Angiotensin AT$_1$ Receptor Subtype as a Cardiac Target of Aldosterone

Role in Aldosterone-Salt–Induced Fibrosis

Valéerie Robert, Christophe Heymes, Jean-Sébastien Silvestre, Abdelkarim Sabri, Bernard Swynghedauw, Claude Delcayre

Abstract—This study tests the hypothesis that aldosterone induces cardiac fibrosis through an increase of cardiac angiotensin II (Ang II) AT$_1$ receptor levels, thereby potentiating the fibrotic effect of Ang II by determining the effects of spironolactone and losartan on cardiac fibrosis, AT$_1$ density, and gene expression in aldosterone-salt–treated rats. Fibrosis was quantified by slot blots of collagen I and III mRNA levels and videomorphometry of Sirius red–stained collagen. AT$_1$ receptor density was determined by $^{125}$I-Sar$_1$-Ile$_8$–Ang II competition binding, and AT$_1$ mRNA levels were analyzed by quantitative reverse transcriptase polymerase chain reaction. One month of aldosterone-salt treatment induced a decrease in plasma Ang II and an increase in blood pressure, left ventricular hypertrophy, and ventricular fibrosis. Spironolactone (20 mg/kg per day) and losartan spironolactone (10 mg/kg per day) had no effect on the first 3 parameters. Losartan was as effective as spironolactone in preventing ventricular collagen mRNA increase and fibrosis. Ventricular density of AT$_1$ receptors increased 2-fold and was accompanied by a 3-fold increase in the corresponding mRNA in aldosterone-salt compared with sham-operated rats. Both spironolactone and losartan prevented the elevation of ventricular AT$_1$ density and that of right ventricular AT$_1$ mRNA levels. These results demonstrate that the mechanism by which aldosterone-salt induces cardiac fibrosis involves Ang II acting through AT$_1$ receptors. They also suggest that the cardiac AT$_1$ receptor is a target for aldosterone. (**Hypertension. 1999;33:981-986.**) 

Key Words: heart ■ aldosterone ■ angiotensin receptor ■ fibrosis

Chronic excess of aldosterone (Aldo) is associated with marked deposition of cardiac interstitial and perivascular collagen.1–3 The Aldo antagonist spironolactone prevents this Aldo-induced cardiac fibrosis, evidence that the intracellular mineralocorticoid receptor is involved in this response.1 The biochemical pathways of Aldo action are, however, still a subject of debate. Brilla et al4 have described a stimulatory effect of Aldo on collagen synthesis in isolated cardiac fibroblasts. In contrast, recent in vitro5 and in vivo6 studies have shown that the Aldo-induced increase in cardiac collagen synthesis involves intermediate steps that remain to be defined.

Angiotensin II (Ang II) is a potent fibrogenic factor.4,5 Besides the well-known effect of Ang II in stimulating Aldo production from the adrenal cortex, a reciprocal interaction has been reported between the hormones. In vivo, mineralocorticoids increase Ang II binding in rat smooth muscle and vessels.7–9 In vitro, incubation of rat vascular smooth muscle cells with Aldo results in an increase of Ang II binding10 and potentiation of the Ang II hypertrophic response.11 Two main subtypes of Ang II receptors, AT$_1$ and AT$_2$, have been identified to date.12 AT$_1$ mediates many of the functions of Ang II, including stimulation of collagen synthesis, whereas the role of AT$_2$ is less well defined. Recent studies, however, have shown that chronic blockade of AT$_2$ in Ang II–induced hypertensive rats has no effect on arterial pressure but antagonizes the Ang II effect on arterial hypertrophy and fibrosis.13,14 An increase in Ang II receptor density has been observed in the heart of Aldo-salt–treated rats.15 Taken together, these results support the hypothesis that Aldo might increase the tissue abundance of Ang II receptors. We therefore hypothesized that Aldo produces fibrosis through an increase of Ang II receptors in myocardium and that such an increase potentiates the fibrogenic action of Ang II in myocardium.

To test this hypothesis, we analyzed the effect of AT$_1$ blockade on cardiac fibrosis in 1-month Aldo-salt–treated rats. Our results show that (1) losartan at very high doses prevents Aldo-induced cardiac fibrosis, (2) Aldo-salt increased cardiac AT$_1$ density and gene expression, (3) spironolactone prevented the increased cardiac AT$_1$ density. Together these results demonstrate that the mechanism of Aldo-induced cardiac fibrosis involves Ang II and upregulation of AT$_1$. 

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Methods

Animals and Drugs

Eight-week-old male Sprague-Dawley rats (Ifia Credo, Lyon, France) weighing 175 g were used. The study was conducted in accordance with both institutional guidelines and those formulated by the European community for use of experimental animals (L358 to 86/609/EEC). Uninephrectomized rats were infused with 0.75 μg/h d-aldosterone (Sigma) through osmotic minipumps (Alzet 2002, Charles River) for 1 month, as described. Aldosterone was dissolved in 0.154 mol/L NaCl 1 and then added to drinking water and did not reprecipitate at these dilutions. Losartan was solubilized in 0.154 mol/L NaCl and then added to drinking water and did not reprecipitate at these dilutions. Losartan was solubilized in 0.154 mol/L NaCl+5 mL/L ethanol. Treated rats drank progressively more over the course of the study: Fluid intake was monitored over the 8-week period of administration and spironolactone concentration adjusted accordingly. Uninephrectomized sham-operated rats were implanted with osmotic minipumps containing vehicle for aldosterone (0.154 mol/L NaCl+5 mL/L ethanol) and received no salt in drinking water. Rats were individually housed in metabolic cages for collection of urine and the kidney and the heart were removed and left ventricle (LV) plus septum was separated after the time of killing, blood was collected for assay of plasma renin activity, Ang II, and corticosterone with the use of radioimmunoas-
stay kits. The cross-reactivity versus Ang I of the anti–Ang II assay by flame photometry.

Collagen Morphometry

Equatorial sections (5 μm) were cut in a cryostat at −20°C and stained with the collagen-specific Sirius red stain, as previously described. Images were digitized on a Macintosh IIx by a gray level camera mounted on Leica ×10 binoculars. Total collagen was quantified by Optilab 2.6.1 image analysis software (Graftek, Velizy, France).

RNA Extraction and Assay

Total RNA was purified according to Chomczynski and Sacchi,16 resuspended in water, and stored at −70°C until use. Samples were then analyzed by Northern and slot blots as previously described2,6: 20 μg of RNA was used for Northern blots and 1, 2, 4, and 10 μg of RNA for slot blots. Membranes were hybridized at 42°C with rat collagen α1 (I) and α1 (III) cDNA probes, and then with rat ribosomal 18S RNA oligonucleotide for normalization of collagen signals. Radioactive signals were analyzed by a computer-based imaging system (Bas 1000, Fuji).

Quantitative Reverse Transcriptase Polymerase Chain Reaction

The sequences of antisense and sense primers (Bioprobe, Paris, France) for AT1, were 5’GCACAATCGCCATAATTACC3’ (extending from base 719 through base 739 of the coding sequence) and 5’CACCATTGTAAGATGCCTTC3’ (extending from base 295 through base 314 of the coding sequence). The expected size of the reverse transcriptase polymerase chain reaction (RT-PCR) product was 444 bp. For preparation of internal standards, the AT1 PCR product was subcloned into a PCR II-vector (TA cloning Kit, Invitrogen, Paris, France), and a 93-bp fragment was removed after double digestion with AccI and SpqI restriction enzymes, providing a 351-bp PCR fragment. The internal standard AT1, RNA was then synthesized by in vitro transcription with T7 RNA polymerase after linearization with HindIII. The transcription reactions were performed in the presence of labeled UTP as a precursor, and the concentration of each transcript was determined after measurement of the radioactivity incorporated into RNA product. Total RNA was then RT-PCR amplified as previously detailed.13 Radioactive signals were analyzed on the Fuji Bas 1000 imaging system.

Quantitative Autoradiography of AT1

Binding analysis of AT1 receptors, incubations were also made in the presence of the AT2 antibodies used in this study was 13.6%. The heart and the kidney were removed and left ventricle (LV) plus septum was separated from the right ventricle (RV). Tissue samples were frozen in liquid nitrogen and stored at −80°C until use.

Anatomical, Urinary, and Plasmatic Data of Rats After 1-Month Treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham-Operated (n=8)</th>
<th>Aldo-Salt (n=8)</th>
<th>Aldo-Salt+ Spironolactone (n=8)</th>
<th>Aldo-Salt+ Losartan (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anatomy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>155±5</td>
<td>196±6*</td>
<td>193±6*</td>
<td>214±8*</td>
</tr>
<tr>
<td>BW, g</td>
<td>338±8</td>
<td>254±20*</td>
<td>295±10*</td>
<td>280±14*</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>3.08±0.04</td>
<td>4.66±0.23†</td>
<td>445±0.35†</td>
<td>4.19±0.25†</td>
</tr>
<tr>
<td>KW/BW, mg/g</td>
<td>4.99±0.26</td>
<td>9.49±0.66*</td>
<td>7.50±0.36§</td>
<td>8.07±59*</td>
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<tr>
<td><strong>Urine</strong></td>
<td></td>
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</tr>
<tr>
<td>Urinary volume, ml/24 hrs</td>
<td>31±5</td>
<td>84±7*</td>
<td>141±12*§</td>
<td>60±8*</td>
</tr>
<tr>
<td>Na+/K+</td>
<td>0.31±0.06</td>
<td>2.87±12*</td>
<td>3.98±0.19*§</td>
<td>2.75±0.23*</td>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
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<tr>
<td>PRA, ng/mL · h</td>
<td>12.88±4.9</td>
<td>0.93±0.33†</td>
<td>0.76±0.17†</td>
<td>1.84±0.98†</td>
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<tr>
<td>Angiotensin II, pg/mL</td>
<td>344±52</td>
<td>85±24*</td>
<td>28±4*</td>
<td>98±22†</td>
</tr>
<tr>
<td>K+, meq/L</td>
<td>4.3±0.3</td>
<td>3.5±0.2</td>
<td>3.4±0.2‡</td>
<td>3.1±0.2‡</td>
</tr>
<tr>
<td>Corticosterone, ng/mL</td>
<td>487±65</td>
<td>132±40‡</td>
<td>218±48‡</td>
<td>169±45‡</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; BW, body weight; HW, heart weight; KW, kidney weight; and PRA, plasma renin activity.

Values are mean±SEM. *p<0.001; †p<0.01; ††p<0.05 vs sham-operated rats. §p<0.05 vs aldo-salt.
antagonist PD 123319 at 10 μmol/L, and signals were analyzed on the Fuji Bas 1000 imaging system.

**Statistical Analysis**

Results are expressed as mean±SEM and differences between groups evaluated by ANOVA comparison with the Scheffé test. A value of $P<0.05$ was considered statistically significant.

**Results**

**Anatomic, Urinary, and Plasma Data**

As shown in the Table, systolic blood pressure increased by 26% ($P<0.001$) after 1 month of Aldo-salt treatment, with a marked decrease in body weight and a 51% ($P<0.001$) increase in heart weight-to-body weight ratio. In Aldo-salt–treated rats, addition of spironolactone (20 mg/kg per day) or losartan (10 mg/kg per day) had no effect on these parameters. The kidney-to-body weight ratio was also elevated by Aldo-salt treatment, and this renal hypertrophy was partially blocked by spironolactone. Aldo-salt treatment produced a marked increase in urine volume and Na$^+/K^+$ ratio. Both parameters were further increased in the spironolactone group compared with Aldo-salt alone, whereas losartan had no effect. Plasma renin activity, plasma Ang II, and corticosterone concentrations were markedly decreased at 1 month of Aldo-salt treatment, and neither spironolactone nor losartan altered these changes. Although no change in plasma potassium was seen in the Aldo-salt group, both spironolactone and losartan decreased plasma potassium.

**Collagen Morphometry and Gene Expression**

Histological examination of the hearts (Figure 1A) showed typical cardiac alterations including interstitial and perivascular fibrosis as previously described after Aldo-salt treatment. Quantification of total collagen volume fraction after Sirius red staining showed a 2-fold elevation ($P<0.001$) in the Aldo-salt group that was totally prevented by spironolactone or losartan (Figure 1B). In parallel, the levels of collagen I and III mRNAs in the LV were analyzed by Northern blot (Figure 2A) and quantified by slot blot. Aldo-salt treatment similarly induced a 2-fold increase ($P<0.001$) of both collagen I and III mRNA levels (Figure 2, B and C, respectively), again totally prevented by either spironolactone or losartan.

**AT$_1$ Autoradiography**

As illustrated in Figure 3A, AT$_1$ binding was uniformly distributed throughout the ventricular myocardium of control (sham-operated) rats. No specific change in distribution was

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**Figure 1.** Analysis of cardiac fibrosis. A, Typical examples of Sirius red–stained ventricular sections from sham-operated and 1-month Aldo-salt–treated rats. Bar represents 1 mm. B, Quantification of collagen volume fraction by videomorphometry of 5-μm Sirius red–stained equatorial sections. n=8 for all groups. Sham indicates sham-operated; Aldo, aldosterone-salt; Spi, spironolactone; Los, losartan. ***$P<0.001$ vs sham-operated group.

**Figure 2.** Analysis of collagen I and III mRNAs in rat LV. A, Northern blot: 20 μg of RNA was used per lane. All rats were treated for 1 month as follows: A, sham-operated; B, Aldo-salt; C, Aldo-salt+spironolactone; D, Aldo-salt+losartan. B, mRNA levels of collagen I and III mRNAs/18 S rRNAs determined by slot blot analysis in rat LV (Spi, spironolactone; Los, losartan). n=8 for all groups. Sham indicates sham-operated; Aldo, aldosterone-salt. ***$P<0.001$ vs sham-operated group.
found with any treatment. AT1 density was elevated by 41% (P, 0.01) in the ventricular myocardium of Aldo-salt–treated rats (Figure 3B). Spironolactone treatment completely prevented the increase of AT1 density, and losartan-treated rats showed a decrease in density compared with sham-operated levels.

AT1 mRNA Determination

Figure 4A shows typical RT-PCR analysis of AT1 mRNA in both ventricles of the rat heart. With the use of quantitative RT-PCR, we found that Aldo-salt treatment induced a 3-fold increase in LV AT1 mRNA level compared with sham-operated rats (Figure 4B) and a 1.5-fold increase in RV AT1 mRNA level (0.43 ± 0.03 vs 0.27 ± 0.02 mol/g total RNA, P < 0.001) (Figure 4C). The Aldo-induced increase of LV AT1 mRNA level was not altered either by spironolactone or losartan; in contrast, the increase of RV AT1 mRNA level was totally prevented by both spironolactone and losartan.

Discussion

The main results of this study on the rat heart are that (1) high-dose losartan prevents Aldo-salt–induced fibrosis and upregulation of collagen types I and III mRNA, (2) 1-month Aldo-salt treatment increases AT1 density and AT1 mRNA accumulation, and (3) spironolactone and losartan prevent the Aldo-salt-induced increase in AT1 density independent of blood pressure and plasma concentration of Ang II.

Role of AT1 in Aldo-Salt Cardiac Fibrosis

We have previously shown in this model of hypertension that cardiac collagen type I and III mRNA levels increase at approximately 15 days of treatment. Such a delay favors an indirect control of Aldo on collagen gene expression and suggests intermediate steps. The main result of the present work is that chronic high-dose losartan administration totally prevented not only elevation in collagen accumulation but also the rise of collagen I and III mRNAs. In this model, elevation of collagen mRNA levels precedes that of protein. Taken together, these results suggest the involvement of Ang II by the AT1 receptor in Aldo-salt–induced cardiac fibrosis.

Such a result is at variance from previous studies showing that captopril1 and losartan18 did not prevent cardiac fibrosis in the same model. There are several possibilities that might explain this discrepancy. (1) angiotensin-converting enzyme (ACE) inhibition decreases not only Ang II synthesis but also bradykinin degradation. Bradykinin modulates fibroblast collagen, and blockade of bradykinin B2 receptors by HOE-140 completely prevents Ang II–induced cardiac fibrosis.20 Given the increased cardiac bradykinin receptors described in Aldo-salt excess,21 these observations may explain that cardiac fibrosis develops despite ACE blockade. (2) In the present study, rats were treated for 4 weeks with 10 mg/kg per day of losartan, that is, at a 3.3-fold higher dose than that used by Young and Funder.18 As it is known that cardiac ACE binding is increased in Aldo-treated rats21 with increased cardiac Ang II formation as a probable consequence and that cardiac AT1 binding is also increased as reported here and as published.
previously by Sun and colleagues, it is likely that a high dose of AT1 inhibitor is required to prevent Ang II action. Diminished cardiac fibrosis by AT1 blockade has been also observed in models of hypertension in which the circulating renin-angiotensin system is not activated, as in the stroke-prone spontaneously hypertensive rat strain or in deoxycorticosterone (DOCA)-salt treatment.

The increase of AT1 density is consistent with a potentiation of Ang II effects. For example, in smooth muscle, the consequence of increased Ang II receptor density is to strongly enhance Ang II hypertrophy. Similarly, in cardiomyocytes from DOCA-salt–treated rats, the increased AT1 density is associated with an increased intracellular calcium response to Ang II. Hence, the rise of cardiac AT1 density seen in the Aldo-salt model might potentiate the well-described fibrogenic effect of Ang II.

Aldosterone and Cardiac AT1 Receptors

These results raise the question of the mechanism whereby Aldo increases the density of AT1. Lowering the plasma concentrations of potassium or Ang II, or increased glucocorticoid levels, has been shown in a variety of models to increase Ang II receptor density. It seems unlikely that these factors are involved here because (1) the plasma concentration of corticosterone fell in all groups, (2) plasma potassium was unchanged in the Aldo-salt group, and (3) Ang II binding normalized in the spironolactone and losartan groups despite plasma concentrations of Ang II remaining low. Direct effects of mineralocorticoids on Ang II receptor density have been demonstrated in vitro in cultured vascular smooth muscle cells in which DOCA or Aldo increase Ang II receptor density in a time- and concentration-dependent manner. Despite the differences in target tissue, the decrease of elevated AT1 levels by spironolactone reported here suggests that the cardiac action of Aldo through mineralocorticoid receptors is to increase the level of AT1.

Mechanisms of Control of AT1 mRNA

Recent sequence analysis of AT1 gene has evidenced the presence of several glucocorticoid-responsive elements in the promoter region. It has been shown that the mineralocorticoid receptor–Aldo complex is able to bind to GRE sequences to activate the transcription of genes containing these regulatory elements. One hypothesis tested in this study is that Aldo-salt treatment stimulates cardiac AT1 gene expression, resulting in increased AT1 density, with quantitative RT-PCR performed on RNA prepared separately from the LV and the RV. Aldo-salt treatment induced increases in both cardiac Ang II binding and AT1 receptor mRNA. In all groups, AT1 binding was homogeneously distributed throughout the ventricular myocardium. However, in the spironolactone and losartan groups, levels of LV AT1 mRNA remained elevated in sharp contrast to RV AT1 mRNA and cardiac Ang II binding, which returned to control levels. In addition, AT1 mRNA levels were increased 3-fold in LV and only 1.5-fold in RV with Aldo-salt, again suggesting a differential response of ventricles in terms of regulation of AT1 mRNA. One possible explanation of these results is that AT1 gene expression may be under the control of both Aldo and hemodynamic factors. The doses of spironolactone and losartan were chosen to have no effect on hypertension, so that the LV but not the RV remained exposed to increased hemodynamic load in all treatment groups. The consequences of chronic Aldo-salt induced hypertension on cardiac structure have been previously described, and both marked hypertrophy and upregulation of atrial natriuretic peptide are consistently observed in LV, not in RV. On this hypothesis, blockade of Aldo action by spironolactone or losartan results in decreased AT1 mRNAs in RV, which remain elevated in LV in response to hemodynamic factors. This idea is consistent with previous studies indicating that mechanical factors trigger the increase of AT1 mRNA level in ventricles during hemodynamic overload. Moreover, an increase of AT1 receptors primarily caused by increased AT1 gene transcription is seen in neonatal rat cardiac myocytes subjected to stretch. Even though conclusions from neonatal isolated cells cannot be extrapolated to the in vivo situation, they are further evidence that cardiac AT1 gene expression may be modified by hemodynamic overload. In the same vein, control AT1 mRNA concentrations were 1.7-fold higher in LV than RV. The reason of this difference is unknown; it may reflect metabolic differences between the RV and the LV under very different conditions of work and load.

These observations support the hypothesis that AT1 mRNA is upregulated by Aldo and is maintained elevated in the LV by hemodynamic factors. The differential response of the mRNA and of protein to blockade of either Aldo or Ang II action suggests that the regulation of AT1 receptors is more complex than anticipated and that posttranscriptional control may play an important modulatory role.

Mineralocorticoids and Fibrosis

Mechanisms for cardiac fibrosis seen in mineralocorticoid-salt excess are still uncertain. Previous results have shown that Aldo-salt or DOCA-salt cardiac fibrosis may be prevented or reduced by several classes of inhibitors, making it difficult to postulate a simple mechanism of fibrosis development. One of the essential contributions of this work is to emphasize the existence of a connection between the actions of Aldo and Ang II leading to increased responsiveness of cardiac cells to Ang II. The growth-promoting effect of Ang II has been well described, and it may thus participate in the stimulation of myofibroblast proliferation observed at sites of fibrosis in Aldo-salt–treated or Ang II–treated animals. Ang II–induced intracellular calcium increase may also be involved in this process because the calcium channel blocker mibebradil prevents Aldo-induced or Ang II–induced cardiac fibrosis. Increased cardiac bradykinin receptor binding is seen in Aldo-salt excess, and blockade of bradykinin B2 receptors prevents the Ang II–induced cardiac fibrosis; the postulated pathway of bradykinin action is also through stimulation of myofibroblast proliferation, possibly mediated by prostaglandin release. The intervention of other fibrogenic hormones such as endothelin-I in Aldo-salt cardiac fibrosis is also likely, given that it is overexpressed in the endothelium of coronary arteries and endocardium of DOCA-salt–treated rats, and the endothelin antagonist bosentan decreases cardiac fibrosis in the same model.
Acknowledgments

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References

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