Variable Renal Atrial Natriuretic Factor Gene Expression in Hypertension

Tsune 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 modulated 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. ANF is mainly of atrial origin, but it is also synthesized in other tissues, including the renal parenchyma. 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, resulted in a significant decrease in renal ANF gene expression. 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. 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 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 mg/kg). Ramipril was administered by daily oral gavage for 6 weeks during the experiment. Five weeks later, blood pressure was measured by tail sphygmomanometry (Narco Bio-Systems), and 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.

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 C18 cartridges (Millipore) that were pretreated 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 3 mL of 60% acetonitrile in 0.1% TFA. Tissue samples were then washed with 20 mL of 0.1% TFA and then eluted with 10 mL of 0.1% TFA. The eluates were evaporated to dryness and stored at −20°C until they were used. The eluates were reconstituted with 0.1% TFA and 10 mL of 0.1% TFA. The eluates were evaporated to dryness and stored at −20°C until they were used.

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 antiserum showed <0.01% cross-reactivity with Ang II peptide, and the Ang II antiserum 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 antiserum 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 Salt/EcoRI fragment of mouse 28S rRNA cDNA. Autoradiographs were scanned with the use of an Ultrascan XL laser densitometer (LKB Producter) and LKB 2400 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)
Renal ANF Gene Expression in Hypertension

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>1.60±0.05</td>
<td>1.70±0.03</td>
<td>2.00±0.03*†‡</td>
</tr>
<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>
</tr>
</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.

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 mg/kg)</th>
</tr>
</thead>
<tbody>
<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>
</tr>
<tr>
<td>BW, g</td>
<td>449±6</td>
<td>455±7</td>
<td>415±4</td>
<td>411±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.
§ 8P<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 ANF99–126 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.14

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 hypertension.
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. The 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.

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References
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