Variable Renal Atrial Natriuretic Factor Gene Expression in Hypertension

Tsuneo Ogawa, Wolfgang Linz, Bernward A. Schölkens, Adolfo J. de Bold

Abstract—We have previously established the existence of atrial natriuretic factor (ANF) gene expression within the renal parenchyma. Neither the role nor the regulation of this extracardiac source of ANF is clearly defined. To determine whether renal ANF gene expression, similar to cardiac expression, is linked to the activity of the renin-angiotensin system (RAS), we compared renal ANF gene expression in rats after suprarenal aortic banding, a hypertension model associated with activation of RAS, and in the deoxycorticosterone acetate (DOCA)–salt model, which is characterized by depression of RAS. Renal ANF mRNA was measured with a quantitative competitive reverse transcription polymerase chain reaction method. DOCA-salt hypertension significantly reduced the expression of renal ANF. In contrast, aortic banding significantly increased renal ANF expression. In both cases, ANF gene expression in the heart increased. Ramipril treatment at 10 μg/kg of aortic-banded rats, a treatment that specifically affects local RAS but maintains hypertension, normalized renal ANF mRNA levels. Altogether, these results suggest that renal ANF gene expression is regulated by local RAS and is independent of circulating RAS and hypertension per se. The marked decrease of renal ANF mRNA in DOCA-salt hypertension suggests a pathogenic role for renal ANF gene downregulation by decreasing the sodium excretory mechanism mediated by the local expression of ANF acting on receptors found in the inner medullary collecting ducts. In aortic banding, renal ANF gene expression upregulation suggests a local compensatory function consistent with the consensus role of natriuretic peptides in the modulation of RAS, thus ameliorating the sodium-retaining effects of renal underperfusion. (Hypertension. 1999;33:1342-1347.)

Key Words: atrial natriuretic factor ■ renin-angiotensin system ■ kidney ■ deoxycorticosterone ■ hypertension, renal

The polypeptide hormone atrial natriuretic factor (ANF) plays a significant role in modulating blood volume and blood pressure through cGMP-mediated actions on several target organs.1 ANF is mainly of atrial origin, but it is also synthesized in other tissues, including the renal parenchyma.2 Recently, we succeeded in reliably measuring ANF mRNA levels in kidney using a quantitative competitive reverse transcription polymerase chain reaction (QC-RT-PCR) and found that 1 week of deoxycorticosterone acetate (DOCA)–salt administration to rats, a treatment known to increase atrial ANF gene expression without modifying blood pressure,3 resulted in a significant decrease in renal ANF gene expression.2 Neither the role nor the regulation of this extracardiac source of ANF is known.

Because the renin-angiotensin system (RAS) and ANF often play counterregulatory roles, we took advantage of the known inhibiting effect of the DOCA-salt treatment on RAS on the one hand and the upregulation of RAS after aortic banding on the other to substantiate the hypothesis that renal ANF gene expression may be influenced by RAS status. Furthermore, the aortic-banded rats were treated with either a low- or a high-dose schedule of the angiotensin-converting enzyme (ACE) inhibitor ramipril. High-dose treatment with ramipril leads to inhibition of both the local and circulating RAS, resulting in normalization of blood pressure, although low-dose treatment inhibits local RAS with persisting hypertension.4 This approach thus allows definition of the differential contributions of high blood pressure and RAS to ANF gene expression. In the present studies we show that renal ANF gene expression is downregulated by DOCA-salt. Conversely, hypertension induced by aortic banding leads to upregulation of renal ANF gene expression. In the latter model, renal ANF mRNA levels were normalized by the ACE inhibitor independently of hypertension. Together, these findings suggest that renal ANF gene expression is RAS dependent but independent of hypertension. They further suggest that the decrease of renal ANF mRNA in DOCA-salt hypertension could play a pathogenic role because it represses a potential sodium excretory mechanism mediated by the local expression of ANF acting on renal receptors. In aortic banding, renal ANF gene expression upregulation suggests a local compensatory function consistent with the consensus role of natriuretic peptides in the modulation of RAS, leading to amelioration of the sodium-retaining effects of renal underperfusion induced by aortic banding.
Methods

DOCA-Salt Experiment

Male Sprague-Dawley rats weighing 100 to 125 g were divided into (1) control, (2) DOCA, (3) salt, and (4) DOCA-salt groups. Treatment of animals was conducted following institutional guidelines. The rats in the DOCA and the DOCA-salt groups were injected subcutaneously with a suspension of DOCA (30 mg/kg; Sigma Chemical Co) dissolved in sesame oil once a week. The rats were killed by decapitation. Trunk blood was collected in chilled tubes containing EDTA and immediately centrifuged at 4°C. After centrifugation, the plasma was stored at −80°C until it was used for radioimmunoassay (RIA). After blood collection, the heart was excised, rapidly weighed, and dissected in cold saline into right and left atrium and right and left ventricle, with the septa as part of the left chambers. Similarly, both kidneys were rapidly removed and rinsed in cold saline. After they were weighed, the tissues were wrapped in aluminum foil and flash-frozen in liquid nitrogen.

Aortic Banding Experiment

The preparation of animals used in these experiments has been previously described. Briefly, adult male Sprague-Dawley rats weighing 270 to 280 g had the aorta constricted above the kidneys. Five groups of animals were used: (1) control, (2) sham operated, (3) aortic banded, (4) aortic banded treated with high-dose ramipril (1 mg/kg), and (5) aortic banded treated with low-dose ramipril (10 μg/kg). Ramipril was administered by daily oral gavage for 6 weeks to rats immediately after the aortic-banding operation. Ramipril dosage was adjusted weekly according to body weight. At the end of the treatment period, the animals were instrumented for measurement of carotid blood pressure because tail sphygmomanometry is not feasible because of the blood pressure drop distal to the aortic coarctation. Both methods of blood pressure measurement give comparable results.

Extraction of Plasma and Tissue Samples

Plasma samples were acidified by adding 100 μL/mL of 1 mol/L HCl and passed through Sep-Pak C_{18} cartridges (Millipore) that were prewetted with 5 mL of 80% acetonitrile in 0.1% trifluoroacetic acid (TFA) and 10 mL of 0.1% TFA. The cartridges with the absorbed peptides were washed with 20 mL of 0.1% TFA and then eluted with 3 mL of 60% acetonitrile in 0.1% TFA. Tissue samples were homogenized in 10 volumes of an extracting mixture consisting of 0.1N HCl, 1.0 mol/L acetic acid, and 1% NaCl and centrifuged at 10 000 g for 30 minutes at 4°C. The supernatants were then extracted with the use of Sep-Pak C_{18} cartridges as described above for plasma, except that elution was 80% acetonitrile in 0.1% TFA. The eluates were freeze-dried and processed for RIA as described below.

Assay Methods

Plasma renin activity (PRA) was measured by determining the level of angiotensin I (Ang I) generated during 1 hour of incubation at 37°C in the presence of 8-hydroxyquinoline. The Ang I concentration was measured by RIA kit (Du Pont). PRA values were expressed as nanograms Ang I synthesized per milliliter per hour. Plasma Ang I and plasma angiotensin II (Ang II) levels were determined with RIA kits (Advanced ChemTech). The Ang I antisera showed <0.01% cross-reactivity with Ang II peptide, and the Ang II antisera showed 1.7% cross-reactivity with Ang I peptide. The concentration of immunoreactive ANF in plasma and tissue samples was determined by RIA as previously described with the use of anti-rat ANF(99–126) Serum from Peninsula Laboratories. The ANF antisera showed <0.01% cross-reactivity with brain natriuretic peptide.

Total RNA Extraction and Northern Blot Analysis

Total RNA extraction and Northern blot analysis were performed as previously described with the following 32P-labeled probes: a 1.4-kb BamHI/HindIII fragment of the rat renin cDNA, a 1.6-kb EcoRI/HindIII fragment of the rat angiotensinogen cDNA, a 3.8-kb EcoRI fragment of the mouse ACE cDNA, a 0.9-kb EcoRI/HindIII fragment of the rat ANF cDNA, a 2-kb BamHI/BglII fragment of the mouse phosphoglycerate kinase cDNA, and a 5-kb SalI/EcoRI fragment of mouse 28S rRNA cDNA. Autoradiographs were scanned with the use of an Ultrascan XL laser densitometer (LKB Products) and LKB 2300 Gelscan XL software package. The scanning values of each mRNA were normalized to 28S rRNA or phosphoglycerate kinase mRNA as internal controls to correct for differences in the amount of RNA applied and transfer efficiency.

Quantitative Competitive Reverse Transcription Polymerase Chain Reaction

A detailed description of the QC-RT-PCR has been previously published. Briefly, RNA samples were reverse transcribed with Super Script II RNase H− Reverse Transcriptase and oligo(dT)_{12-18} primer with the use of a reverse transcription kit (GIBCO BRL). An aliquot of the cDNA product was used for PCR amplification with ANF primers. A dilution series of total RNA (5 μg) aliquots was prepared for each sample. Each dilution was spiked with competitor RNA. After the PCR, aliquots (5 μL) of the PCR product were electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. Photographs were taken with Polaroid 55 film, and the negatives were scanned with the use of an Ultrascan XL laser densitometer and Gelscan XL 2000 software package. The ratio of the density of the competitor RNA to the target RNA was plotted against the amount of the competitor RNA added to each reaction.

Statistical Analysis

All data were expressed as mean±SEM, and a level of P<0.05 was considered significant. ANOVA was performed to determine statistical differences among multiple groups. When significance was obtained by ANOVA, Fisher’s least squares differences post hoc analysis was used to determine pairwise differences.

Results

Systolic Blood Pressure, Body Weight, and Tissue Weight

Both DOCA-salt and aortic-banded rats had systolic blood pressure and kidney weight/body weight ratio significantly increased compared with their appropriate controls (Tables 1 and 2). The 2 models had similar systolic blood pressure differences between treated and control groups. High-dose ramipril normalized systolic blood pressure and kidney weight/body weight ratio in the banded rats. Low-dose ramipril induced similar changes except for blood pressure, which remained elevated.

Plasma RAS

DOCA-salt and salt treatments significantly decreased PRA (Figure 1). Plasma Ang I and Ang II levels followed a pattern similar to that of PRA. The changes of PRA and plasma Ang II levels in the aortic-banding experiments have been previously reported. These consisted of a slight, nonsignificant increase in PRA and a significant increase in Ang II plasma levels in the banded rats.

ANF Plasma Levels

In the DOCA-salt experiments, plasma ANF levels of the DOCA-treated rats (95±7 pg/mL), the salt-treated rats (87±6 pg/mL), and the DOCA-salt–treated rats (117±7 pg/mL)
were significantly higher than those of the control rats (67±5 pg/mL; P<0.01 for all groups versus control). We have previously reported that in the aortic-banding experiments, plasma ANF in the banded rats was significantly higher than those of the control and sham-operated rats. In the banded rats treated with high-dose ramipril, ANF was normalized, but in the banded rats treated with low-dose ramipril, plasma ANF remained higher than that of the control and sham-operated rats although lower than that of the banded rats and untreated animals.

### ANF Concentration and mRNA Levels in Cardiac Tissue

Left atrial ANF was partially depleted by DOCA-salt treatment even though ANF mRNA was significantly higher than those in other groups (Figure 2). Ventricular ANF and ANF mRNA levels in the DOCA-salt–treated rats were higher than those of the control groups. We previously reported that in the aortic-banding experiments, atrial ANF and ANF mRNA levels were similar among the groups. Ventricular ANF and ANF mRNA levels closely paralleled the changes in plasma ANF levels. Both hypertensive models are therefore accompanied by stimulation of cardiac ANF gene expression, although aortic banding is characterized by displaying a ventricular response only.

### Renal RAS

Figures 3 and 4 show Northern blot analysis of renal renin, angiotensinogen, and ACE. DOCA, salt, and DOCA-salt significantly lowered renal renin mRNA. In aortic-banded rats, renal renin mRNA levels were significantly lower than those of the control and sham-operated rats. High-dose ramipril treatment increased renin mRNA levels. Animals treated with low-dose ramipril had renal renin mRNA levels comparable to those of untreated, banded animals. Angiotensinogen mRNA levels and ACE mRNA levels were similar among all aortic-banded groups. ACE mRNA levels in rats treated with high-dose or low-dose ramipril were normalized with respect to control and sham-operated rats.

### Renal ANF Concentration

Renal ANF levels were similar among all groups of the DOCA-salt experiment and aortic-banding experiment (Figure 5).

### Renal ANF mRNA Levels

DOCA-salt treatment significantly depressed renal ANF mRNA levels compared with all other groups. Aortic banding, on the other hand, significantly increased renal ANF mRNA levels over those of the control and sham-operated groups (Figures 5 and 6). High-dose or low-dose ramipril treatment significantly lowered renal ANF mRNA levels in the banded rats to levels similar to those in the control and sham-operated rats.

### Discussion

The findings reported here demonstrate opposite changes in renal ANF gene expression in 2 models of hypertension that differ in the status of RAS. Thus, downregulation of renal ANF mRNA is a feature of DOCA-salt hypertension, whereas hypertension induced by aortic banding is accompanied by upregulation of renal ANF mRNA. These important differences in renal ANF gene expression contrast with cardiac ANF gene expression, which was found to increase in the heart of both hypertensive models in this and in a previous study.

The differences in renal ANF gene expression occur even though the animals in the 2 hypertension models had similar

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### Table 1. Hemodynamic Data and Tissue Weight in DOCA-Salt Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DOCA</th>
<th>Salt</th>
<th>DOCA-Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mm Hg</td>
<td>113±1</td>
<td>122±3</td>
<td>118±2</td>
<td>144±4†‡</td>
</tr>
<tr>
<td>BW, g</td>
<td>391±5</td>
<td>390±9</td>
<td>376±7</td>
<td>374±7</td>
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<tr>
<td>LW/BW, mg/g</td>
<td>1.60±0.05</td>
<td>1.80±0.05</td>
<td>1.70±0.03</td>
<td>2.00±0.03†‡</td>
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<tr>
<td>RW/BW, mg/g</td>
<td>0.35±0.02</td>
<td>0.38±0.02</td>
<td>0.42±0.02</td>
<td>0.46±0.02†</td>
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<tr>
<td>RKW/BW, mg/g</td>
<td>3.6±0.07</td>
<td>3.9±0.07</td>
<td>4.1±0.07</td>
<td>4.9±0.17†‡</td>
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</tbody>
</table>

Values are mean±SEM. BP indicates blood pressure; BW, body weight; VW, ventricular weight; KW, kidney weight; L, left; and R, right. n=8 to 10.

*P<0.01 vs control.
†P<0.05 vs DOCA.
‡P<0.01 vs salt.

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### Table 2. Hemodynamic Data and Tissue Weight in Aortic-Banded Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sham</th>
<th>Banded</th>
<th>Banded + Ramipril (1 mg/kg)</th>
<th>Banded + Ramipril (10 μg/kg)</th>
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<tr>
<td>Systolic BP, mm Hg</td>
<td>122±2</td>
<td>120±3</td>
<td>154±2†</td>
<td>124±7§</td>
<td>151±9</td>
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<tr>
<td>BW, g</td>
<td>449±6</td>
<td>455±7</td>
<td>415±4</td>
<td>411±5</td>
<td>445±5</td>
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<tr>
<td>RKW/BW, mg/g</td>
<td>3.2±0.06</td>
<td>3.0±0.08</td>
<td>3.7±0.25*</td>
<td>3.0±0.13‡</td>
<td>3.2±0.15‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Abbreviations are as defined in Table 1. n=7 to 10.

*†P<0.05, ‡P<0.01 vs control and sham operated.
‡§P<0.05, ‡P<0.01 vs aortic bandings.
||P<0.01 vs aortic banding plus ramipril (1 mg/kg).
systolic blood pressure differences between treated and control groups, resulting in a similar increase in the left ventricular weight/body weight and kidney weight/body weight ratios.

Renal ANF levels were not significantly increased in aortic-banded rats banded for 6 weeks, during which time ANF mRNA levels were also significantly increased. The significance of renal ANF changes is obscured by the fact that high-performance liquid chromatography analysis of ANF extracted from the renal tissue shows that most of the ANF is processed ANF$_{99-126}$ and not ANF$_{1-126}$, which is the expected storage form of ANF. Therefore, it is likely that the former represents mostly blood-borne or receptor-bound ANF.

The decreased values for PRA, plasma Ang I, and Ang II values found in the present study in the DOCA-salt experiments confirm the characteristic downregulation of circulating RAS caused by DOCA-salt treatment. Renal renin mRNA levels in the animals treated with DOCA alone, salt alone, or DOCA-salt were significantly decreased compared with the control rats. This finding is consistent with the expected effect of volume expansion and with the known role of sodium in the regulation of renal renin mRNA.

In aortic-banded rats, we have previously reported a slight increase in PRA and a significant increase in plasma Ang II levels. The latter may be expected to exert a negative feedback inhibition on renin release and a reduction of renal renin mRNA. Although we did not perform a complete evaluation of renal RAS both in terms of message and product activity or concentration, the significant decrease in plasma Ang II levels in the DOCA-salt rats and its increase previously reported in the banded rats may explain the opposite regulation of the renal ANF mRNA levels between these models given that Ang II stimulates ANF gene expression either in culture or in vivo independently of hypertrophy.

An insight into the relative influence of plasma and local RAS on the changes of renal ANF transcript levels found in the present study is provided by the fact that high-dose ramipril normalized renal ANF synthesis together with renal ACE mRNA levels (this study) and plasma ACE and decreased plasma Ang II. Low-dose ramipril also decreased renal ANF gene expression and normalized renal ACE mRNA levels, but, from previous studies, it is known not to decrease plasma ACE activity or plasma Ang II, suggesting that inhibition of local renal RAS is sufficient to prevent
The upregulation of renal ANF synthesis. It remains to be determined whether the effects of ACE inhibition described here are the direct result of interference with Ang II generation or some other process, including kinin formation.

The upregulation of renal ANF gene expression observed after aortic banding suggests a local compensatory role consistent with the consensus role of natriuretic peptides in the modulation of RAS. Thus, it may be hypothesized that intrinsic renal ANF ameliorates the sodium-retaining effects brought about by renal underperfusion. This function of ANF could also partly explain the development of sodium-sensitive hypertension observed in ANF knockout mice.

The decrease in renal ANF mRNA levels in the DOCA-salt–treated rats may contribute to the development of volume expansion and hypertension despite the increased cardiac and plasma ANF levels. DOCA-salt treatment increases proximal tubule neutral endopeptidase activity. This results in increased degradation of filtered ANF, as demonstrated by the fact that administration of neutral endopeptidase inhibitors increases the renal actions of ANF in volume-expanded states, including the DOCA-salt model. This significant decrease of renal ANF mRNA in DOCA-salt hypertension found in the present investigation suggests that this decrease, together with increased degradation of blood-borne ANF, could play a pathogenic role in the development of mineralocorticoid hypertension. Supporting this view is our previous finding that blockade of the natriuretic peptide receptor

impairs the ability of the kidneys to escape the salt-retaining effects of mineralocorticoid administration.

Acknowledgments

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Figure 4. Relative renal renin, angiotensinogen, and ACE mRNA levels in the DOCA-salt experiment and aortic-banding experiments; n = 4 to 5. Top, *P < 0.05 vs control rats; †P < 0.05 vs DOCA-treated rats in the DOCA-salt experiments. Bottom, *P < 0.05, **P < 0.01 vs control and sham-operated rats; ††P < 0.01 vs banded rats; §§P < 0.01 vs banded rats treated with high-dose ramipril (R) in the aortic-banding experiment.

Figure 5. Renal ANF and ANF mRNA levels in DOCA-salt and aortic-banding experiments; n = 3 to 5. Top, *P < 0.05 vs control rats; ‡P < 0.05 vs DOCA-treated rats; §P < 0.05 vs salt-treated rats in the DOCA-salt experiment. Bottom, *P < 0.05 vs control and sham-operated rats; ‡‡P < 0.05 vs banded rats in the aortic-banding experiment. R indicates ramipril.

Figure 6. Top, Ethidium bromide–stained QC-RT-PCR of kidney samples from a sham-operated and an aortic-banded rat. Lanes 1 to 6 are as follows: 5 μg of kidney RNA and the dilution series (10, 5, 2.5, 1.25, 0.63, 0.31 fg) of ANF competitor RNA were added for RT. Lane 7 is a 123-bp DNA ladder. Bottom, The top picture was taken with a negative film, and the density of each band was measured by densitometry. The ANF/ANF competitor ratio of each lane was plotted, thus allowing for the calculation of the quantity of ANF competitor RNA that must be added to achieve an ANF/ANF competitor ratio of 1, which represents the equimolarity between the amount of ANF mRNA and ANF competitor RNA.
Laboratory Medicine, Emory University, Atlanta, Ga) for providing the ACE cDNA. We thank Michelle Stevenson, Amalia Ponce, and Carole Frost for their excellent assistance.

References

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