SUMMARY  Atrial natriuretic factor (ANF) is a potent natriuretic and vasorelaxant agent that also stimulates guanosine 3',5'-cyclic monophosphate (cGMP) excretion in normotensive animals. These properties suggest that ANF may be involved in the regulation of blood pressure. To test a pure preparation of ANF in both normotensive and hypertensive animals, a synthetic 26 amino acid peptide (sANF) contained within endogenous rat ANF was infused intravenously into conscious Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) at doses from 12 to 190 pmol/minute. Mean arterial pressure fell progressively as doses of sANF were increased until maximum responses of —41 ± 5 mm Hg and — 29 ± 5 mm Hg were obtained during infusion of 95 pmol/minute sANF in SHR and WKY, respectively. Heart rate was not significantly affected in either group. At sANF doses of 12 to 50 pmol/minute, urinary electrolyte excretion rose in a dose-related fashion and was similar in WKY and SHR. At infusions of 95 to 190 pmol/minute, the diuretic and saluretic responses were diminished in the hypertensive animals. Only the 190 pmol/minute sANF dose significantly enhanced cGMP excretion in SHR (p < 0.05); however, in WKY urinary cGMP excretion was elevated in a dose-related fashion. At the highest sANF dose, cGMP excretion was approximately 15 times that observed in the pretreatment urine. The differences in the renal and blood pressure responses to sANF in SHR and WKY suggest that the actions of endogenous ANF may be altered in hypertension.

(Key Words: diuresis • natriuresis • cGMP • vasorelaxation • Wistar-Kyoto rats)

Atrial natriuretic factor (ANF), a potent diuretic and natriuretic agent extractable from rat atria, lowered mean arterial pressure (MAP) without affecting heart rate (HR) when injected into anesthetized bioassay rats. In other studies, ANF relaxed in vitro smooth muscle preparations contracted by various agonists. As ANF also increased urinary guanosine 3',5'-cyclic monophosphate (cGMP) excretion and plasma cGMP concentration in anesthetized normotensive rats, this nucleotide has been implicated as a possible mediator of the biological actions of ANF.

Most of the studies performed to date have characterized the biological responses to crude or semipurified ANF. Once the amino acid sequence was determined, a synthetic 26 amino acid peptide (sANF) was produced by solid-phase synthesis of appropriate fragments, which were coupled in solution to produce the following sequence: Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-COOH (disulfide bridge between cysteines). This synthetic peptide has been shown to be natriuretic and vasorelaxant and to elevate urinary and plasma levels of cGMP.

To define the renal and vascular effects of sANF in a model of genetic hypertension, we infused the synthetic peptide intravenously into conscious, normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). The experimental design allowed measurements of MAP, HR, urine flow (V), and excretion rates of sodium (U_sodium V), chloride (U_chloride V), potassium (U_potassium V), and cGMP under steady state conditions in unanesthetized animals.
Materials and Methods

Male SHR and WKY (250–375 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were maintained on Purina rat chow (sodium content, 0.145 mEq/g; Ralston-Purina, St. Louis, MO) and water ad libitum. Before the study began, hypertension was verified in the SHR by the piezocrystal-tail cuff method; systolic pressures averaged 191 ± 4 mm Hg.

After fasting for 12 hours, rats were anesthetized with ether and catheters were inserted in the left femoral vein for infusion of lactated Ringer’s solution and sANF and in the left femoral artery for continuous MAP measurement. A bladder catheter was implanted through a suprapubic incision and secured so that dead space was minimized. The rats were comfortably restrained, and infusion of lactated Ringer’s solution was begun (0.028 ml/min); 160 minutes was allowed for recovery and stabilization. Body temperatures were measured with rectal probes and maintained at 37°C with heat lamps if necessary.

The experimental protocol consisted of six continuous, 20-minute urine collection periods. The MAP was determined by electronic integration at the beginning and midpoint of each period with a transducer (Micron Instruments, Inc., Los Angeles, CA) and an automated data acquisition system (Coulbourn Instruments, Inc., Lehigh Valley, PA; Hewlett-Packard Desktop Computer Division, Fort Collins, CO). The HR was calculated from the undamped pulse trace at the midpoint of each period. Following an initial control period, a single dose of sANF was infused at 0.026 ml/minute during a 20-minute experimental period (lactated Ringer’s infusion was not interrupted). The peptide was administered to five groups of WKY and five groups of SHR at doses of 12, 25, 50, 95, or 190 pmol/minute. Because of the rapid onset and decay of previously observed sANF renal responses (unpublished data from preliminary experiments, 1983), urine collections were made at 10-minute intervals during sANF infusion. The MAP was measured at intervals of 2 minutes during the infusion, while HR was ascertained at the midpoint of each of the 10-minute collection periods. After stopping the infusion, urine was collected through four additional 20-minute recovery periods during which MAP and HR were measured as described previously. Initial vehicle studies, in which saline (0.9% NaCl) was infused in lieu of sANF, verified the stability of all measured variables and the results from five to eight rats. BPM = beats/minute; Veh = vehicle response; and maximum changes from control levels, the vehicle response and for comparisons between WKY and SHR. Differences were considered significant at p < 0.05.

Results

In all rats, MAP began to fall after initiating the sANF infusion, remained depressed for the duration of the infusion, and then recovered to levels that were not significantly different from control within 10 minutes of discontinuing the infusion. When the data were expressed as maximum changes from control levels, the greatest hypotensive responses occurred in both the normotensive and hypertensive animals at 95 pmol/minute (Figure 1, upper panel). At that dose, MAP was lowered from a control level of 134 ± 4 to 110 ± 4 mm Hg in WKY and from 164 ± 5 to 128 ± 6 mm Hg in SHR. (Control values for the measured cardio-

Urine volumes were ascertained gravimetrically, and urinary electrolyte concentrations were determined by ion-selective electrodes (Technicon Corp., Inc., Tarrytown, NY). Levels of cGMP were measured with cGMP radioimmunoassay kits (Amersham Corp., Arlington Heights, IL). All results are given as means ± SEM. Data collected during infusion of a single dose of sANF into each group of WKY or SHR were subjected to analysis of variance, and significant differences among the means of the control, experimental, and recovery samples were identified by the Newman-Keuls test. The maximum response to each treatment (vehicle or each sANF dose) was calculated as the difference between the preinfusion control value and the highest (or lowest) measurement obtained during the sANF infusion. Dunnett’s t test was used to compare maximum responses at each dose level to the vehicle response and for comparisons between WKY and SHR. Differences were considered significant at p < 0.05.

**FIGURE 1.** Maximum changes in blood pressure and HR following sANF infusion into conscious WKY and SHR. The maximum change (Δ) was calculated as the difference between the peak response observed during the sANF infusion and the preinfusion control level. Each data point represents the mean ± SEM of from five to eight rats. BPM = beats/minute; Veh = vehicle responses; * = significant difference from the response of WKY or SHR at the same dose (p < 0.05); † = significant difference from the corresponding vehicle maximum Δ of WKY or SHR (p < 0.05).
vascular and renal variables are shown in Table 1.) No further reduction in MAP occurred on increasing the dose to 190 pmol/minute. The MAP response in SHR was greater than that in WKY at doses of 50 to 190 pmol/minute (Figure 1, upper panel); however, there were no detectable differences between the hypotensive responses of WKY and SHR at 12 and 25 pmol/minute of sANF.

Despite the large decreases in MAP, HR, which averaged 395 ± 9 and 443 ± 6 beats/minute (Table 1) during the control period in WKY and SHR respectively, was essentially unchanged. Although tachycardia was observed during vehicle and sANF infusions (Figure 1, lower panel), the maximum changes in sANF-infused rats were not significantly different from those observed in the vehicle-treated animals.

The renal responses to sANF, expressed as the maximum changes from control values, are presented in Figures 2 and 3. In WKY, stimulation of urinary volume and electrolyte excretion progressively increased over the entire range of sANF doses. In SHR, UrNaV and UClV were enhanced in a dose-dependent manner until a plateau was reached at 50 pmol/minute (Figures 2 and 3). At the higher sANF doses of 95 and 190 pmol/minute, the diuretic, natriuretic, and chloride responses in the hypertensive animals began to dwindle and were significantly less (p < 0.05) at the highest dose than those in the normotensive rats. The UrNaV was increased only moderately in both WKY and SHR (Figure 3, lower panel).

Basal excretions of cGMP measured in 30 SHR (see the grand mean in Table 1) during the initial control period were significantly less (p < 0.05) than that of the 29 WKY. The cGMP excretion was clearly enhanced during infusions of 50, 95, and 190 pmol/min-

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**Table 1. Pretreatment Control Values of Cardiovascular and Renal Variables in Conscious Wistar-Kyoto and Spontaneously Hypertensive Rats Before Administration of Synthetic Atrial Natriuretic Factor**

<table>
<thead>
<tr>
<th>sANF dose (pmol/min)</th>
<th>Strain</th>
<th>Mean arterial pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
<th>Urine flow (ml/10 min)</th>
<th>Sodium excretion (μEq/min)</th>
<th>Chloride excretion (μEq/min)</th>
<th>Potassium excretion (μEq/min)</th>
<th>cGMP excretion (pmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>WKY</td>
<td>132 ± 3</td>
<td>0.14 ± 0.03</td>
<td>0.65 ± 0.20</td>
<td>0.88 ± 0.28</td>
<td>1.43 ± 0.21</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>169 ± 5</td>
<td>0.14 ± 0.02</td>
<td>1.58 ± 0.53</td>
<td>0.82 ± 0.28</td>
<td>0.87 ± 0.14</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>WKY</td>
<td>135 ± 1</td>
<td>0.11 ± 0.02</td>
<td>1.27 ± 0.33</td>
<td>0.75 ± 0.12</td>
<td>0.84 ± 0.10</td>
<td>1.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>161 ± 4</td>
<td>0.23 ± 0.04</td>
<td>2.80 ± 0.20</td>
<td>2.10 ± 0.12</td>
<td>0.99 ± 0.16</td>
<td>0.5 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>WKY</td>
<td>125 ± 4</td>
<td>0.24 ± 0.04</td>
<td>1.72 ± 0.60</td>
<td>1.53 ± 0.35</td>
<td>1.66 ± 0.26</td>
<td>24.4 ± 7.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>164 ± 3</td>
<td>0.16 ± 0.04</td>
<td>1.47 ± 0.37</td>
<td>0.79 ± 0.21</td>
<td>1.02 ± 1.11</td>
<td>5.4 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>WKY</td>
<td>134 ± 4</td>
<td>0.15 ± 0.02</td>
<td>0.80 ± 0.18</td>
<td>1.04 ± 0.18</td>
<td>1.61 ± 0.38</td>
<td>20.1 ± 8.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>164 ± 5</td>
<td>0.15 ± 0.04</td>
<td>1.71 ± 0.84</td>
<td>0.84 ± 0.41</td>
<td>0.67 ± 0.14</td>
<td>11.1 ± 5.4</td>
<td></td>
</tr>
<tr>
<td>190</td>
<td>WKY</td>
<td>131 ± 3</td>
<td>0.18 ± 0.04</td>
<td>1.22 ± 0.34</td>
<td>1.51 ± 0.36</td>
<td>1.87 ± 0.22</td>
<td>36.3 ± 6.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHR</td>
<td>169 ± 4</td>
<td>0.21 ± 0.05</td>
<td>1.85 ± 0.62</td>
<td>1.03 ± 0.51</td>
<td>1.26 ± 0.11</td>
<td>13.7 ± 4.2</td>
<td></td>
</tr>
</tbody>
</table>

**Grand mean:** WKY (29) 131 ± 2 395 ± 9 0.16 ± 0.02 1.13 ± 0.19 1.14 ± 0.16 1.48 ± 0.18 20.5 ± 7.3

SHR (30) 165 ± 2* 443 ± 6* 0.18 ± 0.02 1.88 ± 0.24* 1.13 ± 0.25 0.96 ± 0.10* 7.7 ± 3.0*

*Grand mean of SHR significantly different from WKY (p < 0.05).
Values are means ± SEM.
NA = not available due to loss of the urine samples before analysis; cGMP = guanosine 3',5'-cyclic monophosphate; sANF = synthetic atrial natriuretic factor; SHR = spontaneously hypertensive rats; WKY = Wistar-Kyoto rats.
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Factors responsible for the elevation of MAP in SHR are undefined, but ANF possibly could contribute to this as well as other forms of hypertension. Although this study does not address directly the potential role of ANF in hypertension, it does present evidence that the synthetic form of this endogenous peptide can effectively lower MAP and increase urinary electrolyte excretion in SHR.

The mechanism by which blood pressure was lowered in this study appears to be related to the direct vasorelaxation induced by sANF,7 as blood pressure declined immediately after beginning the sANF infusion and returned toward control levels after the infusion was stopped. This observation is consistent with a direct action on the cardiovascular system rather than an indirect effect secondary to volume depletion. Also, Hirata et al.16 found that atrial extracts reduced MAP in bilaterally nephrectomized rats. Although direct vasodilation appears to be the most likely mechanism for the depressor response, the present data do not eliminate a possible effect on cardiac function.

Despite the depressor effect of sANF, HR was not significantly affected. Although instrumentation limitations allowed only two HR measurements during the sANF infusion, it is unlikely that any transient HR responses were overlooked, as the effect of sANF on MAP was sustained for the duration of the infusion.

Natriuresis, the first biological action of ANF to be described,1 also was demonstrated with infusion of sANF in our study using conscious WKY and SHR. The three lowest doses produced equivalent dose-relat-

Discussion

Several investigators1-3,4 have observed decreases in arterial blood pressure during treatment of normotensive rats with atrial extracts. Although the hypotensive response may have been due to nonspecific effects in at least one in vivo study,2 the potent vasorelaxation observed with the use of atrial, but not ventricular,5 extracts in vitro suggest that blood pressure lowering in the intact animal is a consequence of a specific action of ANF. The present study, which sought to demonstrate the hypertensive and renal actions of sANF in normotensive and hypertensive rats, used a synthetic peptide preparation, which obviated the problem of nonspecific effects caused by impurities in tissue extracts.

Both of the principal actions of ANF (e.g., natriuresis and vasorelaxation) suggest that this substance could be important in blood pressure regulation. The
ed increases in $U_{\text{Na,V}}$ in the normotensive and hypertensive groups. At higher doses in SHR, the saluretic responses declined such that the $U_{\text{Na,V}}$ stimulated by the 190 pmol/minute infusion approached that observed at 25 pmol/minute, which resulted in a bell-shaped, dose-response curve (Figure 2, lower panel). In the WKY the diuretic, natriuretic, and chloruretic responses continued to increase almost linearly throughout the entire dose range of sANF tested. Why the saluretic responses should wane in SHR but not in WKY cannot be explained by the present data. If an increase in renal medullary blood flow contributed to the diuretic and natriuretic action of sANF, as has been suggested for other vasodilators, a disparity in the redistribution of blood between the WKY and SHR may be involved in the observed differences.

These observations suggest that, at high doses, sANF may be a less effective natriuretic agent in SHR than in WKY and raise the possibility that the kidney of the SHR responds differently to ANF, as has been observed in Dahl salt-sensitive rats. Interestingly, Sonnenberg et al. reported that the natriuretic component of atrial extracts from SHR is less than that of WKY, while Winquist et al. observed that the ability of atrial extracts of SHR to stimulate vasorelaxation is greater than that of WKY. Whether the differences in the renal and vascular responses to exogenous sANF observed in the present study could be related to these purported differences in atrial extracts of SHR cannot be determined from the present data.

As expected, increases in $U_{\text{Cl,V}}$ paralleled those of $U_{\text{Na,V}}$. The failure of $U_{\text{Na,V}}$ and $U_{\text{Cl,V}}$ to increase dramatically in WKY at the 190 pmol/minute sANF dose, as did $V$, is paradoxical. Currently, there are inadequate data to explain this apparent dissociation between the $V$ and $U_{\text{Na,V}}$ responses to a high dose of sANF. The modest elevations in $U_{\text{Cl,V}}$ following sANF infusions could be attributed to an increase in tubular flow as well as possible enhanced delivery of sodium to the potassium secretory portion of the distal nephron.

Dibutyryl cGMP has been shown to exert a vasorelaxant effect on smooth muscle from Sprague-Dawley, WKY, and to a lesser extent, SHR. As tissue, plasma, and urinary cGMP levels also increase following ANF treatment, the vasorelaxant effect of sANF could be linked to cGMP stimulation. It would be inviting to propose cGMP as a common mechanism for the renal and vascular effects of ANF if this cyclic nucleotide were to possess diuretic and natriuretic properties in addition to the aforementioned vasorelaxant properties. The ability of a peptide to regulate ion transport is not without precedent, in that a heat-stable enterotoxin of Escherichia coli has been shown to inhibit chloride transport in the gut, an effect mediated by cGMP. Our data demonstrating minimal changes in urinary cGMP excretion in SHR indicate that alterations in urinary cGMP were not essential to the renal or hypotensive effects of sANF. In agreement with our results, Seymour et al. found that unilateral intrarenal infusion of sANF increased cGMP excretion equally from both kidneys of anesthetized dogs, while $U_{\text{Na,V}}$ rose in the sANF-treated kidney only. Although a tubular source of urinary cGMP could not be excluded in the latter study, the dissociation of the cGMP response from the natriuresis suggests that the nucleotide was uninvolved in the sANF-induced natriuresis. Consequently, the potential role of cGMP in the mechanisms of the renal and vascular actions of sANF requires further clarification. Furthermore, our results indicate that in SHR the metabolism or renal handling of cGMP may be different from that of WKY. Whether the failure of cGMP excretion to increase in SHR resulted in modified renal and vascular responses to sANF and is thereby related to the hypertension is unknown.

**Conclusion**

Our results indicate that sANF exerts potent natriuretic and hypotensive effects in normotensive WKY and SHR. The renal and vascular responses of the two groups of rats were comparable at lower doses of sANF. At higher doses of the peptide, however, greater absolute falls in MAP were observed in SHR than in WKY. Additionally, the dose-response curves for ANF-induced renal effects were characteristically bell-shaped in SHR, while those of the WKY were distinguished by dose-related increases. The differential responses of the normotensive and hypertensive rats to sANF suggest the potential importance of this peptide in blood pressure regulation.

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