Effect of Atrial Natriuretic Factor on Blood Pressure, Renin, and Aldosterone in Goldblatt Hypertension

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SUMMARY We previously provided evidence that atrial natriuretic factor (ANF) antagonizes angiotensin II-induced vascular contractility and angiotensin II-stimulated aldosterone production by isolated adrenal cells. To examine the importance of these effects in vivo, synthetic ANF (auriculin A) was administered intravenously (2 μg/kg bolus followed by 0.3 μg/kg/min constant infusion) to conscious, unrestrained two-kidney, one-clip and one-kidney, one-clip rats on normal sodium intake and their sham-operated controls. The one-kidney, one-clip rats also were studied on a sodium-deficient diet. Mean blood pressure, plasma renin activity, and plasma aldosterone levels were measured before and after 60-minute infusion. In saralasin-responsive two-kidney, one-clip rats (n = 10), ANF administration reduced blood pressure (from 187 ± 11 [SE] to 153 ± 11 mm Hg; p < 0.001) and plasma aldosterone levels (from 182 ± 61 to 125 ± 60 ng/dl; p < 0.05), while plasma renin activity increased (from 59 ± 16 to 82 ± 20 ng/ml/hr; p < 0.05). Lesser changes in blood pressure occurred in saralasin-nonresponsive two-kidney, one-clip rats (149 ± 10 to 143 ± 8 mm Hg; n = 5), sodium-replete one-kidney, one-clip rats (183 ± 9 to 170 ± 11 mm Hg; n = 9), two-kidney sham-operated rats (122 ± 3 to 115 ± 4 mm Hg; n = 8), and one-kidney sham-operated rats (117 ± 3 to 112 ± 3 mm Hg; n = 7). Control plasma renin and aldosterone levels were not elevated in these latter groups and did not change significantly with ANF administration. In sodium-depleted one-kidney, one-clip rats, which became saralasin responsive, ANF administration significantly reduced blood pressure (from 184 ± 11 to 156 ± 12 mm Hg; n = 8), plasma aldosterone levels (from 286 ± 41 to 179 ± 36 ng/dl), and plasma renin activity (from 69 ± 19 to 44 ± 13 ng/ml/hr). These data indicate that ANF has potent antihypertensive and aldosterone-suppressing effects in vivo, irrespective of induced changes in plasma renin activity. Both the vascular and adrenal effects of ANF appear to be enhanced when the activity of the renin-angiotensin system is increased.

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KEY WORDS • angiotensin • auriculin • saralasin • rats • renovascular hypertension

MAMMALIAN atrial myocytes contain potent natriuretic polypeptides that appear to be associated with secretory granules. These structurally related forms of atrial natriuretic factor (ANF) have been isolated from rat or human atria. These peptides, which have also been termed cardionatrins, atriopeptins, and auriculins, are derived from a common precursor. Although the precise sequence of the peptide(s) normally produced in vivo has yet to be determined, the smaller peptides ranging between 23 and 33 amino acids in length appear to be similar in potency and have a similar spectrum of biological activities.

Atrial natriuretic factor has marked renal hemodynamic actions that account, at least in part, for its natriuretic and diuretic effects. In addition, ANF antagonizes vascular smooth muscle contraction induced by a variety of vasoactive agents. This latter effect is especially pronounced with angiotensin II-induced contraction of the isolated rabbit aorta.
Several other observations also suggest potentially important interactions of ANF with the renin-angiotensin system. It has been shown that ANF inhibits both basal and agonist-induced aldosterone production by isolated adrenal zona glomerulosa cells. In normal dogs, synthetic ANF reduces blood pressure, renin secretion rate, plasma renin activity (PRA), and plasma aldosterone (PA) levels. In addition, preliminary results suggest an especially pronounced reduction of blood pressure and aldosterone levels in rats with renin-dependent hypertension.

The present study investigated the hemodynamic and hormonal responses to synthetic ANF in two forms of experimental renovascular hypertension in the rat, which provide in vivo models characterized by suppression or stimulation of the renin-angiotensin system. This experimental approach was undertaken to investigate whether, in the intact animal, ANF induces more profound reduction of blood pressure and aldosterone levels when the activity of the renin-angiotensin-aldosterone system is enhanced.

Methods

The synthetic ANF used throughout the study was the 24 amino acid residue peptide auriculain A (molecular weight, 2546), which was produced by solid phase synthesis, as previously described.

Renal hypertension was produced in male Wistar rats weighing between 75 and 125 g. Groups of up to 40 rats were prepared at the same time. Two-kidney, one-clip hypertension was induced by placing a silver clip (0.22 mm inside diameter) across the left renal artery, exposed while the rat was under anesthesia, with the right kidney left untouched. Sham-operated rats served as controls. One-kidney, one-clip hypertension was produced by applying the same surgical procedure to rats that had undergone right nephrectomy 1 week earlier. One-kidney sham-operated rats also were prepared. Following operation, rats were maintained on normal sodium Purina rat chow (0.35% sodium;Ralston Purina Co., Richmond, IN) and were allowed water ad libitum. Blood pressure was monitored continuously through a 60-minute recovery period. Mean blood pressure was monitored continuously throughout the experiment, and heart rate was determined at the end of each experimental phase.

In addition to the experimental groups already described, a group of sodium-depleted one-kidney, one-clip animals was prepared. After hypertension was established, these rats, selected at random, were put on a sodium deficient diet (ICN, Cleveland, OH) for 1 week before instrumentation.

The PRA and PA levels were determined as previously described. Urinary sodium concentration was measured by direct potentiometry (Nova 1, Nova Biomedical, Newton, MA). Mean blood pressure measurements were analyzed at 5-minute intervals throughout the experiment; steady state responses were taken as the average of the last eight determinations in each experimental period.

Statistical analysis was performed by using the BMDP statistical software. Significance of blood pressure responses to ANF among the various groups was evaluated by analysis of variance for repeated measurements. Simultaneous multiple comparisons between pairs of groups were made by the modified t test; the Bonferroni method was used to adjust the significance level. Where no repeated measurements were involved, comparisons within the same group were made using a paired Student’s t test. All values are presented as means ± SEM.

Results

Table 1 shows the mean values for body weight and urinary sodium excretion rate in the different groups of animals on the day before the experiment. No significant difference in these parameters was observed among the groups, except that sodium excretion rate was, as expected, significantly reduced in the sodium-depleted animals (p < 0.05).
TABLE 1  Body Weight and Baseline Sodium Excretion Rate in Renovascular Hypertensive and Sham-Operated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Weight (g)</th>
<th>( U_{Na}V ) (( \mu )Eq/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2K-1C Saralasin responsive</td>
<td>10</td>
<td>342±13</td>
<td>1 47±0 3</td>
</tr>
<tr>
<td>2K-1C Saralasin nonresponsive</td>
<td>5</td>
<td>376±28</td>
<td>1 60±0 4</td>
</tr>
<tr>
<td>1K-1C Sodium-replete</td>
<td>9</td>
<td>344±16</td>
<td>1 23±0 2</td>
</tr>
<tr>
<td>1K-1C Sodium-depleted</td>
<td>8</td>
<td>322±6</td>
<td>0 13±0 08</td>
</tr>
<tr>
<td>2K Sham operated</td>
<td>8</td>
<td>428±29</td>
<td>0 98±0 3</td>
</tr>
<tr>
<td>1K Sham operated</td>
<td>7</td>
<td>417±22</td>
<td>0 98±0 2</td>
</tr>
</tbody>
</table>

Values are means ± SEM

Figure 1 shows the time course of the blood pressure response to synthetic ANF in the two-kidney and one-kidney, one-clip rats and in their respective controls. Analysis of variance for all the groups revealed highly significant changes in blood pressure during the time course of the experiment (\( F = 18.41; p < 0.001 \)). A significant fall in blood pressure was observed within 10 minutes in the two-kidney, one-clip rats (\( p < 0.01 \)), and within 20 minutes in the one-kidney, one-clip group (\( p < 0.05 \)) and was sustained throughout the infusion. A small but significant decrease in blood pressure was detectable in the one-kidney sham-operated group after 20 minutes and was sustained throughout the infusion (\( p < 0.05 \)). In the two-kidney sham-operated animals, blood pressure tended to fall during ANF infusion, but the decrease did not reach statistical significance.

The degree of blood pressure reduction by ANF was most pronounced in the 10 renin-dependent two-kidney, one-clip rats. As shown in Table 2, the percent change in blood pressure from baseline was greatest in this group at each time point during the ANF infusion.

Figure 2 shows the effect of sodium deprivation on the blood pressure response to ANF in the one-kidney, one-clip hypertensive model. Control blood pressure was not significantly affected by sodium depletion.

TABLE 2  Percent Changes in Mean Blood Pressure During Atrial Natriuretic Factor Infusion in Hypertensive and Sham-Operated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>2K-1C</td>
<td>10</td>
<td>2±0</td>
<td>-13±2 2</td>
<td>-18±2 5</td>
<td>-21±3 3</td>
<td>-19±2 8</td>
<td>-20±3 4</td>
</tr>
<tr>
<td>1K-1C Sodium-replete</td>
<td>10</td>
<td>-2±0 6*</td>
<td>-4±1 0*</td>
<td>-6±1 8*</td>
<td>-7±2 0*</td>
<td>-9±2 1*</td>
<td>-6±2 7*</td>
</tr>
<tr>
<td>2K Sham operated</td>
<td>10</td>
<td>0±2 4</td>
<td>-3±2 0</td>
<td>-4±2 5</td>
<td>4±1 7</td>
<td>-6±2 4</td>
<td>-6±2 8</td>
</tr>
<tr>
<td>1K Sham operated</td>
<td>10</td>
<td>3±0 7</td>
<td>-3±0 8</td>
<td>-4±1 6</td>
<td>-6±1 6</td>
<td>-6±1 9</td>
<td>-5±1 7</td>
</tr>
<tr>
<td>1K-1C Sodium-depleted</td>
<td>10</td>
<td>9±1 7</td>
<td>-12±2 4</td>
<td>-17±3 5</td>
<td>-17±2 4</td>
<td>-16±2 3</td>
<td>-17±2 3</td>
</tr>
</tbody>
</table>

Each value represents the mean percent change from baseline (± SEM) in blood pressure at the indicated times of ANF infusion and following a 60-minute recovery period.

\( p \) values were adjusted for multiple simultaneous comparisons of all pairs of means.

There was no significant difference between the sodium-replete one-kidney, one-clip, two-kidney sham-operated, and one-kidney sham-operated groups at any time point.

\( K = \) kidney, \( C = \) clip, \( ANF = \) atrial natriuretic factor

\*\( p < 0.05 \), \( \ddot{p} < 0.001 \) compared with the two-kidney, one-clip group

\( \dddot{p} < 0.05 \), \( \dddot{p} < 0.001 \) compared with the sodium-replete one-kidney, one-clip group
converted the one-kidney, one-clip animals to a renin- 
dependent form of hypertension, led to a significantly 
greater ANF-induced fall in mean blood pressure at 
each time point during the ANF infusion (see Table 2).

Figure 3, which compares the steady state changes 
in blood pressure induced by ANF and saralasin, 
shows that the decrease in blood pressure induced by 
ANF was greatest in the two saralasin-responsive 
groups of animals: -34 ± 5 mm Hg in the two-
kidney, one-clip or -28.5 ± 3 mm Hg in the sodium-
depleted one-kidney, one-clip rats (versus -13.5 ± 4 
mm Hg in the sodium-replete one-kidney, one-clip, 
*p < 0.05, and versus -6.6 ± 3 and -5.9 ± 1 mm 
Hg in the two-kidney and one-kidney sham-operated 
groups respectively, *p < 0.001). Only mild hyperten-
sion developed in 5 of the 15 two-kidney, one-clip 
animals prepared (control mean blood pressure, 149 ± 
10 mm Hg) and was not saralasin responsive (not 
shown in Figure 1). In these five animals ANF admin-
istration reduced blood pressure by only 6.6 ± 3 mm 
Hg, far less than the reduction observed in the 10 
renin-dependent two-kidney, one-clip animals (*p < 
0.001).

No significant change in heart rate was induced by 
ANF in any of the groups (two-kidney, one-clip: 411 
± 17 to 413 ± 15 beats/min, n = 10; one-kidney, 
one-clip: 408 ± 19 to 416 ± 8, n = 9; sodium-
depleted one-kidney, one-clip: 454 ± 12 to 456 ± 
17, n = 8; two-kidney sham-operated: 403 ± 18 to 388 ± 
18, n = 8; one-kidney sham-operated: 423 ± 17 to 
401 ± 14, n = 7).

The individual responses of blood pressure, PRA, 
and PA to ANF in the saralasin-responsive two-kid-

![Figure 3](http://hyper.ahajournals.org/)

**FIGURE 3** Steady state blood pressure responses to synthetic 
ANF and saralasin administration in renovascular hypertensive 
and sham-operated rats. *Sar+* = saralasin responsive, 
*Sar−* = saralasin nonresponsive (see Methods for definitions) 
Multiple comparisons of the saralasin-induced changes in mean 
blood pressure showed a significantly greater response in the 
high-renin two-kidney, one-clip (2K-1C) and the sodium-depleted 
one-kidney, one-clip (1K-IC) rats compared with the re-
maining groups (*p < 0.001; Bonferroni test). No significant 
differences were observed in the saralasin response among the 
one-kidney, one-clip, normal renin two-kidney, one-clip, 
one-kidney sham-operated (1K-sham), or two-kidney sham-operat-
ed (2K-sham) rats.

![Figure 4](http://hyper.ahajournals.org/)

**FIGURE 4** Individual blood pressure and plasma renin and 
aldosterone responses to synthetic ANF in saralasin-responsive 
two-kidney, one-clip hypertensive rats. *C* = control, *E* = ex-
perimental, *R* = recovery periods.

Discussion

These results provide evidence that synthetic ANF 
reduces blood pressure in vivo, which indicates that 
the blood pressure lowering effect previously observed 
with crude atrial extracts is due specifically to ANF. 
Not surprisingly, blood pressure fell to a greater extent 
in hypertensive than in normotensive animals. Our 
study clearly shows, however, that, despite similar 
degrees of hypertension, ANF induced greater falls in 
blood pressure in saralasin-responsive animals with 
higher baseline values of renin and aldosterone.

![BLOOD PRESSURE COUNCIL SUPPL HYPERTENSION, Vol 7, No 3, MAY-JUNE 1985](http://hyper.ahajournals.org/)
The greater responsiveness of renin-dependent animals may depend on the dose of ANF used. In earlier unpublished experiments we observed that the blood pressure lowering effect of large doses of crude atrial extract was similar in two-kidney, one-clip and one-kidney, one-clip rats. In additional preliminary experiments we have found that a threefold increase in the dose of synthetic ANF (i.e., 6 μg/kg bolus followed by 0.9 μg/kg/min infusion) markedly enhances the antihypertensive effect in one-kidney, one-clip rats and appears to abolish the differences in blood pressure response observed herein between renin-dependent and non-renin-dependent hypertensive rats.

This increased blood pressure sensitivity to low-dose ANF infusion in renin-dependent hypertensive rats may be directly related to the increased circulating levels of angiotensin II that presumably exist in these animals. Thus, although ANF has generic actions on vascular smooth muscle in vitro, it is possible that angiotensin II-induced increases in vascular resistance may be more susceptible to antagonism by ANF in vivo. At higher doses, ANF might exhibit less specificity by antagonizing a variety of vasoconstrictor mechanisms. This hypothesis would be consistent with our earlier observations in isolated aortic rings. Other possible explanations cannot, however, be excluded. For instance, ANF might induce greater blood pressure reduction in volume-contracted animals, and both two-kidney, one-clip and sodium depleted one-kidney, one-clip rats probably represent relatively volume-contracted models, especially compared with sodium-replete one-kidney, one-clip animals. An enhanced blood pressure response in the volume-depleted state might reflect a nonspecific response to vasodilatation, or alternatively, it might reflect the lower endogenous circulating levels of ANF that would hypothetically exist in such a state. Further work is needed to distinguish among these and other possibilities.

The fall in blood pressure, even when marked, was not associated with the expected reflex increase in heart rate. This observation may be consistent with the hypothesis that ANF has vagal stimulating activity. Our study is not conclusive in this respect, however, since a transient increase in heart rate during the first minutes of the infusion may have been missed. Moreover, a direct effect of ANF on baroreceptors cannot be ruled out.

Another important finding of our study is the marked aldosterone-suppressing effect of ANF observed in the renin-dependent hypertensive rats. The PA levels tended to fall in the other groups of rats as well, although the change was not statistically significant. The fact that PA levels fell irrespective of whether PRA increased or decreased suggests a direct adrenal effect of ANF. Although it is possible that divergent responses of PA and PRA (as occurred in the two-kidney, one-clip animals) could result from inhibition of angiotensin-converting enzyme, this seems to be an unlikely explanation as we have found that ANF, at concentrations up to 10^-6M (2.5 μg/ml), does not inhibit converting enzyme activity in vitro (unpublished data, 1984). Thus, the response of aldosterone to ANF in the present study is in keeping with previous reports showing that aldosterone production is antagonized by ANF in vitro. In bovine adrenal cells, auriculin has been shown to inhibit aldosterone production in the basal state and following stimulation by angiotensin II, dibutylryl cyclic adenosine monophosphate, and potassium. Each agonist is able to partially overcome the inhibitory effect. In isolated rat adrenal cells, however, unlike other agonists, angiotensin II is unable to overcome the inhibitory effect of auriculin on aldosterone production (G Aguilera, personal communication, 1984).

Our finding that PRA generally increased in two-kidney, one-clip hypertensive rats after 1 hour of constant ANF infusion contrasts with our previous finding that ANF decreased PRA and renin secretion rate in normal dogs. Suppression of renin release by ANF might result from an increased load of sodium chloride to the macula densa. If so, then the failure of ANF to decrease PRA in two-kidney, one-clip hypertensive

**Figure 5. Effects of ANF on blood pressure, plasma renin activity, and plasma aldosterone levels in the different experimental groups.** Shaded bars represent the mean values obtained in the control period, and solid bars the values obtained at the end of the ANF infusion. In the saralasin nonresponsive two-kidney, one-clip (2K-IC) rats (not shown), baseline plasma renin activity was 2.4 ± 1 ng/ml/hour and did not change significantly after ANF administration. * = p < 0.05; 1K-1C = one-kidney, one-clip rats; 1K-sham = one-kidney sham-operated rats, 2K-sham = two-kidney sham-operated rats.
rats, at least acutely, might be due to an impaired ability of the peptide to increase distal sodium chloride delivery promptly in the ischemic, rennin-secreting kidney. In the acute response of the ischemic kidney, therefore, other factors, such as a reduction in renal perfusion pressure or, hypothetically, direct effects on the juxtaglomerular cells, might predominate, which would result in net stimulation of rennin release. If this hypothesis is correct, then the consistent suppression of PRA in the sodium-depleted one-kidney, one-clip animals may be difficult to explain. It is quite likely, however, that when the macula densa mechanism is markedly stimulated by sodium depletion, even a small increase in distal sodium supply may cause marked inhibition of rennin release.

In conclusion, the results of the present study indicate that ANF reduces blood pressure in vivo and that its hypotensive effect appears to be enhanced in the presence of high circulating levels of angiotensin II. Similarly, the ability of ANF to reduce PA levels in vivo appears to be especially pronounced when angiotensin II and basal aldosterone secretion are elevated. These studies reveal major interactions with the rennin-angiotensin-aldosterone system in vivo that are of potential importance in the presumed physiological actions of ANF.

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References


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