Infusion of atrial natriuretic hormone in DOCA/salt and spontaneously hypertensive rats

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Abstract. The effects of a 6-day infusion of atrial natriuretic hormone (ANH) on blood pressure and urinary sodium excretion were determined in conscious DOCA/salt and spontaneously hypertensive rats. The DOCA/salt rats were randomly divided into two groups after 4 weeks and either infused by osmotic minipump with 32.5 pmol/h of ANH in 0.1% gelatin vehicle or sham operated with emplacement of a blind cannula. Thirteen-week-old spontaneously hypertensive rats were studied in a similar fashion. The baseline systolic blood pressure prior to the infusion was 176 ± 7 mmHg (x ± SEM) in the ANH group and 169 ± 5 mmHg in the sham group of DOCA/salt animals. The ANH infusion in the DOCA/salt animals dropped their blood pressure to 160 ± 10 mmHg (p < 0.01) compared to that in the sham controls which continued to rise to 200 ± 7 mmHg. The blood pressure response to ANH infusion in the spontaneously hypertensive rats was slightly greater, with a blood pressure of 192 ± 5 mmHg in the sham group and 132 ± 3 mmHg in the ANH-infused animals. ANH infusion produces a qualitatively similar blood pressure response in the DOCA/salt rat as well as the other hypertensive models. This response is relatively less on a quantitative basis than that observed in the spontaneously hypertensive rats and is not related to changes in sodium balance or volume contraction.

The cardiac hormone atrial natriuretic hormone (ANH) appears to be one of several hormones which regulates blood pressure and fluid homeostasis (1,2). Chronic ANH infusion by osmotic minipumps has reduced blood pressure in several models of hypertension in rats. Blood pressure was significantly decreased by chronic ANH infusion at 32.47 pmol/h in spontaneously hypertensive rats (SHR) (3), one-kidney, one-clip hypertensive rats (1-K, 1-C rats) (4), and two-kidney, one-clip hypertensive rats (2-K, 1-C rats) (5). In DOCA/salt hypertensive rats (DOCA rats), however, ANH infused at 32.5 pmol/h did not reduce blood pressure (6) although it did lower the blood pressure in 2-K, 1C hypertensive rats in a parallel experiment. Much larger dosages of ANH have apparently been utilized to produce such an effect (7,8). Two possible mechanisms might be operative in producing this decreased responsiveness to exogenous ANH in DOCA rats. ANH vascular receptors have been shown to be down-regulated by ANH (9,10). Since endogenous plasma ANH concentrations are increased in DOCA rats, the density of ANH vascular receptors was likely decreased and thereby contributed to the observed poor responsiveness to exogenous ANH (6,11,12). Secondly, as the plasma ANH concentrations at the end of the experiment in the DOCA rats with ANH infusion at 32.47 pmol/h were not higher than those in DOCA rats with a sham-infusion (6), there is a strong possibility that the amount of ANH actually infused was significantly lower than expected in this model as well as in the parallel experiments.

In a preliminary study, we observed that 0.1% gelatin in 0.9% NaCl was a superior vehicle for ANH and led to an increased recovery of peptide during the infusion when compared to the 0.9% NaCl solution alone. We have therefore used the 0.1% gelatin solution as the vehicle to re-examine the effect of a 6-day ANH infusion on blood press-
ure and urinary sodium excretion in DOCA rats. Additional balance studies were performed on SHR rats which served as a hypertensive control group.

Materials and Methods

Male Wistar and 13-week-old SHR rats (Charles River Laboratories, Wilmington, MA) were maintained at a constant temperature of 24°C and 60% relative humidity on a normal 12-h light-dark cycle. Rats had free access to a regular laboratory chow (Wayne Roden Blox, Wayne Laboratories, Chicago, IL) containing 0.39% Na and 0.96% K by weight and tap water ad libitum. The Wistar rats, weighing 225 to 250 g were subjected to unilateral left nephrectomy under light ether anesthesia. Following nephrectomy, 14 days were allowed for compensatory renal hypertrophy to occur before DOCA treatment was begun. A 50 mg DOCA tablet (Yoshitomi Pharmaceutical Industries, Ltd, Osaka, Japan) was implanted ip at the beginning of the experiment and again after three weeks. A 1% NaCl solution was given as drinking water ad libitum from the beginning of the experiment. The rats were randomly divided into two groups at four weeks for either ANH infusion or sham surgery as controls. The SHR were obtained and used at 13 weeks when their blood pressure was elevated.

Synthetic ANH (Ser 99-Tyr 126, Sigma Chemical Corporation, St, Louis, MO) was infused by means of an osmotic minipump (Model 2ML2, Alza, Palo Alto, CA). Preliminary studies were performed to compare a 0.9% NaCl solution to the same solution with 0.1% gelatin (type 1, Sigma) as a vehicle for ANH infusion by osmotic minipumps. [125]I-ANH (Amersham, Inc, Arlington Heights, IL) was purified by high-pressure liquid chromatography just before the experiments. The amount of [125]I-ANH infused into a test tube for a 24-h period was determined using an identical osmotic minipump which was immersed in physiological saline. The effect of incubation at 37°C on ANH degradation in an osmotic minipump was also determined using the [125]I-ANH. At the end of each incubation (7 and 14 days) a 100-μl sample was aspirated by syringe and applied to a C18 μ Bondapak column (0.39 × 30 cm) (Waters, Milford, MA). The radioactive material was eluted over 45 min using a linear gradient of 10–80% acetonitrile containing 0.1% trifluoroacetic acid. The flow rate was 1 ml/min and 1-ml fractions were collected into tubes. These studies indicated significant binding and degradation of [125]I-ANH to the minipumps using 0.9% NaCl as a vehicle compared to the 0.1% gelatin. Therefore, we chose to use the 0.1% gelatin for our subsequent experiments.

Following light ether anesthesia, osmotic minipumps were implanted in the neck of Wistar rats (N = 10). The osmotic minipumps were filled with synthetic ANH dissolved in the 0.1% gelatin diluent at concentrations calculated to release 32.5 pmol/h of the peptide. The pumps were connected to the left jugular vein by a polyethylene catheter (PE-60, Clay Adams, Parsippany, NJ). A second group of DOCA rats (N = 10) were also anesthetized and the left jugular vein was cannulated with a blind PE-60 catheter to serve as controls.

The blood pressure was determined weekly (0–4 weeks) before ANH infusion, and at 3 and 6 days during the peptide infusion. Indirect systolic blood pressure was measured by means of the tail cuff method (Narco Biosystems, Houston, TX, connected with a polygraph (Model 7C, Grass, Quincy, MA)) in conscious, prewarmed (37°C for 10 min) rats. Body weight and water intake, urinary volume (U) and urinary sodium (U Na) excretion for 24 h were determined at 4 weeks, (day 0) and at day 6 of the ANH infusion. At the end of the experiment the rats were sacrificed by decapitation and the truncal blood was collected for measurement of plasma ANH, plasma renin activity (PRA), and hematocrit. Two additional studies were performed as controls. SHR rats were similarly catheterized and infused with either ANH 32.5 pmol/h or given a blind catheter during a sham operation. Six rats were used in each group. Daily balance studies were performed on these rats beginning on the day after implantation. At the end of the experiment, blood samples were obtained from the abdominal aorta under pentobarbital anesthesia. A third control group of SHR (N = 3) rats were implanted with an osmotic minipump and infused with the 0.1% gelatin vehicle as above for 6 days.

Blood samples were immediately separated and stored at −70°C until analyzed. Plasma ANH was measured by a modification of the radioimmunoassay by Shenker et al. (13) using 0.5 ml of plasma. The intra-assay coefficient of variance (CV) was 10.6% (X = 26.6 pmol/l, N = 10) and the inter-assay CV was 17.9% (X = 31.8 pmol/l, N = 57). The sensitivity was consistently 2.6 fmol/tube or better. PRA was also determined by radioimmunoassay (14). Urinary sodium concentrations were measured by automatic analyzer (E2A analyzer, Beckman, CA).

The results were expressed as mean ± SEM. Statistical evaluation was performed on an IBM Model 3081 computer using the statistical analysis system (SAS) package. Single comparisons were performed using the unpaired Student’s t-test. One-way analysis of variance and Duncan’s multiple range test were used for multiple comparisons.

The animal studies were approved by the appropriate institutional committee and conform to National Institutes of Health and Veterans Administration Medical Center guidelines.

Results

A preliminary study showed that 97% of [125]I-ANH was recovered following 24 h of incubation at
Reverse phase high-pressure liquid chromatography pattern of the $^{125}$I-ANH (Ser 99-Tyr 126) after incubation in an osmotic minipump at 37°C. The recorder gain was turned up $\times 2$ for the 0.1% gelatin solution and $\times 4$ for the 0.9% NaCl for day 14 to better show the intact ANH and its metabolites.

37°C in an osmotic minipump when 0.1% gelatin was used as the vehicle. However, 50% was obtained when 0.9% NaCl alone was used as the vehicle. The effect of incubation in an osmotic minipump at 37°C on ANH degradation is shown in Fig. 1. A significant deterioration of the labelled material occurred at 7 days and especially following 14 days when the 0.9% NaCl solution was used as the vehicle. In contrast, there was no significant deterioration of the label when the 0.1% gelatin vehicle was used. The elution pattern of the labelled ANH in the 0.1% gelatin solution at 14 days was identical to that of the nonlabelled reference ANH (Ser 99-Tyr 126). Moreover, the material in the peak fractions was demonstrated to bind in high concentrations with the ANH antibody. On the basis of these findings, the peak fractions in the 0.1% gelatin were considered to show the iodinated intact ANH.

The mean blood pressure gradually increased in both groups of DOCA rats in the pre-infusion period (data not shown). There was no significant difference in blood pressure between the two groups through this 4-week period. The blood pressure in the DOCA + ANH rats decreased within 3 days after the initiation of ANH infusion and remained significantly lower than that in DOCA rats for the remainder of the infusion period (Fig. 2).

There was no significant difference in body weight, water intake, $U_{\text{V}}$ or $U_{\text{Na}}$ excretion between the two DOCA groups before and on day 6 of the

Fig. 2.

Effect of chronic ANH infusion on blood pressure in DOCA salt hypertensive (DOCA) and spontaneously hypertensive (SHR) rats. Mean ± SEM. •• DOCA rats (N = 10), ○○○ DOCA + ANH rats (N = 10), ▲▲ ▲▲ SHR (N = 6), △△△△ SHR + ANH (N = 6). * $p < 0.05$, ** $p < 0.01$ compared to DOCA or SHR rats.
ANH infusion (Table 1). There also was no significant difference in hematocrit between the two groups (Table 1). As expected, PRA values were low in both groups. Basal plasma ANH levels were 63 ± 18 pmol/l in the DOCA animals. The guillotine decapitation sectioned the functioning catheter in 7 of 10 of the ANH-infused animals leading to artifactually and variably elevated plasma levels so these plasma ANH concentrations are not included.

Similar data were obtained in SHR rats whose basal blood pressure was within 10 mmHg of the DOCA animals. The ANH-infused animals had a significant decrease in blood pressure at 3 and 6 days after the infusion (Fig. 2). The mean decrement of blood pressure at 6 days in the ANH-infused SHR was 60 mmHg, while that for the DOCA animals was 40 mmHg. Daily balance studies were performed beginning the day after minipump implantation. There were no significant differences in body weight, water intake, urinary volume, and urinary sodium excretion from days 2 through 6 (Table 2). Basal plasma ANH concentrations obtained via the aorta were 31.5 ± 3 pmol/l in the basal stated and rose to 55 ± 6.5 pmol/l at the end of 6 days of ANH infusion. The mean plasma concentration during the ANH infusion in the SHR was not significantly different from the basal ANH concentrations in the DOCA hypertensive rats. In a separate experiment, 6 SHR rats had either a blind catheter implanted (N = 3) or were infused with 0.1% gelatin by an osmotic minipump for 6 days (N = 3). There was no effect of the 0.1% gelatin vehicle or placement of the osmotic minipump upon tail blood pressure in the infused animals compared to the sham operated group (Table 3).

Table 1.
Effects of chronic ANH infusion (6 days) on body weight (BW), water intake (WI), urine volume (U_{v}), urinary sodium excretion (U_{Na}), plasma ANH, hematocrit, and plasma renin activity (PRA) in DOCA (N = 10) and DOCA + ANH (N = 10) rats. Mean ± SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before ANH infusion</th>
<th>6 days after ANH infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DOCA rats</td>
<td>DOCA+ANH rats</td>
</tr>
<tr>
<td>BW, g</td>
<td>391 ± 10</td>
<td>380 ± 16</td>
</tr>
<tr>
<td>WI, ml/24 h</td>
<td>110 ± 21</td>
<td>90 ± 25</td>
</tr>
<tr>
<td>U_{v}, ml/24 h</td>
<td>90 ± 20</td>
<td>80 ± 21</td>
</tr>
<tr>
<td>U_{Na}, mmol/24 h</td>
<td>20.5 ± 4.1</td>
<td>19.5 ± 4.0</td>
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<tr>
<td>Plasma ANH, pmol/l</td>
<td>63 ± 18</td>
<td></td>
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<tr>
<td>Hematocrit, %</td>
<td>44.7 ± 2.0</td>
<td>42.9 ± 2.5</td>
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<tr>
<td>PRA, ng·l^{-1}·sec^{-1}</td>
<td>&lt; 0.3</td>
<td>&lt; 0.3</td>
</tr>
</tbody>
</table>

Table 2.
Effects of chronic ANH infusion (6 days) on body weight (BW), water intake (WI), urine volume (U_{v}), and urinary sodium excretion (U_{Na}) in SHR (N = 6) and SHR + ANH (N = 6) rats. Mean ± SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Days of ANH infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>BW, g</td>
<td>SHR</td>
<td>246 ± 3</td>
</tr>
<tr>
<td></td>
<td>SHR + ANH</td>
<td>249 ± 3</td>
</tr>
<tr>
<td>WI, ml/24 h</td>
<td>SHR</td>
<td>31 ± 2</td>
</tr>
<tr>
<td></td>
<td>SHR + ANH</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>U_{v}, ml/24 h</td>
<td>SHR</td>
<td>15 ± 2</td>
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<tr>
<td></td>
<td>SHR + ANH</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>U_{Na}, mEq/24 h</td>
<td>SHR</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>SHR + ANH</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>
Discussion

The present study demonstrates that blood pressure in DOCA/salt rats is significantly decreased by a chronic ANH infusion of 32.5 pmol/h similar to that used to test blood pressure responsiveness of other animal models. Although technical factors prevented measurement of plasma ANH concentrations after ANH infusion in the DOCA/salt rats, a similarly performed experiment in SHR demonstrated a significant increment over baseline that persisted for at least 6 days using this infusion system. Such an effect has not been observed in studies not protecting against adsorption or degradation of the peptide. The present study suggests that the elevated plasma ANH concentration induced by chronic infusion exerted its hypotensive action in DOCA rats. The maximal decrement in mean blood pressure after chronic ANH infusion of 40 mmHg in our study was only slightly less than that reported in other similarly infused animal models (SHR \( \Delta = -44 \) mmHg (3), 1-K, 1-C rats \( \Delta = -48 \) mmHg (4), 2-K, 1-C rats \( \Delta = -48 \) mmHg (5)). It is of interest that Seymour et al. (15) produced a blood pressure drop of approximately 45 mmHg in their DOCA/salt rats with an acute ANH infusion of 380 pmol/min for 20 min which was comparable to or greater than the response observed in their 1K–1C and 2K–1C animals with the same infusion rate. Our study would deliver on a chronic basis a calculated maximal infusion rate of 0.54 pmol/min, yet the decrement of blood pressure was in the range observed by Seymour et al. In a second experiment of the present study, the comparable chronic ANH infusion rate caused a \( \Delta = -60 \) mmHg in equally hypertensive SHR rats. This slightly lower responsiveness in the DOCA rats may well be due to receptor down-regulation secondary to elevated endogenous ANH production when compared to the other animal models (6,9,10). Alternative explanations would include the difference between the levels of other vasoactive hormones and of sodium intake between the different animal models of hypertension.

Since no apparent differences were found in either urinary sodium excretion, urine volume, body weight or hematocrit between ANH-infused and non-infused DOCA rats, it may be assumed that the hypotensive response induced by chronic ANH infusion is not due to a circulatory volume contraction. This study did not incorporate a daily balance in the DOCA animals, and might have missed an early natriuresis. A detailed balance, however, was performed in the SHR rats and no difference was observed between the ANH-infused rats and their non-infused controls. In a separate experiment, neither the gelatin vehicle nor the presence of the osmotic minipump was shown to have a hypotensive effect.

A reduction in blood pressure induced by acute ANH infusion has been reported to be accompanied with a decrease in cardiac output in DOCA/salt rats (16,17) and in SHR (18). The reduction in cardiac output in rats was principally due to a fall in stroke volume (16–18), and might be accounted for by venodilation, increased resistance to venous return, a direct negative inotropic effect of ANH, and/or a reduction in blood volume (17–20). The lack of any natriuresis, loss of body weight, increased water intake, or change in hematocrit during the 24-h collections up to day 6 would suggest that diuresis is not the likely explanation. A full balance study with simultaneous measurements of plasma and extracellular volumes would be necessary to answer this question.

In summary, a six-day infusion of ANH significantly decreased blood pressure in DOCA/salt fed rats at infusion levels which produced plasma concentrations of ANH 75% greater than baseline.

### Table 3.

**Effect of chronic 0.1% gelatin vehicle infusion on blood pressure (BP) in SHR rats.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Days of ANH infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>BP mmHg</td>
<td>SHR (N = 3)</td>
<td>170 ± 11</td>
</tr>
<tr>
<td></td>
<td>SHR + gelatin (N = 3)</td>
<td>165 ± 10</td>
</tr>
</tbody>
</table>
There was a significant loss of the ANH when 0.9% NaCl was used as vehicle but not in the presence of gelatin. This observation may explain the decreased effectiveness of ANH on BP in some previously reported studies. This infusion system also produced a greater hypotensive effect in the SHR and lends credence to this possibility. Since the hypotensive response in our DOCA/salt rats still did not reach the magnitude of that found in the SHR, it would appear that the DOCA/salt model is less responsive to ANH infusion.

Acknowledgments

Dr Andrew Cucchiara of the University of Oklahoma Health Sciences Research and Education Computing Services provided helpful consultations.

We thank Sally Berg and Gek Choo Ngan for excellent technical assistance.

This work was supported in part by a Paul and Doris Travis International Visiting Professorship and by grants from the VA Medical Center and the Presbyterian Foundation.

References


Received April 4th, 1989.
Accepted June 30th, 1989.

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