Passive Mechanical Stretch Releases Atrial Natriuretic Peptide From Rat Ventricular Myocardium

Päivi Kinnunen, Olli Vuolteenaho, Paavo Uusimaa, and Heikki Ruskoaho

Ventricular hypertrophy is characterized by augmentation of synthesis, storage, and release of atrial natriuretic peptide (ANP) from ventricular tissue, but the physiological stimulus for ANP release from ventricles is not known. We determined the effect of graded, passive myocardial stretch on ANP release in isolated, arrested, perfused heart preparations after removal of the atria in 13–20-month-old Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). By this age, ANP gene expression was increased in the hypertrophic ventricular cells of SHR, as reflected by elevated levels of immunoreactive ANP and ANP mRNA and the increased ANP secretion (SHR, 93±14 pg/ml, n=22; WKY rats, 22±2 pg/ml, n=20; p<0.001) from perfused ventricles after removal of the atria. The release of ANP from ventricles was examined at two levels of left ventricular pressure by increasing the volume of the intraventricular balloon for 10 minutes. Stretching of the ventricles produced a rapid but transient increase in ANP secretion. As left ventricular pressure rose from 0 to 14 and 26 mm Hg in WKY rats and from 0 to 13 and 27 mm Hg in SHR, increases in ANP release into the perfusate of 1.4±0.1-fold and 1.5±0.2-fold (p<0.05) in WKY rats and 1.1±0.1-fold and 1.6±0.2-fold (p<0.05) in SHR, respectively, were observed. There was a highly significant correlation between the left ventricular pressure level and the maximal concentration of ANP in the perfusate during stretching (p<0.001, r=0.59, n=42), as well as between the maximal ANP concentrations in perfusate during stretching and the ventricular weight/body weight ratios of the corresponding animals (r=0.38, p<0.05, n=42). High performance liquid chromatographic analysis revealed that the ventricles both before and during stretch primarily released the processed, active, 28-amino acid ANP-like peptide into the perfusate. These results indicate that stretching is a direct stimulus for ventricular ANP release and show that ANP is also a ventricular hormone. (Circulation Research 1992;70:1244–1253)

KEY WORDS • atrial natriuretic peptide • mechanical stretch • cardiac hypertrophy • spontaneously hypertensive rats • intraventricular balloon

Increased work load of the heart causes ventricular hypertrophy, molecular changes in contractile proteins, and a complex reprogramming of cardiac gene expression, including ventricular reexpression of the gene for atrial natriuretic peptide (ANP), a hormone that regulates blood volume and pressure. Increased ventricular ANP synthesis has been demonstrated in patients with congestive heart failure and hypertension and in numerous animal models of cardiac hypertrophy such as spontaneously hypertensive rats (SHR). Recent in vitro studies show that ANP is released from ventricular tissue and that the ventricular source substantially contributes to the circulating level of ANP. Ventricular cardiocytes in culture secrete ANP into the incubation medium, and when the perfused heart preparation is used, ANP is released into the perfusion fluid; the amount of the peptide released depends on the degree of ventricular hypertrophy.

In severe ventricular hypertrophy, ANP release can be stimulated by phorbol ester, thus demonstrating the ability of mature ventricular tissue to secrete ANP in response to a secretagogue. Basal ventricular release of ANP from the human ventricle has also been demonstrated in individuals with congestive heart failure. However, the physiological stimulus for ventricular ANP release is not known.

In the atria, the principal stimulus controlling the acute release of ANP is atrial wall stretch. Interestingly, in vivo physical exercise has been shown to cause release of ANP from the hypertrophic ventricle with depletion of endocardial left ventricular stores, thus suggesting that stretch might also be a stimulus for ANP release from ventricular myocardium. To test the hypothesis that stretch releases ANP from ventricles, we determined the ability of normal and hypertrophic ventricular tissue in the isolated perfused heart preparation to secrete ANP in response to varying degrees of stretch produced by an intraventricular balloon. In addition, immunoreactive ANP (IR-ANP) and ANP mRNA levels in atria and ventricles were determined, and high performance liquid chromatographic (HPLC)
analysis of the perfusate was done. These studies demonstrate for the first time that stretching of the left ventricle in arrested, isolated, perfused rat hearts after removal of the atria causes a stimulus-dependent increase in perfusate ANP concentration. The ANP-like immunoreactive material secreted corresponded to the processed, active, low molecular weight peptide found in plasma.

Materials and Methods

Materials

The chemicals used in this study were HPLC chemicals from J.T. Baker, Deventer, Holland; heparin from Medica, Helsinki, Finland; and synthetic rat ANP-(1-28) and bovine serum albumin from Sigma Chemical Co., St. Louis, Mo. Unless otherwise stated, other chemicals were from Sigma.

Animals

Male SHR of the Okamoto-Aoki strain (F67) and Wistar-Kyoto (WKY) rats (F65) from the Department of Pharmacology and Toxicology colony at the University of Oulu, Finland, were used. Both strains were originally obtained from Mollegaards 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 on–6 PM off environmental light cycle was maintained. The animals were examined at ages ranging from 13 to 20 months. The experimental design was approved by the Animal Experimentation Committee of the University of Oulu.

Isolated Perfused Rat Hearts

The isolated perfused rat heart preparation used in this study was a modification of that previously described.14 Briefly, the rats were given heparin (500 units/kg body wt i.p.) and were decapitated 20 minutes later. The abdominal cavity was immediately opened, the diaphragm was transected, lateral incisions were made along both sides of the rib cage, and the heart was cooled with perfusion fluid (4–10°C). The aorta was cannulated above the aortic valve, and retrograde perfusion was begun with a modified Krebs-Henseleit bicarbonate buffer, pH 7.4, equilibrated with O2-CO2 (95:5) at 37°C. Final concentrations of the salts in the buffer were (mM) NaCl 113.8, NaHCO3 22.0, KCl 4.7, KH2PO4 1.2, MgSO4×7H2O 1.1, CaCl2×2H2O 2.5, and glucose 11.

Variations in perfusion pressure, arising from changes in coronary vascular resistance, were recorded on a polygraph (model 7DA, Grass Instrument Co., Quincy, Mass.) with a pressure transducer (model MP-15, Micron Instruments, Los Angeles) situated on a side arm of the aortic cannula. The heart rate was counted from the perfusion pressure curve driven with a higher level of sensitivity. The hearts were stimulated (10 V, 0.5 msec) with a stimulator (model S88, Grass Instruments) to increase heart rate 15–20% above the spontaneous beating rate. During the equilibration period (60 minutes), the hearts were perfused with a peristaltic pump (Minipuls 3, model 312, Gilson, Villiers, France) at flow rate of 8 ml·min⁻¹ and then switched to constant flow of 5 ml·min⁻¹.

Each experiment was started by perfusing hearts for 10 minutes at the constant flow rate. Next, all atrial tissue was carefully cut away along the ventricular surface, and a Swan-Ganz catheter (Arrow International Inc., Reading, Pa.) with a compliant latex balloon was placed across the mitral valve into the left ventricle and secured to the aortic cannula. The catheter was connected to the pressure transducer (model MP-15) for the measurement of left ventricular pressure (LVP), and variations in pressure were recorded on the Grass polygraph. To arrest the heart, KCl at a concentration of 27 mM was added into the aortic perfusion cannula as a continuous infusion via an infusion pump (Secan PSA 55, Sky Electronics S.A., Grenoble, France) at a rate of 0.5 ml·min⁻¹. Then, the ventricles were perfused for 10 minutes with the balloon empty. By increasing the volume of the intraventricular balloon, we studied hearts at LVPs of 13–14 (stretch 1 group) and 26–27 (stretch 2 group) mm Hg. The ventricles were perfused with this higher LVP for a period of 10 minutes, which was followed by a 10-minute recovery period with the balloon emptied. Control experiments were run with an empty intraventricular balloon throughout the test period. The coronary venous effluents were collected at 2-minute intervals before and after (control, stretch, and recovery periods) removal of the atria and were immediately placed on dry ice and stored at −20°C until assayed.

Preparation of Tissues

After perfusion, the aorta and pulmonary artery were carefully cut close to the ventricular surface. The right and the left ventricles were not separated, because only approximately 0.9% and 2.8% of the IR-ANP released from the ventricles in SHR and WKY rats, respectively, are generated from the right ventricle.14,17 To avoid the possible contamination of the ventricular sample by atrial tissue, ventricles were cut into superior (−15–20% of total weight) and inferior parts, the latter being used for ventricular ANP determinations. Ventricular tissue, as well as previously removed atrial tissue, was blotted dry, weighed, and stored at −70°C until assayed. To rule out the possibility that changes in cardiac weights were nonspecific, both kidneys were removed and weighed.

ANP mRNA Determination

RNA was isolated from fresh ventricular tissue of SHR and WKY rats by the guanidine thiocyanate–LiCl method.21 For the RNA dot blot analysis, 20-μg samples of the RNA from ventricles were transferred to the nitrocellulose membrane (BAS 85, Schleicher & Schuell, Dassel, Germany).22 The full-length rat ANP cDNA probe, Car-55,23 a generous gift from Dr. Peter L. Davies, Queen’s University, Kingston, Canada, was labeled with [32P]dCTP (deoxyctydine-5′-triphosphate, Amersham, Buckinghamshire, England) with a random-primed labeling kit (Boehringer-Mannheim GmbH, Mannheim, FRG). The hybridizations, washings, exposures of membranes to film, and scanning of the optical density were done as described by Kinnunen et al.17 To quantify the amount of RNA loaded on agarose gels, a 32P-labeled oligonucleotide probe complementary to rat 18S ribosomal RNA was hybridized to all filters as
described by Albretsen et al.24 To estimate tissue ANP mRNA concentration, the ANP/18S ratio was determined by dividing the absorbance corresponding to the ANP probe by the absorbance corresponding to 18S probe hybridization.

**Assay of IR-ANP in the Perfusate and Tissues**

For the radioimmunoassay, the atrial and ventricular guanidine thiocyanate extracts were diluted 5×10²-fold and 1,200-fold, respectively. Tissue ANP is expressed either as concentration per milligram wet weight or as content per organ. For the ANP radioimmunoassay, the tissue extracts as well as the unextracted perfusate samples were incubated in duplicates of 100 μl, with 100 μl of the middle specific rabbit ANP antiserum at a final dilution of 1:25,000.25 Synthetic rat ANP-(1-28), ranging from 0 to 500 pg per tube, was incubated as a standard. The ANP tracer was rat [¹²⁵I]ANP-(1-28) from Amersham, Buckinghamshire, UK. After incubation for 48 hours at +8°C, the immunocomplexes were precipitated with sheep antiserum against rabbit γ-globulin in the presence of 500 μl of 1.2 M ammonium sulfate, pH 7, followed by centrifugation for 40 minutes at 3,000g.

The sensitivity of the assay was 0.8 pg per tube. The 50% displacement of the standard curve was at 20 pg per tube. The interassay and the intra-assay variations were 14% and 5%, respectively. Serial dilutions of perfusate and tissue extracts showed parallelism to the synthetic ANP standard.

**HPLC and Assay of Lactate Dehydrogenase in the Perfusate**

Two-milliliter samples of the perfusates were lyophilized, dissolved in 0.4 ml of 40% acetonitrile/0.1% trifluoroacetic acid in water, and passed through a 0.45-μm filter (Millipore Corp., Bedford, Mass.). The samples were then applied to a 7.8×300-mm Protein-Pak-125 gel filtration HPLC column (Waters Chromatography Division, Milford, Mass.) and eluted with the same solvent. The flow rate was 1 ml/min, and fractions of 0.5 ml were collected. The fractions were dried with 0.1 mg bovine serum albumin in a Savant Speed Vac concentrator and redissovled in buffer for radioimmunoassay. The column was calibrated with bovine serum albumin (V₀), rat pro-ANP (purified from rat auricles), rat ANP-(1-28), and radioiodine (Vₘₐₓ) (Amersham). In addition, 200–500 cpm radiolabeled rat ANP-(1-28) and radioiodine were run with each sample as internal standards. The recovery of IR-ANP from the column was 60–95%. The cytosolic marker enzyme, lactate dehydrogenase, in the perfusate was assayed as described by Bergmeyer and Bernt.26

**Statistical Analysis**

The results are expressed as mean±SEM. The data were analyzed with two- or one-way analysis of variance (ANOVA). For the comparison of statistical significance between two groups, Student’s t test for unpaired or paired data was used. For the multiple comparison, one-way ANOVA followed by Bonferroni’s t test was used. Differences at the 95% level were considered statistically significant.

## Results

**Body, Cardiac, and Kidney Weights of SHR and WKY Rats**

The left ventricular mass of SHR increases progressively with increasing age, usually resulting in impairment of myocardial performance and heart failure in rats at the age of 1 year or more.27,28 In the present study, ventricular ANP release was examined in 16–20-month-old WKY rats and 13–14-month-old SHR (Table 1). To confirm the degree of ventricular hypertrophy in the hypertensive rat strain, the hearts were weighed at the end of perfusion. As expected, ventricular weight and ventricular weight/body weight ratios were 31% and 42% (p<0.001) higher, respectively, in SHR (n=22) than in WKY (n=20) rats (Table 1). WKY rats were slightly heavier than SHR (p<0.001), whereas atrial and kidney weights were not significantly different in the two strains.

**Ventricular and Atrial Levels of IR-ANP and ANP mRNA in SHR and WKY Rats**

Ventricular hypertrophy was associated with marked augmentation of ventricular ANP synthesis in SHR, as reflected by elevated levels of IR-ANP. Ventricular IR-ANP content (in nanograms per ventricle) and concentration (in picograms per milligram wet weight) were 3.7- and 3.0-fold higher (p<0.01), respectively, in SHR than in WKY rats (Table 2). The induction of the ANP
TABLE 2. Atrial Natriuretic Peptide mRNA and Immunoreactive Atrial Natriuretic Peptide Levels in Ventricles and Atria of Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY (n=5)</th>
<th>SHR (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular ANP mRNA</td>
<td>0.30±0.05</td>
<td>0.84±0.14*</td>
</tr>
<tr>
<td>(pg/mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular IR-ANP</td>
<td>112±13</td>
<td>339±47†</td>
</tr>
<tr>
<td>(pg/ventricle)</td>
<td>191±28</td>
<td>708±123†</td>
</tr>
<tr>
<td>Atrial IR-ANP</td>
<td>156±13</td>
<td>105±16*</td>
</tr>
<tr>
<td>(ng/mg)</td>
<td>33±2</td>
<td>26±2*</td>
</tr>
<tr>
<td>(μg/atrium)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; ANP, atrial natriuretic peptide; IR, immunoreactive. ANP mRNA and IR-ANP levels were measured from unstretched control hearts (see Tables 1 and 3). Ventricular ANP mRNA is the ratio of the absorbance corresponding to the ANP probe to 18S probe hybridization (arbitrary densitometric units). Atrial IR-ANP was measured from total atrial tissue. *p<0.05 and †p<0.01 vs. WKY by Student’s unpaired t test.

coding gene in the hypertrophic ventricular tissue of hypertensive rats was also seen when cardiac ANP mRNA levels were measured by dot blot analysis. The ventricular ANP/18S ratios were 2.8-fold higher in SHR than in WKY rats (p<0.05, Table 2), analyzed from unstretched control hearts. The Northern blot analysis with a full-length rat ANP cDNA probe identified a single 0.9-kb mRNA species in the ventricles.

Higher concentrations of IR-ANP were found in the atria than in the ventricles in both strains (Table 2). IR-ANP concentration (in nanograms per milligram) and content (in micrograms per atrium) were 48% and 28% higher, respectively, in WKY rats when compared with SHR.

Effects of Stretch on Hemodynamics in the Isolated Perfused Hearts of SHR and WKY Rats

Ventricular stretch was produced by filling the intraventricular balloon with two different volumes of water: 0.18±0.04 and 0.63±0.08 ml in WKY rats and 0.23±0.05 and 0.48±0.06 ml in SHR. LVP was measured to estimate the degree of the stretch (Figure 1). When the balloon was filled, LVP increased rapidly and remained constant during the stretch. When the balloon was emptied, LVP quickly returned to the level preceding the stretch. The values of LVP obtained in each group are shown in Table 3. No significant differences between the corresponding groups of SHR and WKY rats were noted (Figure 1, Table 3).

As we have already reported using these experimental conditions, the basal perfusion pressure was significantly higher in SHR than in WKY rats (p<0.001, Table 3). The basal heart rates were 194±16 (control), 167±6 (stretch 1), and 163±8 (stretch 2) beats per minute in WKY groups and 159±7, 182±7, and 162±7 beats per minute in SHR, respectively. Addition of 27 mM KCl into the perfusion fluid after removal of the atria during perfusion of the ventricles at a constant flow caused rapid cardiac arrest and led to marked coronary vasoconstriction, as reflected in the continuous rise in perfusion pressure in all groups (Figure 2). Stretch of the ventricles with intraventricular balloon further elevated perfusion pressure. Perfusion pressure increased immediately from the beginning of the stretch period and terminated rapidly as the balloon was emptied. During the recovery period, the pressure continued to rise in a manner similar to the rise observed in control experiments (Figure 2). The perfusion pressure values before stretch and maximal values obtained during stretch are shown in Table 3.

Effect of Stretch on IR-ANP Release in the Isolated Perfused Hearts of SHR and WKY Rats

The basal (before removal of the atria) ANP release varied substantially even between individual animals from the same strain (Table 3). However, ANP release after removal of the atria did not differ significantly between the groups in either of the strains. The concentration of IR-ANP in the perfusate both before and after removal of the atria was significantly higher in SHR than in WKY rats (p<0.001) (Table 3), reflecting the increased release of ANP from hearts with myocardial hypertrophy. The ventricles contributed 15±3% and 9±3% of the total ANP secreted by the hearts in SHR and WKY rats, respectively.

Mechanical stretch of the ventricular tissue by the intraventricular balloon stimulated ANP release both in SHR and WKY rats (Figure 3). As LVP rose from 0 to 14 and 26 mm Hg in WKY rats and from 0 to 13 and 27 mm Hg in SHR, increases in ANP release into the

FIGURE 1. Left ventricular pressure in isolated, arrested, perfused hearts of Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). After removal of the atria, the ventricles were perfused for 10 minutes with the intraventricular balloon empty. Then, the balloon was filled with various volumes of water for 10 minutes (between arrows), which was followed by a recovery period of 10 minutes. Line graphs (from top to bottom) represent control, stretch 1, and stretch 2 groups. For the number of rats in each group, see Table 1. Results are expressed as mean±SEM.
TABLE 3. Effect of Stretch on Hemodynamic Variables and Immunoreactive Atrial Natriuretic Peptide Release in Wistar-Kyoto and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th></th>
<th>WKY Control (n=6)</th>
<th>Stretch 1 (n=8)</th>
<th>Stretch 2 (n=6)</th>
<th>All WKY (n=20)</th>
<th>SHR Control (n=8)</th>
<th>Stretch 1 (n=7)</th>
<th>Stretch 2 (n=7)</th>
<th>All SHR (n=22)</th>
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<tbody>
<tr>
<td>LVP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stretch</td>
<td>0 ± 1</td>
<td>14 ± 1</td>
<td>26 ± 1</td>
<td>19 ± 1</td>
<td>0</td>
<td>13 ± 1</td>
<td>27 ± 2</td>
<td>0</td>
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<tr>
<td>Recovery</td>
<td>1 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Perfusion pressure (mm Hg)</td>
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<td></td>
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<tr>
<td>Basal</td>
<td>19 ± 1</td>
<td>20 ± 1</td>
<td>18 ± 1</td>
<td>19 ± 1</td>
<td>36 ± 1</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>29 ± 1*</td>
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<tr>
<td>Control</td>
<td>74 ± 5</td>
<td>99 ± 6</td>
<td>112 ± 8</td>
<td>96 ± 5</td>
<td>114 ± 12</td>
<td>117 ± 7</td>
<td>116 ± 11</td>
<td>116 ± 6†</td>
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<tr>
<td>Stretch</td>
<td>88 ± 5</td>
<td>109 ± 9</td>
<td>139 ± 7</td>
<td>...</td>
<td>124 ± 12</td>
<td>136 ± 8</td>
<td>150 ± 14</td>
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<tr>
<td>Recovery</td>
<td>88 ± 6</td>
<td>104 ± 6</td>
<td>104 ± 10</td>
<td>...</td>
<td>129 ± 12</td>
<td>129 ± 8</td>
<td>138 ± 19</td>
<td>...</td>
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<tr>
<td>IR-ANP (pg/ml)</td>
<td></td>
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<tr>
<td>Basal</td>
<td>198 ± 6</td>
<td>411 ± 23</td>
<td>350 ± 6</td>
<td>319 ± 25</td>
<td>533 ± 9</td>
<td>758 ± 16</td>
<td>623 ± 15</td>
<td>638 ± 26*</td>
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<td>Control</td>
<td>23 ± 3</td>
<td>17 ± 4</td>
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<td>104 ± 22</td>
<td>69 ± 8</td>
<td>106 ± 35</td>
<td>93 ± 14*</td>
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<tr>
<td>Stretch</td>
<td>29 ± 4</td>
<td>25 ± 5</td>
<td>52 ± 10</td>
<td>...</td>
<td>108 ± 21</td>
<td>86 ± 10</td>
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<td>40 ± 9</td>
<td>...</td>
<td>87 ± 17</td>
<td>97 ± 18</td>
<td>142 ± 31</td>
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</table>

Values are mean ± SEM. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; stretch 1, at left ventricular pressures of 13–14 mm Hg; stretch 2, at left ventricular pressures of 26–27 mm Hg; LVP, left ventricular pressure; control, means of the values of the last 2-minute period before stretching; stretch, peak values observed during the 10-minute stretching period; recovery, means of the values of the first 2-minute period after stretching; basal, mean values for perfusion pressure and immunoreactive atrial natriuretic peptide (IR-ANP) before removal of atria. For details of stretched groups, see “Materials and Methods” and Figures 1–3.

*p < 0.001 and †p < 0.01 vs. all WKY by unpaired Student’s t test.

Perfusion of 1.4 ± 0.1-fold (p = NS) and 1.5 ± 0.2-fold (p < 0.05) in WKY rats and 1.1 ± 0.1-fold (p = NS) and 1.6 ± 0.2-fold (p < 0.05) in SHR, respectively, were observed (Figure 4). When a regression analysis for the data in Figure 4 using original values from each experiment was performed, there was a statistically significant correlation between LVP levels and the maximal stimulation of ANP release (p < 0.001, r = 0.59, n = 42). The maximal increase in ANP release into the perfusate was seen during the first 2 minutes of the stretch (Figure 3). Then, toward the end of the stretch period, ANP release gradually returned to the prestretch level. When the balloon was emptied, another temporary stimulation of ANP release was seen (Figure 3). The maximal IR-ANP concentrations during the stretch (Table 3) significantly correlated with the ventricular weight/body weight ratios of the corresponding animals (Figure 5).

As seen in Figure 4, no significant differences were noted in the SHR and WKY rats in the relative stimulation of ANP release (i.e., the ratio of ANP concentration during stretch to the ANP concentration before stretch).

**HPLC Analysis**

HPLC analysis of the perfusates was performed in order to exclude the possibility that the stimulation of the ANP release observed was due to tissue destruction. This showed that the ANP immunoreactivity in the perfusates was almost completely due to processed, active, ANP-(1-28)–sized material, both before (Figure 6A) and after (Figure 6B) removal of the atria and during stretch (Figure 6C). Atrial stretching has been shown to stimulate the release of this same low molecular weight peptide. To further examine the possibility that nonspecific tissue damage could be involved in the pressure-induced changes in ANP release, we performed cytosolic marker enzyme, lactate dehydrogenase, determinations of the perfusates. Lacate dehydrogenase was detectable in only a few of the samples at concentrations near the detection limit of the assay (data not shown), and there was no correlation between lactate dehydrogenase and ventricular pressure or increase in ANP release, showing that the increase in ANP secretion during stretching was not likely caused by either myocardial tissue damage or ischemia.

**Discussion**

Ventricular hypertrophy is characterized by augmentation of ventricular synthesis, storage, and release of ANP, but the physiological stimulus for ventricular ANP release is not known. In the present study, we used a modification of our isolated perfused heart preparation, which permitted mechanical distension of the left ventricle. We found an increase in LVP caused by the dilatation of a balloon in the left ventricle to be a direct stimulus for ventricular ANP release both in normotensive and spontaneously hypertensive rats. The HPLC analysis of the perfusates showed that the ANP-like immunoreactive material released corresponded to the processed, active, low molecular weight peptide found in plasma. Thus, ANP is also a ventricular hormone stimulated by increased cardiac filling pressure.

The animals used in this study were old SHR and WKY rats. The SHR strain is a particularly suitable experimental model for examining pathophysiological alterations in ventricular hypertrophy. Unlike other models of cardiac hypertrophy, the condition is spontaneous, and the gradual and progressive increase in cardiac mass in response to chronically elevated blood pressure occurs as a function of age. As expected, ventricular IR-ANP content and concentration were substantially higher in the ventricles of the hypertensive
rats than in the ventricles of the normotensive control rats. The ANP mRNA level was also significantly higher in the ventricles of SHR, demonstrating the induction of the ANP coding gene. This was further indicated by the augmented ANP secretion from the ventricles into the perfusate in SHR. In contrast, atrial ANP content and concentration were significantly lower in SHR than in WKY rats. Similar results have been reported in these rat strains.12–14,17,18

In the atria, the release of ANP is primarily regulated by atrial stretch.20 Atrial wall stretch in isolated perfused hearts29–32 and atria,33–35 as well as osmotic stretch of isolated rat myocytes,36 stimulates the secretion of ANP. Recent in vitro studies show that ANP is
ANP biosynthesis. 

Taken together, these observations suggest that stretch might also be a common hemodynamic stimulus for the expression and release of ANP within ventricular myocardium.

To test this hypothesis, we evaluated the ability of ventricular cardiomyocytes in the isolated perfused heart preparation after removal of the atria to secrete ANP in response to direct passive mechanical stretch produced by an intraventricular balloon. In isolated canine ventricles, myocardial stretch has been shown to cause arrhythmias, and ventricular dilatation enhances the susceptibility to stretch-induced arrhythmias. Therefore, to study the effect of stretch on ventricular ANP release in the absence of interfering spontaneous cardiac myocytic activity, we arrested the hearts after removal of the atria with an infusion of potassium chloride. The amount of ventricular ANP release of the total ANP secreted by the hearts in the present study (15% and 9% in SHR and WKY rats, respectively) was similar to that seen in our studies using beating hearts and suggests that the infusion of potassium chloride into the perfusate did not affect the basal release of ANP from the ventricular tissue.

Our present results demonstrate that stretching of the ventricular tissue in arrested, isolated, perfused rat hearts after removal of the atria caused a pressure-dependent rapid increase in ANP release into the perfusate. The LVP required for ANP release was comparable with that used in previous studies using different experimental models. In a recent study by Capasso et al., left ventricular end-diastolic pressure was approximately 5–6 mm Hg. The peak systolic pressure reached 130–140 mm Hg in normal rats, and left ventricular end-diastolic pressure increased to approximately 25 mm Hg when these rats were subjected to surgical constriction of the left renal artery. In SHR, left ventricular peak systolic pressure values as high as 187–199 mm Hg have been observed. Thus, the LVP may only be high enough to cause ANP release from ventricular tissue under conditions of pressure and volume overload. This could
released by emptying the intraventricular balloon, there was a second temporary stimulation of ANP release, which was more marked when higher LVPs were used. This second increase in perfusate ANP concentration was probably due to peptide already secreted during stretch that only appeared in the perfusate after the flow in myocardial vasculature returned to the prestretch level. This interference in myocardial flow, however, did not cause myocardial ischemia, as reflected by undetectable or very low lactate dehydrogenase concentrations in the perfusates. Further, it is unlikely that residual atrial tissue could have contributed to the pressure-induced changes in ANP release. First, if this were the case, one would expect the basal release as well as the increase in ANP release in response to increased LVP to be higher in WKY rats than in SHR, because atrial ANP concentration was significantly higher in the WKY strain. In fact, the absolute increase in perfusate IR-ANP concentration (expressed as picograms per milliliter) was substantially higher in the SHR strain (see below). Second, stretch-stimulated ANP secretion did not correlate with atrial concentrations of ANP, but a statistically significant correlation was found between ventricular hypertrophy and ANP release (see below). Third, when ventricles were stretched in the presence of whole atrial tissue, perfusate ANP concentration increased similarly (from 247 to 390 pg/ml, i.e., a 1.5-fold increase) as observed when ventricles were stretched after removal of the atria.

The time course for the stimulated ANP release differed markedly from that observed in atria. We found that the maximal stimulation of ventricular ANP release occurred during the first 2 minutes of stretching, and this was followed by a gradual decrease in ANP release during the 10-minute stretch period and reached control values by the end of it. In the atria, as in the ventricles, the ANP release increases rapidly in response to stretching but remains constant or continues to rise during stretching. This difference probably reflects the more limited ANP storage capacity of the ventricular tissue and differences in mechanisms of ANP secretion between atria and ventricles. Thus, a 32-fold increase in IR-ANP but a 100-fold increase in ANP mRNA were reported from hamster ventricles that developed severe congestive heart failure, which suggests that ANP is secreted more rapidly after synthesis from ventricular cardiocytes. The transient ANP secretory response to stretch further suggests that the ventricles make a greater contribution to the circulating ANP level at the onset of cardiac pressure or volume overload. Indeed, an association between ventricular ANP content and increase in plasma ANP levels in response to acute volume expansion in the conscious hypertensive rat strain was observed.

A significant correlation between ventricular weight and ANP release from ventricles has been found in vitro, indicating that the amount of the peptide released depends on the degree of ventricular hypertrophy. In severe congestive heart failure in the hamster, as much as 74% of ANP released into the perfusion fluid is produced in the ventricles. In the present study, the maximal ANP concentrations obtained during stretch periods correlated significantly with the degree of cardiac hypertrophy. Thus, the stimulated release of ANP from the hypertrophied ventricles is also higher in SHR.
than in normal rat hearts. However, the relative increase in ANP release (i.e., the ratio between the maximal ANP concentration during stretch to control ANP concentration in the perfusate) at identical degrees of stretch was similar between these two strains. These observations agree with our previous report\(^4\) showing that, although the amount of ANP released (in picograms per milliliter) during swimming was greater in SHR, the percent increase in plasma ANP was similar in WKY rats (240%) when compared with that in SHR (233%). Thus, although myocardial stretch caused by elevated ventricular pressure contributes to the increased plasma IR-ANP levels in patients with congestive heart failure and in experimental models of cardiac hypertrophy, the hypertrophic process in SHR does not seem to alter the ability of ventricular cells to secrete ANP in response to stretch.

The release of ANP from the intact heart (both in vivo and in vitro) is accompanied by immediate selective proteolytic cleavage of prohormone into the C-terminal peptide ANP-(1-28) and the N-terminal fragment.\(^4\) A 56-amino acid dimer of ANP-(1-28) (\(\beta\)-ANP) has been found in patients with hypertrophic cardiomyopathy.\(^48\) We found only one major ANP-like peptide in the perfusate by the HPLC analysis. Its concentration increased after stretching of the left ventricle. The ANP-like immunoreactive material secreted both before and after removal of the atria and during stretch corresponded to the processed, active, low molecular weight peptide also found in plasma.\(^4\) This suggests that the release of the peptide by ventricular distension is a physiological mechanism and is not the result of nongenetic tissue destruction. Otherwise the high molecular weight peptide pro-ANP, which is the tissue storage form of ANP,\(^25\) would also have appeared in the perfusate. The observation that only one major peptide was released from hypertrophied ventricles of SHR contrasts with the results obtained from experiments on normal and cardiomyopathic hamsters, in which a relatively large portion of pro-ANP was released intact by the ventricles.\(^10\) Thus, ANP processing in both ventricles and atria may be species dependent.

How stretching of ventricular myocytes stimulates ANP secretion remains to be established. Originally, Bloch et al\(^15\) suggested that neonatal ventricular cells use a constitutive pathway for ANP release because they lack the secretory granules characteristic of atrial cardiocytes, which store the peptide before secretion. However, the presence of ANP granules in the hypertrophied ventricular tissue\(^5,15,18,36,49\) suggests that a secretagogue would enhance ANP release from hypertrophied ventricular cells in a manner similar to that occurring in atria.\(^50\) In fact, we have recently shown that phorbolesters that mimic the action of diacylglycerol by acting directly on protein kinase C stimulate ANP release from severely hypertrophied SHR ventricles but not from normal rat myocardium.\(^18\) These results demonstrated the ability of mature ventricular tissue to secrete ANP in response to a secretagogue and suggested a possible role for calcium-activated protein kinase C in the regulation of basal ANP secretion from ventricular cells. Phorbolesters have been shown to stimulate basal ANP secretion in isolated perfused hearts (for review, see Reference 50) and cultured myocytes.\(^51-53\) The observations that right atrial dilata-

tion stimulates the formation of inositol-1,4,5-triphosphate\(^54\) and that phorbolesters enhanced atrial stretch-stimulated ANP secretion from isolated perfused heart\(^46\) further suggest that the activation of phosphatidylinositol turnover and protein kinase C may contribute to stretch-induced ANP release. However, cellular stretch modifies the intracellular contents of several other signaling compounds, such as Ca\(^{2+}\), Na\(^+\), K\(^+\), cAMP, and proto-oncogenes,\(^55\) and a major challenge is to clarify the role of these intracellular signals in the mechanical stretch-induced ventricular ANP release and synthesis.

In conclusion, in ventricular hypertrophy ANP is synthesized, stored, and released from ventricular and atrial cardiocytes. As occurs in the atria, we have now shown that increases in cardiac filling pressure that increase ventricular myocardial wall stretch will also lead to ANP release. The kinetics of the ANP secretory response to stretch suggest that the ventricles make a greater contribution to the circulating level of ANP at the onset of cardiac pressure or volume overload. However, if the overload persists, the atrial contribution to ANP release becomes more important. The ANP-like immunoreactive material secreted in response to ventricular stretch corresponded to the processed, active, low molecular weight peptide already identified in plasma, showing that atrial natriuretic peptide also is a ventricular hormone.

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