A Comparison of Synthetic Rat and Human Atrial Natriuretic Factor in Conscious Dogs

ANDREA A. SEYMOUR, SHALER G. SMITH, ELAINE K. MAZACK
AND EDWARD H. BLAINE

SUMMARY The renal and hypotensive responses to intravenous infusions of 10, 50, 100, and 200 pmol/kg/min of synthetic rat atrial natriuretic factor (Arg₁₀¹-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile₁₁⁰-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly₁₁⁰-Cys-Asn-Ser-Phe-Arg-Tyr; disulfide bond between cysteines) were compared with those produced by synthetic human atrial natriuretic factor (Met₁₁⁰) in five conscious dogs. Increasing doses of rat or human atrial natriuretic factor lowered mean arterial pressure in a dose-related manner. At 200 pmol/kg/min, the maximally effective dose for both peptides, mean arterial pressure was reduced from 116 ± 4 to 96 ± 5 mm Hg and from 117 ± 5 to 100 ± 3 mm Hg (p < 0.01), respectively. Neither peptide affected heart rate. Fractional sodium excretion increased from 0.69 ± 0.22 to 3.95 ± 1.23% and from 0.69 ± 0.16 to 4.62 ± 0.72% during infusions of 200 pmol/kg/min of rat and human atrial natriuretic factor, respectively. Urine volume and fractional chloride excretion rose during infusions of rat or human atrial natriuretic factor in a manner that resembled the elevation in sodium excretion. The stimulation of fractional potassium excretion by both rat and human peptides was more variable and not as clearly dose-dependent. Glomerular filtration rate was enhanced by both rat and human atrial natriuretic factor, while neither peptide significantly changed renal plasma flow. Arterial plasma renin activity fell from 1.4 ± 0.4 to 0.6 ± 0.2 ng/ml/hr during administration of 200 pmol/kg/min of rat atrial natriuretic factor and from 0.8 ± 0.4 to 0.2 ± 0.2 ng/ml/hr during 100 pmol/kg/min of human atrial natriuretic factor. In conclusion, synthetic rat and human atrial natriuretic factor (101-126) peptides appear to elicit comparable degrees of hypotension, saluresis, diuresis, and renin inhibition in conscious dogs. (Hypertension 8: 211-216, 1986)

KEY WORDS • natriuresis • diuresis • mean arterial pressure • heart rate • glomerular filtration rate • renal plasma flow • plasma renin activity

THE saluretic, diuretic, and hypotensive actions of atrial natriuretic factor (ANF) were first demonstrated by injection of homologous atrial extract into anesthetized rats. Later, extracts of atria from several vertebrates were shown to elicit the biological activities attributed to ANF, even when tested in a nonhomologous species. Recently, several related peptides were isolated from rat atria, and their amino acid sequences were determined. A synthetic peptide corresponding to a 26 amino acid sequence (101-126) located near the C-terminal of the rat ANF precursor (Arg₁₀¹-Arg-Ser-Ser-Cys-Phe-Gly-Gly-Arg-Ile₁₁⁰-Asp-Arg-Ile-Gly-Ala-Gln-Ser-Gly-Leu-Gly₁₁⁰-Cys-Asn-Ser-Phe-Arg-Tyr; disulfide bond between cysteines) was synthesized and shown to possess the natriuretic and vasorelaxant properties of the ANF derived from endogenous sources. Furthermore, this synthetic rat ANF increased sodium excretion and lowered mean arterial pressure in conscious rats and anesthetized dogs. Human ANF (α-human atrial natriuretic peptide), which was isolated as a 28 residue peptide, differed from the sequence of the rat peptide synthesized in our laboratory by the addition of Ser-Leu at the N-terminal and the substitution of methionine for isoleucine at position 110. The synthetic α-human ANF produced natriuretic and hypotensive responses in anesthetized rats and dogs and in conscious dogs.

Elucidation of the complementary DNA sequence that encodes for the precursor ANF proteins found in rats and humans has revealed that these synthetic peptides represent a highly conserved segment located near the C-terminal. Although the reported activities of the rat and human ANF appear to be similar, to our knowledge no formal comparison of peptides of equal lengths has been made using a single experimental protocol. In addition, none of the earlier studies...
determined full dose-response relationships. Since the natriuretic profile often assumes a bell shape, use of a single dose may not offer the best opportunity for accurate comparisons among peptides. Therefore, to systematically compare our rat ANF (101-126) with a synthetic human peptide of the same length, increasing doses of each compound were infused into conscious dogs prepared for renal and cardiovascular measurements.

**Materials and Methods**

Catheters were implanted under sterile conditions in an iliac artery and vein of each of five mongrel dogs anesthetized with a halothane (1%), nitrous oxide (2 L/min), oxygen (1 L/min) mixture. The animals were allowed to recuperate from the surgical procedures for approximately 1 week. During that time, each dog was trained to stand quietly in a nylon mesh support sling (Alice King Chatham, Los Angeles, CA, USA).

On the day of the experiment, a sterile Foley catheter was inserted into the urinary bladder for timed urine collections. Priming doses of creatinine, 50 mg/kg, and para-aminohippurate (PAH), 8 mg/kg, were administered intravenously followed by a constant (1 ml/min) intravenous infusion of creatinine, 1 mg/kg/min, and PAH, 0.3 mg/kg/min. A Micron pressure transducer (Micron Instruments, Los Angeles, CA, USA) was connected to the iliac artery catheter. The pulsatile pressure signal triggered a cardiotachometer for the determination of heart rate and was electronically averaged for measurement of mean arterial pressure (MAP).

After an equilibration period of at least 45 minutes, the urinary bladder was rinsed with 20 ml of sterile distilled water and two 20-minute control clearance samples were obtained. During the next four periods, either 0.9% saline (vehicle) or doses of 10, 50, 100, and 200 pmol/kg/min of synthetic rat ANF or synthetic human ANF were infused intravenously. During the final 40 minutes of the experimental protocol, two recovery periods were observed. Each dog received the vehicle as the first test in the series, then the two ANF peptides were administered in random order. At least 1 day of recovery was allowed between experiments.

Urine was collected in graduated tubes throughout each 20-minute period. Blood samples were drawn at the midpoint of each sampling interval, and the plasma was separated by centrifugation. Urinary and plasma creatinine, PAH, and electrolyte concentrations were ascertained using standard automated procedures (Technicon Instruments, Tarrytown, NY, USA). The renal clearances of creatinine and PAH were calculated as estimates of glomerular filtration rate (GFR) and effective renal plasma flow, respectively. Fractional excretion of sodium, potassium, and chloride was calculated using the standard methods.

Arterial blood samples were obtained during the final minute of each period and expressed into chilled test tubes containing sodium ethylenediaminetetraacetic acid. The plasma was separated by centrifuga-

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Significant changes from a single control value (the average of the two 20-minute samples obtained before treatment) were detected using Dunnett’s t test. Differences among the responses to equivalent doses of rat and human ANF and saline treatments were identified by analysis of variance and application of Newman-Keuls test for instances in which the populations were normally distributed or the Kruskal-Wallis procedure for samples in which normality could not be demonstrated.

The rat ANF was synthesized by the Medicinal Chemistry Department of Merck Sharp & Dohme Research Laboratories (West Point, PA, USA). The human ANF peptide was purchased from Bachem (Torrance, CA, USA). Purity (≥ 95%) of each peptide was verified by Dr. Ruth Nutt and associates. Each compound was dissolved and diluted in sterile 0.9% saline. Both peptides remained intact at room temperature for longer than 24 hours (J. P. Draper, R. G. Bergstrom, personal communication, 1985).

**Results**

Infusion of the saline vehicle had no significant effect on blood pressure, heart rate, PRA, or renal function in the five conscious dogs used in this study (Table 1). Both rat and human ANF significantly reduced MAP in the same dose-related manner (Figure 1). During administration of 100 and 200 pmol/kg/min, blood pressure was significantly less than that measured in saline-treated animals and was less than the pretreatment values in each group. The hypotensive effect of each peptide persisted throughout the 40-minute recovery period when compared with baseline levels.

The natriuretic responses to synthetic rat and human ANF peptides were identical in these conscious dogs. Sodium excretion rose in a dose-related manner from a baseline of 53 ± 20 to a peak of 351 ± 93 μEq/min during infusion of 200 pmol/kg/min of the rat ANF and from 48 ± 14 to 389 ± 71 μEq/min during treatment with the same dose of human ANF. The GFR increased significantly in response to both rat and human ANF (Table 2). Although the rise in GFR improved sodium delivery into the tubules, that increase probably did not account for the full natriuretic effect since fractional sodium excretion was also significantly elevated above pretreatment values by infusions of 50, 100, and 200 pmol/kg/min (see Figure 1). During administration of 100 and 200 pmol/kg/min of either rat or human ANF, fractional sodium excretion was also significantly different from that measured in saline-infused dogs, which indicates that these changes did not represent spontaneous fluctuations.

Fractional chloride excretion followed a similar response pattern, increasing from 1.1 ± 0.3% to a maximum of 5.4 ± 1.6% during treatment with 200 pmol/kg/min of rat ANF and from 1.0 ± 0.3 to 6.6 ± 1.3% during human ANF infusion. Rat and human
TABLE 1. Effects of Saline Infusion in Five Conscious Dogs

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>ERPF (ml/min)</th>
<th>GFR (ml/min)</th>
<th>UV (ml/min)</th>
<th>FE_{Na} (%)</th>
<th>FE_{K} (%)</th>
<th>PRA (ng/ml/hr)</th>
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<tbody>
<tr>
<td>20</td>
<td>116±6</td>
<td>89±8</td>
<td>175±26</td>
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<td>8.1±2.7</td>
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<td>176±20</td>
<td>54±5</td>
<td>0.41±0.10</td>
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<td>183±28</td>
<td>57±5</td>
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<td>0.7±0.3</td>
<td>6.8±1.6</td>
<td>1.1±0.3</td>
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<td>117±5</td>
<td>86±6</td>
<td>182±21</td>
<td>54±5</td>
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<td>85±6</td>
<td>197±37</td>
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<td>0.9±0.3</td>
<td>8.5±2.5</td>
<td>0.9±0.3</td>
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</table>

Values are means ± SEM. MAP = mean arterial pressure; HR = heart rate; ERPF = effective renal plasma flow; GFR = glomerular filtration rate; UV = urine volume; FE_{Na} = fractional sodium excretion; FE_{K} = fractional potassium excretion; PRA = plasma renin activity.

ANF also stimulated equivalent levels of diuresis (see Table 2). Fractional potassium excretion increased progressively in response to doses of 10, 50, and 100 pmol/kg/min of human ANF but reached a maximum during infusion of 50 pmol/kg/min of the rat ANF (see Table 2). Since these responses were highly variable, there were no significant differences between the two groups. However, fractional potassium excretion during infusions of 50 pmol/kg/min of rat and human ANF and 100 pmol/kg/min of human ANF was significantly different from that measured in the saline-infused dogs.

Although MAP fell by almost 20 mm Hg in both experiments, no reflex tachycardia occurred (see Table 2). In addition, effective renal plasma flow did not change significantly during infusion of either peptide, although it did tend to decline once the ANF treatments were ended.

Both rat and human ANF peptides significantly reduced arterial PRA; the three lowest doses of the rat and human ANF peptides produced parallel decreases in PRA (see Figure 1). The suppression of renin levels was reversed when the ANF treatment was terminated.

Discussion

Atrial natriuretic factor peptides were isolated from both rat-11 and human atria15 and, by comparing the nucleotide sequences of the larger precursor proteins, were found to represent a highly conserved region of the C-terminal of the precursor.21 A portion of this homologous site, which corresponds to the Arg^{101}-Tyr^{126} fragment of rat ANF precursor, contains an isoleucine residue at position 110 of the rat sequence and methionine in the analogous human ANF.

This single amino acid substitution appeared to have no effect on the biological activities measured in conscious dogs in the present study. The natriuretic responses were statistically indistinguishable and appeared to begin to level off between doses of 100 and 200 pmol/kg/min. The diuretic, chloruretic, and kaliuretic responses to rat ANF also were similar to those produced by the human peptide in the conscious dogs. As observed in the earlier studies using the same synthetic rat ANF in anesthetized dogs14 and in conscious rats,16 the increases in urine volume and chloride excretion closely followed the natriuretic response while the rises in potassium excretion were less dramatic and much more variable.

Effective renal plasma flow did not change significantly in response to either rat or human ANF, although small or transient responses may not have been detected using the PAH clearance technique. Both
peptides enhanced GFR and, therefore, increased the filtered sodium load. However, since fractional sodium excretion (urinary sodium excretion/filtered sodium) was significantly elevated by both peptides, the rise in GFR was not sufficient to account for the full natriuretic response, unless glomerulotubular balance was disturbed so that the increased sodium load was not entirely reabsorbed. Conceivably, the peptides could have induced natriuresis by changing intrarenal hemodynamics, by affecting peritubular physical factors, or by directly inhibiting sodium reabsorption. Since the current study did not directly address these issues, we were unable to select from among these possibilities a precise mechanism for the ANF-induced natriuresis. The present data confirmed earlier observations made in anesthetized\(^{14}\) and conscious\(^{13}\) dogs that anesthesia, which has many recognized effects on cardiovascular and renal function, can also influence the natriuretic response to ANF.

The rat and human ANF peptides elicited virtually identical hypotensive responses in the conscious dog. The blood pressures measured during infusion of 200 pmol/kg/min (96 ± 5 and 100 ± 3 mm Hg with rat and human ANF, respectively) were slightly higher than the MAP of 87 ± 5 mm Hg measured during intra-renal administration of 156 pmol/kg/min in anesthetized dogs.\(^{14}\) Once again, the greater effect of the synthetic rat ANF in anesthetized animals may relate to a difference in the amount of peptide degradation or to the anesthesia. Interestingly, in conscious normotensive rats, the optimal hypotensive dose of 95 pmol/min of synthetic rat ANF reduced blood pressure by 20 mm Hg to 97 ± 3 mm Hg. Despite the species difference there was an excellent agreement between the hypotensive floor achieved in rats and dogs. Therefore, apparently both rat and human ANF produced hypotensive responses in conscious dogs that were similar to those obtained with rat ANF in anesthetized dogs\(^{14}\) and conscious rats.\(^{13}\)

As in our previous study of anesthetized dogs,\(^{14}\) blood pressure did not return to baseline as quickly as did renal function (see Figure 1). The reason for that difference cannot be ascertained in the present study and may include such diverse possibilities as a more rapid renal degradation,\(^{27}\) different sensitivities of renal and vascular tissue to metabolites of the 26 amino

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Dose (pmol/kg/min)</th>
<th>HR (beats/min)</th>
<th>ERPF (ml/min)</th>
<th>GFR (ml/min)</th>
<th>UV (ml/min)</th>
<th>FEK (%)</th>
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<tr>
<td>20</td>
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<td>rANF 0</td>
<td>97±13</td>
<td>170±27</td>
<td>57±8</td>
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</table>

Values are means ± SEM. rANF = rat synthetic atrial natriuretic factor; hANF = human atrial natriuretic factor. See Table 1 for key to other abbreviations.

*p < 0.05, compared with baseline value at 20 minutes; †p < 0.01, compared with baseline value at 20 minutes.
acid peptide, or secondary effects on the hormonal or neural systems that modulate blood pressure.

Finally, rat ANF significantly suppressed arterial PRA in a dose-related manner in the conscious dogs. In two of the five animals, PRA was at the lower limit of the assay sensitivity (0.2 ng/ml/hr) during treatment with 200 pmol/kg/min of the rat ANF. In two of the five dogs treated with human ANF, baseline renin values were less than 0.2 ng/ml/hr so that no additional reductions were evident in those animals. Nevertheless, PRA fell in the other three dogs and reached the lowest detectable level during infusions of 100 and 200 pmol/kg/min in all but one experiment. Despite these limitations, a significant attenuation of PRA was revealed. Arterial PRA returned to control levels within 20 minutes after infusion of either rat or human ANF ended, which indicates that the inhibition was fully reversible. These data agree with an earlier report that a single dose of the synthetic rat ANF (101–126) reduced renin secretion. In addition, the present study found that both rat and human ANF decreased circulating renin levels in a dose-related fashion.

As noted in an earlier study, PRA fell despite a decrease in MAP, a condition that usually stimulates renin release. The present data are consistent with either a direct action of ANF on the juxtaglomerular apparatus to reduce renin secretion or inhibition of release through the macula densa mechanism. The latter possibility would require an enhancement of the sodium load to the macula densa, presumably by increasing the filtered sodium load or reducing sodium reabsorption, or both, at some site proximal to the thick ascending loop of Henle. From the present studies, in which fractional sodium excretion was elevated by both rat and human ANF, it would appear that the enhanced distal sodium delivery resulting from the rise in GFR is supplemented by some other, as yet undefined pathway. These observations suggest some interesting areas for further investigations of the mechanism by which ANF lowers renin activity.

Our findings indicate that responses to rat and human ANF are indistinguishable in a third species. Unfortunately, the sequence of dog ANF has not been published, so it is not possible to ascertain the degree of homology that canine ANF shares with either the rat or human peptide. Regardless, the hypotensive responses of the dogs to rat and human synthetic ANF were remarkably similar to that produced by the rat sequence in conscious rats. It therefore appears that the canine vascular or renal receptors, or both, are equally sensitive to peptides with either isoleucine or methionine at position 110. Whether these responses are the same as those produced by endogenous ANF in the dog remains to be determined.

To summarize, two 26 amino acid peptides corresponding to the active portion of the precursors of rat and human ANF produced equivalent saluresis and diuresis in conscious dogs. These responses were dose-related and approached their peaks at doses of 100 to 200 pmol/kg/min. Significant increases in GFR may have contributed to the enhancement in electrolyte excretion but were not sufficient to account for the full saluretic response. The dose-dependent reductions in MAP produced by rat and human ANF were nearly identical in the conscious dogs and were not associated with any reflex tachycardia. Finally, rat and human ANF peptides elicited parallel decreases in arterial PRA. In conclusion, analogous human and rat ANF peptides reversibly stimulated electrolyte excretion and lowered MAP and PRA with equal effectiveness in conscious dogs.

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