Synthetic Atrial Natriuretic Factor in Conscious Normotensive and Hypertensive Rats

ANDREA A. SEYMOUR, ELIZABETH A. MARSH, ELAINE K. MAZACK, INEZ I. STABILITO, AND EDWARD H. BLAINE

SUMMARY Synthetic atrial natriuretic factor (Arg-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Phe-Arg-Tyr-COOH [disulfide bond between cysteines]) was infused intravenously into conscious normotensive and deoxycorticosterone, one-kidney, one-clip, and two-kidney, one-clip hypertensive rats. Mean arterial pressure, urine volume, and electrolyte excretion rates were measured during a 20-minute infusion of a single dose (ranging from 0-1520 pmol/min) into each animal; 95 to 380 pmol/minute of synthetic atrial natriuretic factor maximally reduced mean arterial pressure by $-20 \pm 4$, $-29 \pm 2$, and $-39 \pm 7$ mm Hg in normotensive, one-kidney, one-clip, and two-kidney, one-clip hypertensive rats, respectively. In deoxycorticosterone rats, a dose of 760 pmol/minute was required to produce the largest depressor response ($-58 \pm 12$ mm Hg). Sodium excretion increased to $8.8 \pm 2.5 \mu$Eq/minute at 760 pmol/minute in normotensive rats, to $6.5 \pm 1.1 \mu$Eq/minute at 50 pmol/minute in deoxycorticosterone rats, and to $5.8 \pm 1.5 \mu$Eq/minute at 95 pmol/minute in one-kidney, one-clip animals. The natriuretic effect was consistently greater at all doses of synthetic atrial natriuretic factor in the two-kidney, one-clip hypertensive model, in which the maximum response was $15.3 \pm 4.7 \mu$Eq/minute at 190 pmol/minute. The changes in urine volume and excretion rates of potassium and chloride tended to parallel the increases in sodium excretion in each model. Interestingly, the maximally effective hypotensive dose of synthetic atrial natriuretic factor was different from the maximally effective natriuretic dose in all four groups. Even though its exact mechanisms of action remain unclear, synthetic atrial natriuretic factor appears to be an effective hypotensive and natriuretic agent in several models of conscious hypertensive rats. (Hypertension 7 [Suppl I]: I-35-I-42, 1985)

Key Words • renovascular hypertension • mineralocorticoid hypertension • natriuresis • diuresis • hypotensive agent

In 1981 de Bold et al.1 described the biological actions of atrial extracts in anesthetized rats and reported that atrial natriuretic factor (ANF) induced a natriuretic and a hypotensive response. Shortly thereafter, a direct vasorelaxant effect of atrial preparations was demonstrated in isolated vascular smooth muscle strips.2-4 In addition, urinary guanosine cyclic 3',5'-monophosphate (cGMP) excretion and plasma cGMP concentration were elevated following injection of atrial extracts in anesthetized rats.5 A closely related group of active peptides was later isolated from rat6-11 and human12 atria, and a peptide containing a 26 amino acid sequence was synthesized 13. The structure of the synthetic ANF is as follows: Arg-Arg-Ser-Cys-Phe-Gly-Gly-Arg-Ile-Asp-Arg-Ile-Gly-Ala-Gln-Gly-Leu-Gly-Cys-Asn-Ser-Phe-Arg-Tyr-COOH (disulfide bond between cysteines). As this is the only synthetic peptide we will discuss, it will be referred to simply as sANF.

The sANF has been shown to enhance sodium excretion in anesthetized rats7 and to relax isolated vascular smooth muscle preparations8 in a dose-related manner comparable to that of arterial extracts. In addition, intrarenal infusion of the synthetic peptide in anesthetized dogs lowered blood pressure at doses that elevated sodium excretion only in the sANF-infused kidney.9

Both the natriuretic and vasodilatory actions of sANF indicated that the peptide might be particularly effective as an antihypertensive agent. To explore that potential use, sANF was infused in conscious normotensive rats and in models of mineralocorticoid and renovascular hypertension. Synthetic ANF was found to reduce blood pressure and increase sodium excretion in normotensive and in deoxycorticosterone (DOC), one-kidney, one clip (1K-1C), and two-kidney, one-clip (2K-1C) hypertensive rats.
Methods

Six-week-old male Sprague-Dawley rats weighing 150 g (Charles River Breeding Laboratories, Wilmington, MA) were housed in rooms in which temperature and lighting cycles were controlled. Food and tap water were allowed ad libitum except as noted.

Fifty-six of the rats were rendered hypertensive by weekly subcutaneous injections of 20 mg of deoxycorticosterone pivalate (Percorten) and substitution of 0.9% sodium chloride for drinking water. After 3 weeks of treatment, systolic blood pressure was determined with the piezocrystal tail cuff method and those animals in which hypertension was verified were selected for sANF treatment.

One hundred additional rats (150 g) were anesthetized with ether, and the left kidneys were exposed through a flank incision. In each of 47 animals, a silver clip with an internal diameter of 0.15 mm was placed around the renal artery to induce 2K-1C hypertension. In the remaining 53 rats, 1K-1C hypertension was initiated by unilateral nephrectomy and constriction of the remaining renal artery with a 0.2-mm silver clip. Three to four weeks later, when systolic blood pressure (measured by the tail cuff method) exceeded 180 mm Hg, these hypertensive rats were infused with sANF.

Fifty-four male Sprague-Dawley rats (weighing about 300 g) were obtained from Charles River and subjected to sANF testing. At the time of the experiment, these animals were approximately the same age as the hypertensive rats and served as the normotensive control group.

All experiments were performed in conscious rats prepared for study in the following manner. The test animals fasted for approximately 12 hours and then were anesthetized with ether for placement of vascular and urinary bladder catheters. Each animal was then comfortably restrained and allowed to recover from the surgical procedures for at least 160 minutes Lactated Ringer’s solution was continuously infused at 0.028 ml/minute through a polyethylene catheter inserted into the left femoral vein. Arterial pressure was measured using a Micron (Los Angeles, CA) pressure transducer attached to a catheter implanted in the left femoral artery. The pulsatile pressure signal was electronically integrated by a Colburn (Lehigh Valley, PA) data acquisition system for recording of mean arterial pressure (MAP). The pulsatile pressure trace was displayed periodically for determination of heart rate.

Urine was collected in preweighed tubes through a catheter implanted directly in the urinary bladder through a suprapubic incision. The lower end of the bladder was tied off so that the luminal space was minimized. The volume of urine collected during each timed period was determined gravimetrically, and concentrations of sodium, potassium, and chloride were ascertained with an ion-selective electrode (Technicon, Tarrytown, NY). The cGMP concentrations were measured in selected urine samples with a radioimmunoassay kit (Amersham, Arlington Heights, IL). Electrolyte and cGMP excretion rates were calculated by the standard formula.

Before beginning the infusion of sANF, urine was collected throughout a 20-minute pretreatment period. During the next 20 minutes, a single dose of sANF was infused intravenously while urine was obtained at 10-minute intervals. Twenty minute sampling periods were resumed on completion of the sANF treatment and continued for an additional 80 minutes.

Each dose of 0 (vehicle), 12, 25, 50, 95, 190, 380, 760, or 1520 pmol/minute of sANF was infused into normotensive animals (n = 6 per dose) and into separate groups of DOC, 1K-1C, and 2K-1C hypertensive rats. The peak responses to sANF were calculated as the difference between baseline levels and the maximum (or minimum) values measured during sANF infusions. The responses of each hypertensive model to a single dose of the peptide were compared with those of the normotensive rats by Dunnett’s t test. Within each group of hypertensive or normotensive rats, the responses to all doses of sANF were compared with the effects of vehicle infusion by analysis of variance and significant differences were identified by application of Newman-Keuls’s test. Data are presented as the mean ± SEM.

The 26 amino acid peptide was synthesized by Dr. Nutt and associates (Merck Sharp and Dohme Research Laboratories) using a combination of classical solution and solid phase techniques. The lyophilized peptide was dissolved and diluted in saline for infusion into the rats.

Results

During vehicle infusions (dose = 0; Figure 1) MAP was relatively stable in normotensive rats and in all models of hypertension. Administration of the lower doses of sANF (12–50 pmol/min) gradually reduced pressure to its nadir, which was achieved by the end of the 20-minute treatment. At the higher doses, MAP began to decrease rapidly and dropped most sharply during the first 5 minutes of sANF administration. After stopping the sANF infusion, blood pressure rose rapidly toward control values. These trends were most readily seen in the hypertensive rats.

There seemed to be a lower limit to which blood pressure would fall in each model. Once that floor was reached higher doses of sANF did not reduce MAP any further. In normotensive animals, a nadir of 101 ± 3 mm Hg was produced by infusion of 95 pmol/minute. The lowest pressures measured in the renovascular hypertensive models (134 ± 5 mm Hg during infusion of 190 pmol/min in 1K-1C and 132 ± 6 mm Hg during administration of 380 pmol/mm in 2K-1C) were still elevated above the control measurements in normotensive rats. In contrast, MAP dropped to normotensive levels (119 ± 9 mm Hg) during infusion of 380 pmol/minute in DOC rats. In that model, a higher dose of 760 pmol/minute dropped pressure from 181 ± 7 to 123 ± 12 mm Hg, a change of −58 ± 12 mm Hg.

To account for differences among the initial pressures of the normotensive and the hypertensive rats, the depressor responses were expressed as the difference between the lowest pressure observed during
The maximal reductions in MAP achieved at each level of sANF infusion into DOC, 1K-1C, and 2K-1C hypertensive rats. The responses to vehicle infusion are given at dose = 0. The depressor responses in normotensive animals are included in each panel for the sake of comparison. The pretreatment MAP was 117 ± 1 (n = 54) in normotensive (NT), 162 ± 2 (n = 55) in DOC, 166 ± 3 (n = 53) in 1K-1C, and 162 ± 2 (n = 47) mm Hg in 2K-1C rats.

sANF infusion and the pretreatment level measured in each rat. The decreases in MAP produced by each dose of sANF in the DOC, 1K-1C, and 2K-1C rats were compared with the response to the same treatment in the normotensive animals (see Figure 1). In the normotensive rats, the maximal hypotensive response of −20 ± 4 mm Hg was achieved during administration of 95 pmol/minute of sANF, higher doses had no additional effect on pressure. Even though the pretreatment pressures of 1K-1C and 2K-1C rats were significantly greater than that of the NT rats (p < 0.05; Table 1), the depressor responses to sANF obtained in both renovascular hypertensive models were not significantly different from those measured in the normotensive animals (see Figure 1). In contrast, sANF (≥190 pmol/min) was more effective in DOC hypertensive rats than in normotensive animals.

The attendant changes in heart rate were generally small and not apparently related to the sANF dose (see Table 1). The sANF produced bradycardia in DOC and 2K-1C rats during infusions of 760 and 12 pmol/minute respectively, 95 pmol/minute of sANF increased heart rate in normotensive rats.

Sodium excretion rose in 1K-1C rats much as it had during administration of the same doses in normotensive animals (Figure 2). In the 2K-1C rats, however, the natriuretic responses to infusions of 12, 25, 95, and 190 pmol/minute of sANF were significantly greater than those elicited by the same doses in the normotensive rats (p < 0.05). Interestingly, in both the normotensive and the hypertensive rats, the doses of sANF required to produce the natriuretic responses were clearly different from those needed for the maximal depressor responses. In the normotensive rats, the
Table 1. Peak Responses to 20-Minute Infusions of Synthetic Atrial Natriuretic Factor into Conscious Rats

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment levels</th>
<th>Synthetic atrial natriuretic factor dose (pmol/kg, i.v)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
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<tr>
<td>Δ Heart rate (beats/min)</td>
<td>NT (n = 54)</td>
<td>446 ± 6</td>
</tr>
<tr>
<td></td>
<td>DOC (n = 55)</td>
<td>419 ± 6†</td>
</tr>
<tr>
<td></td>
<td>1K-1C (n = 53)</td>
<td>463 ± 5</td>
</tr>
<tr>
<td></td>
<td>2K-1C (n = 47)</td>
<td>461 ± 6</td>
</tr>
<tr>
<td>Δ Urine volume (µL/min)</td>
<td>NT (n = 54)</td>
<td>30 ± 2</td>
</tr>
<tr>
<td></td>
<td>DOC (n = 56)</td>
<td>24 ± 2</td>
</tr>
<tr>
<td></td>
<td>1K-1C (n = 53)</td>
<td>28 ± 2</td>
</tr>
<tr>
<td></td>
<td>2K-1C (n = 47)</td>
<td>43 ± 3†</td>
</tr>
<tr>
<td>Δ Chloride excretion (µEq/min)</td>
<td>NT (n = 54)</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>DOC (n = 56)</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1K-1C (n = 53)</td>
<td>2.4 ± 0.2</td>
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<tr>
<td></td>
<td>2K-1C (n = 47)</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Δ Potassium excretion (µEq/min)</td>
<td>NT (n = 54)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>DOC (n = 53)</td>
<td>0.5 ± 0.1†</td>
</tr>
<tr>
<td></td>
<td>1K-1C (n = 53)</td>
<td>1.8 ± 0.1</td>
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<tr>
<td></td>
<td>2K-1C (n = 47)</td>
<td>2.1 ± 0.1†</td>
</tr>
<tr>
<td>Δ cGMP excretion (pmol/min)</td>
<td>NT (n = 32)</td>
<td>2006 ± 876</td>
</tr>
<tr>
<td></td>
<td>DOC (n = 34)</td>
<td>31 ± 8†</td>
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<tr>
<td></td>
<td>1K-1C (n = 43)</td>
<td>829 ± 128</td>
</tr>
<tr>
<td></td>
<td>2K-1C (n = 39)</td>
<td>704 ± 185</td>
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</tbody>
</table>

Values are means ± SEM.

NT = normotensive rats (body weight, 283 ± 4 g), DOC = deoxycorticosterone rats (265 ± 9 g), 1K-1C = one-kidney, one-clip rats (260 ± 5 g), 2K-1C = two-kidney, one-clip rats (281 ± 7 g), cGMP = guanosine cyclic 3',5'-monophosphate.

*p < 0.05 compared with dose = 0.

†p < 0.05 compared with the response of normotensive rats to the same dose.

The greatest increase in sodium excretion was produced by 760 pmol/minute (see Figure 2), an infusion rate that far exceeded the first fully active hypotensive dose (95 pmol/min). In contrast, the most effective natriuretic doses of sANF in DOC, 1K-1C, and 2K-1C rats (50, 95, and 190 pmol/min respectively) were lower than the treatment required to maximally reduce MAP in the same models (760, 380, and 380 pmol/min).

During the lower infusions of sANF in all rats, sodium excretion rose throughout the 20-minute treatment (Figure 3). At the higher infusions, the maximal increase in sodium excretion occurred during the first 10-minute interval then declined during the last 10 minutes of sANF administration.

The diuretic and chloruretic dose-response curves paralleled the corresponding change in sodium excretion (see Table 1) in all animals. Because the slight kaliuresis stimulated by sANF was less consistent, no clear dose-response relationships were described for either the normotensive or the hypertensive rats.

Urinary cGMP excretion was low in the normotensive and the DOC hypertensive animals and did not increase significantly at any of the sANF doses at which urine was available for analysis (see Table 1). In the renal hypertensive rats, control cGMP excretion was higher and there was a general tendency for the cGMP responses to rise with increasing doses of sANF.

Discussion

In 1981 de Bold et al.1 initiated a new area of research with the report that a natriuretic factor present in atrial extracts increased sodium excretion and lowered MAP. Several laboratories subsequently have verified the natriuretic activity in conscious rats,16 in isolated perfused rat kidneys,17 18 in anesthetized rats,16 19-21 rabbits,22 and monkeys.23 In addition, ANF was shown to lower MAP in anesthetized rats,1 16 21 to decrease vascular resistance in isolated perfused rat kidneys,17 18 24 25 and to relax isolated vascular smooth muscle preparations precontracted with a variety of spasmodgens.2-4 24 25 Finally, ANF was found to elevate cGMP levels in the urine and plasma of anesthetized rats and in renal minces and primary cultures of tubular cells.5
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To evaluate the biological activity of a single peptide, a 26 amino acid peptide was synthesized. The natriuretic and vasorelaxant activities of the sANF were verified, and the compound was studied in conscious spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). In the latter experiment, lower doses of sANF elevated sodium excretion equally in both normotensive and hypertensive animals but produced a greater hypotensive effect in SHR. Additional data gathered during infusion of sANF through one renal artery of anesthetized dogs demonstrated that MAP declined in a dose-related manner while sodium excretion rose only in the treated kidney. Finally, the increase in plasma and urinary cGMP levels in those dogs appeared to be dissociated from the natriuretic, but not the hypotensive, responses to sANF.

The present study extended our investigations into the effects of sANF on blood pressure and electrolyte and cGMP excretion to include conscious normotensive rats and several additional models of hypertension. The experimental design allowed examination of the responses to sANF infusions without the changes in basal blood pressure and renal function induced by anesthesia. By infusing the peptide, the effects of sANF could be determined at nearly steady state conditions.

In conscious normotensive and hypertensive animals, the lower doses of sANF progressively diminished MAP throughout the entire 20-minute infusion period. At those low levels of treatment, the small, gradual falls in MAP were accompanied by a modest natriuresis that continued to develop until sANF was withdrawn. During infusions of higher doses of sANF, MAP declined rapidly during the first 10 minutes of treatment. The concurrent increase in sodium excretion appeared to depend on the magnitude of that initial depressor response — that is, the greatest natriuretic responses to sANF were achieved at doses that reduced MAP by no more than 15 mm Hg during the initial 10 minutes of infusion. At sANF doses that lowered MAP by more than 15 mm Hg within the first 10 minutes (190-1520 pmol/min in DOC rats, 380-1520 pmol/min in 1K-1C and 2K-1C rats, and 1520 pmol/min in normotensive animals), the natriuretic responses were diminished.
Theoretically, the reduction in natriuretic activity during administration of higher doses of sANF may result from the development of tachyphylaxis. As the depressor responses were sustained throughout each sANF infusion, however, receptor tachyphylaxis appears improbable unless separate renal and vascular ANF receptors exist. A simpler explanation is that substantial reductions in MAP induced by the higher doses of sANF compromised renal perfusion pressure to such an extent that the natriuretic response was attenuated.

The latter suggestion would also account for the differences in the most effective natriuretic and hypotensive doses found within each model. In all hypertensive animals, the greatest natriuresis stimulated by sANF was observed at doses lower than those required to produce the best depressor effect. At sANF doses larger than those necessary to elicit the peak natriuretic responses in DOC, 1K-1C, and 2K-1C rats, there were increases in both the magnitude of the depressor response and the initial rate at which MAP fell. In the normotensive rats, infusions of 95 to 760 pmol/minute yielded similar hypotensive responses but increasing degrees of natriuresis. At 1520 pmol/minute, the natriuretic response in the normotensive rats waned, and for the first time, MAP fell more than 15 mm Hg during the first 10 minutes of treatment. These observations are consistent with the suggestion that the magnitude of the natriuretic response was dependent not only on the size of the hypotensive response but also on the rate at which it developed.

An interesting observation that cannot be explained by the present data was the enhanced natriuresis elicited by sANF in 2K-1C rats. The peak increase in sodium excretion in 2K-1C animals exceeded that measured in normotensive, DOC, and 1K-1C rats. In the
present study and the maximal response reported in SHR. In addition, DOC rats appeared to be more susceptible to the hypotensive effects of sANF than did the normotensive or other hypertensive animals, including the SHR. Interestingly, sANF produced a significant bradycardia at the most hypotensive dose in DOC rats and during a nonhypotensive infusion in 2K-1C animals. In only one case (95 pmol/min in normotensive rats) did sANF increase heart rate. These results are similar to the effects of sANF found in conscious SHR and in anesthetized dogs. Although the lack of reflex tachycardia, or a frank bradycardia, during hypotensive cGMP in the biological responses to ANF.

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To conclude, the hypotensive and natriuretic effects of sANF were demonstrated in normotensive and in DOC, 1K-1C and 2K-1C hypertensive rats. The maximal depressor response was observed in the DOC rats, while natriuresis was greatest in 2K-1C animals. These results provide evidence of the antihypertensive effects of sANF.

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