 ± 0.09</td>
<td>3.85 ± 0.21</td>
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<tr>
<td>Atria (mg)</td>
<td>213 ± 14</td>
<td>181 ± 9</td>
<td>272 ± 17</td>
</tr>
<tr>
<td>RA (mg)</td>
<td>28 ± 2</td>
<td>21 ± 1†</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>LA (mg)</td>
<td>27 ± 2</td>
<td>18 ± 1</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Hypothalamus (mg)</td>
<td>44 ± 2</td>
<td>32 ± 2†</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>1,711 ± 69</td>
<td>1,655 ± 25</td>
<td>2,462 ± 58</td>
</tr>
<tr>
<td>Kidney weight/BW (mg/g)</td>
<td>3.63 ± 0.04</td>
<td>3.66 ± 0.05</td>
<td>3.06 ± 0.04</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; VW, ventricular weight; RA, right auricle; LA, left auricle.

*p<0.05; †p<0.01, and ‡p<0.001 SHR vs. age-matched WKY rats (two-way analysis of variance followed by Bonferroni t test.)

Mean ± SEM of 6–9 rats.

§Atria is total weight of atrial tissue including weight of both auricles and nonauricle part of the atrium.
Table 2. Effect of Treatment With α-Methyldopa and Minoxidil on Body Weight, Blood Pressure, and Heart Rate in Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>402 ± 8</td>
<td>132 ± 6</td>
<td>294 ± 11</td>
</tr>
<tr>
<td></td>
<td>Methyldopa</td>
<td>400 ± 8</td>
<td>127 ± 2</td>
<td>260 ± 12</td>
</tr>
<tr>
<td></td>
<td>Minoxidil</td>
<td>433 ± 5*</td>
<td>119 ± 3†</td>
<td>301 ± 9</td>
</tr>
<tr>
<td>Spontaneously hypertensive rats</td>
<td>Control</td>
<td>399 ± 5</td>
<td>212 ± 4</td>
<td>308 ± 7</td>
</tr>
<tr>
<td></td>
<td>Methyldopa</td>
<td>365 ± 6†</td>
<td>188 ± 5†</td>
<td>308 ± 7</td>
</tr>
<tr>
<td></td>
<td>Minoxidil</td>
<td>418 ± 7</td>
<td>176 ± 3†</td>
<td>278 ± 9</td>
</tr>
</tbody>
</table>

*p<0.05; †p<0.01; ‡p<0.001 treated rats vs. control group (one-way analysis of variance followed by Bonferroni test). Mean ± SEM of 6 rats.

Results

Blood Pressure, Heart Rate, and Body Weight in SHR and WKY

In WKY, the systolic blood pressure as measured by a tail-cuff method remained below 140 mm Hg throughout the observation period. An increase in systolic blood pressure was seen in SHR by the age of 4 weeks compared with WKY, and thereafter, SHR had consistently higher systolic blood pressures (180–220 mm Hg) than WKY. The heart rate of SHR was significantly higher than that of their age-matched WKY controls from 1 to 4.5 months of age.

Body, Ventricular, and Kidney Weights in SHR and WKY

The body weight was lower in SHR than in WKY (Table 3). The absolute left, right, and total ventricular weights of SHR and WKY did not differ significantly at the age of 2 months. However, thereafter, the left ventricular, as well as total ventricular, weight was significantly higher in SHR than in WKY, while their right ventricular weights did not differ significantly. Left-ventricular and ventricular-weight-to-body-weight ratios were significantly higher in SHR than in WKY from 2 to 12 months of age (Table 3). No significant differences could be found in the right-ventricular-weight-to-body-weight ratio between SHR and WKY at any age. The left-to-right-ventricular-weight ratio was greater in SHR than in WKY (Table 3). Both the kidney weight and kidney-weight-to-body-weight ratio were greater in 12-month-old SHR than in age-matched WKY (Table 3), while no differences were observed in 2- and 6-month-old rats.

Effect of α-Methyldopa and Minoxidil on Blood Pressure, Heart Rate, and Body Weight

The systolic blood pressure of SHR before treatment at the age of 4.5 months was 197 ± 2 mm Hg (n = 18) and that of WKY was 136 ± 3 mm Hg (n = 18, p<0.001). Minoxidil treatment reduced significantly blood pressure in both strains (Table 2). Six weeks' treatment with α-methyldopa decreased systolic blood pressure in SHR, while the difference was not significant in WKY. The body weight increased significantly

Table 3. Effects of Treatment With α-Methyldopa and Minoxidil on Ventricular and Kidney Weights in Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methyldopa</td>
<td>Minoxidil</td>
</tr>
<tr>
<td>LVW (mg)</td>
<td>925 ± 21</td>
<td>1,122 ± 26‡</td>
</tr>
<tr>
<td>LVW/BW (mg/g)</td>
<td>2.31 ± 0.03</td>
<td>2.59 ± 0.04‡</td>
</tr>
<tr>
<td>RVW (mg)</td>
<td>223 ± 4</td>
<td>288 ± 9†</td>
</tr>
<tr>
<td>RVW/BW (mg/g)</td>
<td>0.56 ± 0.02</td>
<td>0.84 ± 0.11*</td>
</tr>
<tr>
<td>VW (mg)</td>
<td>1,148 ± 20</td>
<td>1,487 ± 61‡</td>
</tr>
<tr>
<td>VW/BW (mg/g)</td>
<td>2.87 ± 0.03</td>
<td>3.42 ± 0.13†</td>
</tr>
<tr>
<td>LVW/RVW (mg/mg)</td>
<td>4.15 ± 0.14</td>
<td>3.32 ± 0.36*</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>2,467 ± 39</td>
<td>2,446 ± 0.03</td>
</tr>
<tr>
<td>Kidney weight/BW (mg/g)</td>
<td>3.09 ± 0.02</td>
<td>2.82 ± 0.03‡</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; VW, ventricular weight.

*p<0.05; †p<0.01, and ‡p<0.001 methyldopa-treated vs. minoxidil-treated rats (Student’s paired t test). Mean ± SEM of 6 rats.
FIGURE 1. Ventricular immunoreactive atrial natriuretic peptide (IR-ANP) concentration and content of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). □, WKY; ■, SHR. Statistical significance tested by two-way ANOVA followed by the Bonferroni t test for individual pairs. Numbers below the bars refer to ages of animals. *p<0.05, **p<0.01, ***p<0.001 SHR versus WKY. Mean ±SEM of 6–9 rats.

in minoxidil-treated WKY, while the body weight did not differ significantly between minoxidil-treated and control SHR (Table 2). α-Methyldopa treatment decreased the body weight in SHR but had no effect on body weight in WKY. No significant differences in heart rate were observed between treated and control SHR and WKY (Table 2).

Effect of α-Methyldopa and Minoxidil on Ventricular and Kidney Weights

As shown in Table 3, left-, right-, and total-ventricular-weight-to-body-weight ratios were significantly greater in minoxidil-treated rats than in methyldopa-treated rats in both strains. The absolute left and right ventricular weights as well as total ventricular weight were also higher in minoxidil-treated rats compared with those of methyldopa-treated rats. The left-to-right-ventricular-weight ratio was significantly lower in both minoxidil-treated SHR and WKY (Table 3). These changes in ventricular weights induced by antihypertensive drugs were not associated with similar change in kidney weight. There was no significant difference between methyldopa-treated and minoxidil-treated rats with respect to kidney weight, and kidney-weight-to-body-weight ratio was lower in minoxidil-treated rats (Table 3).

Changes in Ventricular Levels of Immunoreactive Atrial Natriuretic Peptide During Development of Cardiac Hypertrophy

As shown in Figure 1, left ventricular IR-ANP concentration increased from 80 ±11 pg/mg in 2-month-old WKY to level of 313 ±31 pg/mg at 12 months (F =114.2, p<0.001). Left ventricular IR-ANP concentration displayed a slower increase in SHR compared with WKY, the difference between the two strains being significant at 12 months. Right ventricular IR-ANP concentration was 61 ±9 pg/mg in WKY at the age of 2 months and increased to a level of 100 ±9 pg/mg in 12-month-old rats (F =18.3, p<0.001). The IR-ANP concentration of the right ventricle was lower in SHR at the age of 2, 6, and 12 months (Figure 1). Left-to-right-ventricular-ANP-concentration ratio (LV: RV, Table 4) increased with age in both strains and was greater in SHR compared with WKY (F =92.3, p<0.001).

The left ventricular IR-ANP content (nanograms per ventricle) increased 7.5-fold from age 2 months to age 12 months in WKY and 4.3-fold in SHR (Figure 1). The difference in IR-ANP content between the two strains was significant at the age of 12 months. A smaller increase with increasing age in right ventricular IR-ANP content was observed in both strain diets (F =21.4, p<0.001). Although the IR-ANP content of the right ventricle was lower in SHR, the difference between the strains was not significant at the age of 12 months (Figure 1). The total amount of left ventricular IR-ANP (left ventricular:right ventricular content, Table 4) was 3–5 times higher than that of the right ventricle.

Effect of α-Methyldopa and Minoxidil on Ventricular Levels of IR-ANP

The greater left ventricular weight of minoxidil-treated rats compared with α-methyldopa–treated rats was associated with a substantially higher left ventricular IR-ANP concentration (88% and 49% higher in WKY and SHR, respectively) and left ventricular IR-ANP content (125% and 82%, respectively) (Figure 2). In contrast, no significant difference in IR-ANP concentration could be detected in the right ventricle between treated groups in WKY, while the IR-ANP...
Table 4. Tissue Immunoreactive Atrial Natriuretic Peptide Levels in Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Age (months)</th>
<th>WKY</th>
<th>SHR</th>
<th>WKY</th>
<th>SHR</th>
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<tr>
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<td>2</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Atria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IR-ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg/atria)</td>
<td>23.8±1.2</td>
<td>20.0±0.9*</td>
<td>23.5±1.3</td>
<td>22.0±0.9</td>
<td>21.8±2.8</td>
</tr>
<tr>
<td>RA IR-ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg/auricle)</td>
<td>10.6±0.9</td>
<td>8.8±0.6</td>
<td>10.7±1.3</td>
<td>10.0±0.6</td>
<td>9.5±1.1</td>
</tr>
<tr>
<td>RA IR-ANP</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mg)</td>
<td>382±35</td>
<td>431±39</td>
<td>273±19</td>
<td>312±19</td>
<td>276±29</td>
</tr>
<tr>
<td>LA IR-ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µg/auricle)</td>
<td>4.6±0.4</td>
<td>3.3±0.2</td>
<td>8.0±1.0</td>
<td>6.7±0.5</td>
<td>9.3±4.6</td>
</tr>
<tr>
<td>LA IR-ANP</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mg)</td>
<td>172±10</td>
<td>189±13</td>
<td>187±22</td>
<td>184±13</td>
<td>269±29</td>
</tr>
<tr>
<td>RA/LA IR-ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(content)</td>
<td>2.5±1.1</td>
<td>2.8±0.9</td>
<td>1.9±1.0</td>
<td>1.9±0.6</td>
<td>1.1±0.6</td>
</tr>
<tr>
<td>RA/LA IR-ANP</td>
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<td></td>
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<tr>
<td>(concentration)</td>
<td>2.3±0.7</td>
<td>2.4±0.9</td>
<td>1.6±0.6</td>
<td>1.8±0.5</td>
<td>1.1±0.4</td>
</tr>
<tr>
<td>Ventricles</td>
<td></td>
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<tr>
<td>IR-ANP</td>
<td></td>
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</tr>
<tr>
<td>(ng/ventricle)</td>
<td>54±7</td>
<td>50±3</td>
<td>123±13</td>
<td>111±8</td>
<td>361±30</td>
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<td>LV/RV IR-ANP</td>
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<td>(content)</td>
<td>4.7±0.5</td>
<td>8.2±1.0</td>
<td>5.3±0.5</td>
<td>14.5±2.2</td>
<td>10.8±2.3</td>
</tr>
<tr>
<td>LV/RV IR-ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(concentration)</td>
<td>1.3±0.2</td>
<td>2.1±0.2*</td>
<td>1.3±0.1</td>
<td>2.8±0.4†</td>
<td>3.1±0.5</td>
</tr>
<tr>
<td>Atria/Ventricles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/V (content)</td>
<td>497±75</td>
<td>411±34</td>
<td>204±26</td>
<td>204±14</td>
<td>60±10</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR-ANP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/hypothalamus)</td>
<td>1.2±0.2</td>
<td>1.7±0.1‡</td>
<td>0.9±0.1</td>
<td>2.1±0.2†</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>IR-ANP (pg/mg)</td>
<td>27±5</td>
<td>54±5†</td>
<td>20±2</td>
<td>52±7†</td>
<td>29±3</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; IR-ANP, immunoreactive atrial natriuretic peptide; RA, right auricle; LA, left auricle; LV, left ventricle; RV, right ventricle; A, atria; V, ventricle.

* p<0.05; ‡ p<0.01, and †p<0.001 SHR vs. age-matched WKY rats (two-way analysis of variance followed by Bonferroni t test). Mean±SEM of 6-9 rats.

Concentration in the right ventricle was significantly lower in minoxidil-treated than in methyldopa-treated SHR. The IR-ANP content of right ventricle was slightly elevated in minoxidil-treated WKY but decreased in SHR compared with that of methyldopa-treated rats (Figure 2).

Atrial Levels of IR-ANP in SHR and WKY

Right auricular IR-ANP concentration was over two times higher than that of left auricle at the age of 2 months in both strains (Table 4). Right auricular IR-ANP concentration decreased significantly from the age of 2 months to 12 months in SHR and WKY ($F=20.2$, $p<0.001$), while the concentration of IR-ANP in the left auricle increased with age ($F=5.9$, $p<0.02$). Thus, the relation of right auricular to left auricular IR-ANP content (expressed as µg/auricle) and concentration (ng/mg) decreased with increasing age (Table 4). No substantial differences were noted in auricular ANP concentration between WKY and SHR (Table 4).

The total atrial IR-ANP content was over 400 times higher than that of ventricles at the age of 2 months, but the ratio of atrial to ventricular ANP content (atria:ventricle content, Table 4) decreased to 60-80 at the age of 12 months due to an increase in ventricular IR-ANP concentration and mass. Total atrial IR-ANP content (µg/atria, $F=9.4$, $p<0.005$) as well as left ($F=6.3$, $p<0.02$) and right ($F=5.9$, $p<0.02$) auricle IR-ANP contents were lower in SHR rats compared with WKY (Table 4).

IR-ANP in Hypothalamus and Effect of Antihypertensive Treatment

Hypothalamic IR-ANP concentration was about 25 pg/mg wet wt in WKY at the age of 2 months and decreased slightly with age (Table 4). Hypothalamic IR-ANP concentration and IR-ANP content were consistently elevated in SHR. In SHR, α-methyldopa and minoxidil caused 37% and 41% decreases in the IR-ANP concentration of the hypothalamus, respectively; hypothalamic IR-ANP concentration was lower in minoxidil-treated than in methyldopa-treated STR.
Heart rate did not show any significant changes. The right atrial pressure increased significantly in both strains (Figure 3).

The resting values of plasma IR-ANP concentration were similar in 2- and 6-month-old WKY and SHR (Figure 3), but IR-ANP concentration in conscious 12-month-old SHR was significantly higher than that of WKY (300 ± 18 pg/ml versus 200 ± 20 pg/ml, p<0.05, n = 9). One minute after saline infusion, plasma IR-ANP concentration increased significantly in WKY as well as in SHR at all ages (Figure 3). The increase of plasma IR-ANP concentration was smaller in SHR than in WKY, the difference being greatest at the age of 12 months (Figure 4). The theoretical ANP increase in response to volume loading corresponding to the 2-mm Hg increase in the right atrial pressure increased in WKY from 1.82 at the age

Effect of Volume Expansion on Plasma ANP and Hemodynamics in SHR and WKY

The basal mean arterial pressure, as measured directly in chronically cannulated conscious rats, was significantly higher in SHR than in WKY at the ages of 2, 6, and 12 months (Figure 3), but their heart rates and right atrial pressures did not differ significantly. Figure 3 shows the effect of acute saline infusion on hemodynamics in WKY and SHR. Mean arterial blood pressure increased maximally by 6.9 ± 1.8, 16.8 ± 2.2, and 15.1 ± 2.1 mm Hg in WKY and by 6.9 ± 1.5, 12.8 ± 2.6, and 14.0 ± 2.4 mm Hg in SHR at the ages of 2, 6, and 12 months, respectively. However, the increase in blood pressure was significant only in 6-month-old WKY (Figure 3).

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2 mo

E

2 4

0 1 2 4

△RAP (mm Hg)

FIGURE 4. Relation between plasma immunoreactive atrial natriuretic peptide (ANP) and right atrial pressure (RAP) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). ANP max, plasma ANP concentration 1 minute after infusion of 1.1 ml/100 g body wt of 0.9% saline; ANP basal, plasma ANP concentration prior infusion; RAP, change in RAP during infusion; mo, months. Numbers above broken lines refer to theoretical ANP increase in response to volume loading corresponding to 2 mm Hg increase in RAP. *p<0.05 SHR versus WKY (one-way ANOVA followed by Bonferroni t test). Mean ± SEM of 6-9 rats.

of 2 months to 2.4 at the age of 12 months, while that in SHR was about 1.6 at all ages.

Discussion

The spontaneously hypertensive rat model of hypertension is particularly suitable for studying the sequential alterations in hypertension and ventricular hypertrophy. SHR exhibit a slowly developing, progressive increase in arterial pressure accompanied by a proportional increase in left ventricular mass compared with rats in which hypertension is induced by surgical intervention or pharmacological means.\(^{30,31}\) The present study confirms our earlier observations\(^{25,32}\) and those of others\(^{26,31,33,34}\) that the absolute left ventricular mass of SHR increased with increasing age and that the adult rats of the SHR strain had an increased left ventricular weight compared with normotensive WKY. Left-ventricular-weight-to-body-weight ratio, the most commonly used index of the degree of hypertrophy, was greater in SHR compared with WKY throughout the observation period. Although we did not study cardiac performance in the present experiment, other investigators have reported that with severe hypertension and left ventricular hypertrophy, myocardial performance in SHR becomes impaired, and heart failure develops.\(^{35}\)

Recently, high plasma levels of IR-ANP have been reported in patients with heart failure\(^{36-38}\) and hypertension.\(^{39}\) Accordingly, increased plasma ANP levels have also been reported in the anesthetized,\(^{40,41}\) decapitated,\(^{42,43}\) or conscious SHR.\(^{44,45}\) However, Haass et al\(^46\) (in 16-week-old rats) and Petterson et al\(^47\) (in rats weighing 200–250 g) did not find such a difference between the two strains in conscious rats. We measured plasma concentrations of IR-ANP in chronically cannulated, freely moving SHR and WKY during basal conditions, and our results in the younger SHR confirm those of both Haass et al\(^46\) and Petterson et al.\(^47\) We found plasma IR-ANP concentration in SHR compared with WKY was significantly higher only at the age of 12 months. This agrees with the previous findings that severe hypertension in SHR eventuates in a marked depression of cardiac performance that is not observed in younger SHR\(^33\) and that increasing severity of heart failure is associated with higher plasma ANP levels.\(^{47}\)

The most likely explanation for the increased resting plasma ANP levels in SHR is that the atria secrete more ANP in response to chronically increased pressure as shown in acute studies.\(^{44,45}\) Although continuous increase of ANP could reduce atrial ANP concentration, this is not necessarily the case; the elevated release might induce the formation of more peptide, resulting in the same or even a higher ANP level in the atria. This may be why the measurements of atrial ANP content in SHR are so variable; some studies have reported reduced atrial ANP concentrations in SHR,\(^{30,41,44,45}\) while others have found no difference between SHR and WKY\(^38\) or have even reported an increase in atrial ANP concentrations.\(^{41,42}\) In our study, left and right auricular IR-ANP concentrations from age 2 to 12 months in the two strains were similar. However, total atrial, left auricular, and right auricular IR-ANP content (expressed as micrograms per atrium or auricle) were reduced in SHR. This decrease of atrial ANP content agrees with the previous reports\(^{44,50,51}\) and may be associated with changes in plasma IR-ANP levels, but other possibilities also exist.

Our present study results suggest a significant role for both ventricles in the release of ANP, at least in this model of hypertension. The IR-ANP concentration of ventricles was 2,500 (left ventricle) to 10,000 (right ventricle) times lower than that of the corresponding auricles in 2-month-old rats. Since ventricular ANP concentration and ventricular weight increased with age in both WKY and SHR, the proportion of ventricular ANP in total cardiac ANP increased mark-
The ventricular ANP concentration was reduced in both left and right ventricles of SHR hearts compared with their normotensive counterparts, the reduction in left ventricular IR-ANP being greatest in old SHR, which also developed the most severe ventricular hypertrophy. Proportionally, the depletion of ANP content in SHR was greater in ventricles than in atrial tissue. Thus, this study clearly demonstrates that ventricular, as well as atrial, ANP responds to increased pressure overload. In view of elevated plasma ANP levels and reduced ventricular peptide content, it is possible that chronic stimulation of ANP release from the left ventricle may be responsible for the high levels of plasma IR-ANP found in old SHR.

These results differ from those recently reported by Takayanagi et al16 in SHR and WKY. They found that the IR-ANP concentration of the apical portion of ventricles was three times higher in 15-week-old SHR than in age-matched WKY. The reason for the discrepancy is not clear; however, differences in radioimmunoassay techniques, in rat strains, in preparation of ventricles, and in degree and duration of hypertension are all possibilities.

The reduced levels of ventricular ANP found in the present study suggest that either local synthetic mechanisms or storage or release of ANP is altered in ventricular tissue of SHR leading to depleted levels of ANP. There is some evidence that ventricular as well as atrial ANP mRNA increases in models of acute and chronic volume overloading and that the rate of ANP secretion by the neonatal ventricular cardiocytes and atrial cardiocytes is approximately equal. However, neonatal ventricular cardiocytes secrete ANP rapidly after synthesis and differ in their storage of ANP, suggesting that the mechanisms of ANP release may be different in atria and ventricles.13

In the atria, the release of ANP is thought to be regulated predominantly by atrial distension caused by a variety of stimuli, including volume loading.3,4,35,36 We have studied the effects of acute volume expansion on ANP release in conscious rats to investigate the capacity of heart to release ANP. Acute volume expansion with saline in hypertensive as well as normotensive rats resulted in marked increases in right atrial pressure and plasma IR-ANP concentration, while heart rate and arterial pressure remained essentially unchanged. Interestingly, the ANP-versus-right-atrial-pressure curve tended to shift to the left in the WKY rats with age, indicating that a similar increase in right atrial pressure causes a greater increase in plasma ANP in older rats. This is of particular significance since ventricular but not atrial concentration, and especially content, also increased with age. In addition, SHR had reduced ventricular ANP levels and attenuated ANP release to volume loading as shown by the shift of the ANP-versus-right-atrial-pressure curve to the right in SHR compared with WKY. Thus, for a given increase of right atrial pressure, smaller amounts of IR-ANP were released in SHR than in WKY. This association of low ventricular IR-ANP levels and reduced capacity of the heart to release ANP in SHR suggests that ventricular ANP may contribute to the plasma ANP levels under acute volume expansion.

To further evaluate the relation between ventricular ANP and pressure load and the cardiac hypertrophy, we studied the effects of two antihypertensive drugs on blood pressure and ventricular hypertrophy. As reported previously,32,33 ventricles of SHR hypertrophied to a marked difference in ventricular mass between minoxidil-treated and methyldopa-treated rats was noted despite a similar reduction in blood pressure by these drugs. The increased left ventricular mass was associated with increased left ventricular IR-ANP concentration in both strains, although the percent increase in SHR was lower than in WKY. In contrast, right ventricles were unable to respond with increasing ANP concentration, and the IR-ANP concentration was lower in minoxidil-treated SHR than in methyldopa-treated rats. At present, the reason for these disparate responses is not clear. Taken together, our results show that the increase of left ventricular weight occurring with age or secondary to minoxidil treatment is accompanied by higher ventricular ANP concentration. Since the concentration of ANP was markedly lower in highly hypertrophied left ventricles of SHR compared to that of WKY, it is tempting to speculate that ANP itself may have an inhibitory effect on the development of hypertrophy, i.e., the depletion of ANP results in increased growth. However, the causal interrelationship, if any, between ANP and myocardial hypertrophy remains to be determined in different kinds of experimental models.

Recently, several laboratories have reported the existence of immunoreactive ANP in the brain.10-31 It was shown that dietary changes, such as high salt diet or water deprivation,17,30,60 resulted in the change of amount of ANP in the brain, and centrally infused ANP attenuated salt appetite in SHR.30 We examined the ANP concentration in the hypothalamus to see if ANP in the hypothalamus has any correlation with the development of hypertension. The ANP levels in SHR were consistently increased from the age of 2 to 12 months compared with WKY. These results are consistent with previous observations in SHR and WKY.62 This increased IR-ANP concentration in the hypothalamus of SHR is in contrast to the decreased levels in the ventricles and shows that hypothalamus and ventricle respond differently to hypertension. It is possible that elevated hypothalamic ANP represents a homeostatic response to increased arterial pressure to reduce the increase in blood pressure. The fact that antihypertensive treatment reduced hypothalamic ANP concentration supports the conclusion that ANP in the hypothalamus is involved in the central regulation of blood pressure.

In conclusion, old SHR with severe ventricular hypertrophy had increased plasma IR-ANP concentration and reduced ventricular IR-ANP concentration and content, while the atrial IR-ANP content was consistently reduced and hypothalamic IR-ANP concentration increased in SHR at all ages. The association of low ventricular ANP content, increased plasma ANP,
and reduced response to acute volume overload in SHR shows that ventricular ANP production may play a significant physiological role in blood pressure and volume regulation. The increased IR-ANP concentration in the hypothalamus in SHR may represent a compensatory response to increased arterial pressure, as suggested by the observation of decreased levels after lowering of blood pressure. Finally, the findings that increased ventricular weight occurring with age or secondary to a hypertrophic stimulus is associated with increased ventricular ANP and that decreased ventricular ANP concentration in SHR is associated with the most severe ventricular hypertrophy suggest that ANP in ventricles is not only synthesized for circulation as a hormone but might have an influence locally on the development of ventricular hypertrophy.

Acknowledgments
We thank Ms. Tuula Lumijärvi, Mrs. Anna-Maija Ruonula, and Mrs. Tuula Räisänen for expert technical assistance.

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doi: 10.1161/01.RES.62.2.384

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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