Increased Carotid Wall Elastic Modulus and Fibronectin in Aldosterone-Salt–Treated Rats
Effects of Eplerenone

Patrick Lacolley, MD, PhD; Carlos Labat; Alex Pujol; Claude Delcayre, PhD; Athanase Benetos, MD, PhD; Michel Safar, MD

Background—Previous studies have demonstrated the development of cardiac fibrosis in aldosterone (Aldo)–salt hypertensive rats. Our aim was to determine the effects of Aldo and the Aldo receptor antagonist eplerenone (Epl) on in vivo mechanical properties of the carotid artery using echo-tracking system.

Methods and Results—Aldo was administered (1 μg/h) in uninephrectomized Sprague-Dawley rats (SD) receiving a high-salt diet from 8 to 12 weeks of age. Uninephrectomized control SD rats received a normal salt diet without Aldo. Three groups of Aldo-salt rats were treated with 1, 10, or 30 mg/kg·d of Epl by gavage. Elasticity was measured by elastic modulus (Einc)-wall stress curves using medial cross-sectional area (MCSA). The structure of the arterial wall was analyzed by histomorphometry (elastin and collagen), immunohistochemistry (EIIIA fibronectin, Fn), and Northern blot (collagens I and III). Aldo produced increased systolic arterial pressure, pulse pressure, Einc, MCSA, and EIIIA Fn with no change in wall stress or elastin and collagen densities compared with controls without Aldo. No differences in collagen mRNA levels were detected between groups. Epl blunted the increase in pulse pressure in Aldo rats and normalized Einc-wall stress curves, MCSA, and EIIIA Fn. These effects were dose dependent and not accompanied by a reduction in wall stress.

Conclusions—Aldo is able to increase arterial stiffness associated with Fn accumulation, independently of wall stress. The preventive effects of Epl suggest a direct role for mineralocorticoid receptors in mechanical and structural alterations of large vessels in rat hyperaldosteronism. (Circulation. 2002;106:2848-2853.)

Key Words: arteries ■ hypertension ■ elasticity ■ pharmacology
opment of hypertension. The second objective was to determine the dose-dependent preventive effects of the Aldo receptor antagonist eplerenone (Epl) compared with Aldo-salt rats.

Methods

Eight-week-old Sprague-Dawley (SD) male rats (n = 66) weighing 180 to 200 g were obtained from Iffa Credo (France). The rats were divided into 5 groups. In the first group, Aldo-salt rats were uninephrectomized at 8 weeks of age and were given a subcutaneous aldosterone model (1 μg/h) via osmotic minipumps with high-sodium diet (1% NaCl in the drinking water) from 8 to 12 weeks of age. In the second group, control SD rats were uninephrectomized and received a normal salt diet without Aldo administration.11 In other groups, uninephrectomized Aldo-salt-Epl rats received treatment with either 1, 10, or 30 mg/kg orally by gavage of Epl from the age 8 to 12 weeks. All procedures were in accordance with institutional guidelines for animal experimentation.

We simultaneously recorded arterial diameter (left CA) and blood pressure (right CA) in pentobarbital-anesthetized rats. Internal arterial diameter (D) was measured with an ultrasonic echo-tracking device (NIUS-01, Asulab SA). We determined arterial distensibility (Dist), incremental elastic modulus (Einc), and circumferential wall stress (σ) as previously described.10 The relationship between the pressure AP and the lumen cross-sectional area (LCSA) was fitted using an arctangent function and 3 optimal-fit parameters (α, β, and γ), as follows:

\[
\text{LCSA} = \left(\frac{\pi D^2}{4}\right) = \alpha \left[\frac{\pi}{2} \tan^{-1}\left(\frac{P - \beta}{\gamma}\right)\right]
\]

Arterial cross-sectional Dist, σ, and Einc are given by the following equations:

\[
\text{Dist}(P) = \frac{1}{\text{LCSA}} \frac{\delta \text{LCSA}}{\delta P}
\]

\[
\sigma = \frac{2 \text{LCSA} \times P}{\text{MCSA}}
\]

\[
\text{Einc} = \frac{3}{\text{Dist}(P)} \left(1 + \frac{\text{LCSA}}{\text{MCSA}}\right)
\]

where MCSA is the media cross-sectional area.

Elastin, collagen, and MCSA were quantified in 4% formaldehyde-fixed CA and thoracic aorta by histomorphometry. Immunohistochemistry of EIIIA Fn10 and Northern blot of mRNA procollagen I and III1 from arch to thorax aorta as previously described. For EIIIA Fn staining, 5-μm-thick freeze-dried paraffin-embedded aortic sections were treated with the mouse anti-EIIIA Fn antibody (clone IST-9, Sera-Laboratory). For Northern blots, samples of 20 μg of RNA were denatured and electrophoresed in a 1% agarose gel. Blots were subsequently hybridized with the following cDNA probes: a 24-mer oligonucleotide specific to the rat 18S RNA, a rat α1-I collagen cDNA of 1600 bp complementary to the carboxy-terminal propeptide, and a rat α1-III collagen cDNA containing 1300 bp of the 3’ noncoding and coding regions. The relative amounts of mRNAs were quantified on slot blots by dividing the optical densities by the optical density measured using the 18S probe.

Results

Table 1 shows that body weight was similar in both control and Aldo-salt groups. In the Aldo-salt group, heart weight was significantly increased compared with controls. Systolic AP and pulse pressure were higher in Aldo-salt group than in control group, with minor change in heart rate and diastolic...
and mean AP. The distensibility-AP curve in Aldo-salt group was shifted in the prolongation of the distensibility-AP observed in the control group (Figure 1). No differences in arterial diameter and distensibility at mean arterial pressure (MAP) were detected between the 2 groups (Table 2). Carotid and aortic MCSA were significantly increased in Aldo-salt group compared with control rats (Table 3). The Einc-wall stress curve of the Aldo-salt group was significantly shifted upward compared with that of control rats (Figure 2). The mean shift of Einc of Aldo-salt group compared with the control group was 497 kPa (Table 2). At mean AP, the increase in Einc was significant when it was represented in terms of Einc to wall stress ratio. There was no difference in elastin and collagen densities or in collagen and elastin ratio between the 2 groups (Table 3). No significant differences in aortic collagen I and III mRNA were observed between the 2 groups (data not shown). The Aldo-salt rats had significantly increased EIIIA Fn density (3-fold, Figure 3) compared with control rats.

Table 1 shows that heart rate, blood pressure, and heart weight did not differ significantly between the different groups. Pulse pressure was only reduced in the Aldo-salt-Epl 30-mg group compared with the Aldo-salt group. Figure 2 shows that Epl did not modify the distensibility-pressure curves compared with Aldo-salt group. In the Aldo-salt-Epl groups, MCSA was significantly reduced in a dose-dependent manner compared with the Aldo-salt group (Table 3). The Einc-wall stress curves in rats receiving Eplerenone were significantly shifted downward in a dose-dependent manner compared with those of the Aldo-salt group (Figure 3). At mean AP, wall stress and Einc were not different between Epl-treated rats and Aldo-salt rats. Eplerenone did not affect collagen and elastin compared with Aldo-salt rats (Table 3). Aortic collagen I and III messenger RNA were not affected by Eplerenone administration (data not shown). EIIIA aortic Fn density was smaller in Epl group than in Aldo-salt group (Figure 3).

**Discussion**

Aldosterone-treated rats under high-salt diet have been previously described to determine the role of aldosterone on cardiac structure and function.1,5,12 The present study investigated the effects of aldosterone and the aldosterone receptor antagonist eplerenone on the mechanical properties of large

### Table 2. Effects of Aldosterone and Eplerenone on Mechanical Properties of the Carotid Artery

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Aldo-Salt</th>
<th>Aldo-Salt Epl 30 mg</th>
<th>Aldo-Salt Epl 10 mg</th>
<th>Aldo-Salt Epl 1 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Systolic diameter, mm</td>
<td>1.23±0.03</td>
<td>1.25±0.04</td>
<td>1.23±0.04</td>
<td>1.26±0.04</td>
<td>1.30±0.07</td>
</tr>
<tr>
<td>Diastolic diameter, mm</td>
<td>1.09±0.04</td>
<td>1.11±0.04</td>
<td>1.08±0.04</td>
<td>1.09±0.05</td>
<td>1.17±0.07</td>
</tr>
<tr>
<td>Distensibility, mm Hg⁻¹</td>
<td>5.64±0.46</td>
<td>4.16±0.65</td>
<td>5.27±0.55</td>
<td>5.75±0.59</td>
<td>3.75±0.53</td>
</tr>
<tr>
<td>Wall stress, kPa</td>
<td>215±12</td>
<td>190±11</td>
<td>205±20</td>
<td>192±21</td>
<td>220±22</td>
</tr>
<tr>
<td>Einc, kPa</td>
<td>535±63</td>
<td>699±134</td>
<td>599±82</td>
<td>538±95</td>
<td>861±182</td>
</tr>
<tr>
<td>Einc/wall stress</td>
<td>2.47±0.16</td>
<td>3.63±0.59*</td>
<td>2.97±0.15</td>
<td>2.81±0.27</td>
<td>3.84±0.65</td>
</tr>
<tr>
<td>Mean shift of Einc vs control, kPa</td>
<td>...</td>
<td>497</td>
<td>20</td>
<td>113</td>
<td>391</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<0.05 Aldo-salt vs control.

### Table 3. Effects of Aldosterone and Eplerenone on Structure of the Carotid Artery and Thoracic Aorta

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Aldo-Salt</th>
<th>Aldo-Salt Epl 30 mg</th>
<th>Aldo-Salt Epl 10 mg</th>
<th>Aldo-Salt Epl 1 mg</th>
</tr>
</thead>
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<td>10</td>
<td>11</td>
<td>21</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Carotid artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCSA, mm²</td>
<td>0.167±0.006</td>
<td>0.217±0.018*</td>
<td>0.177±0.008†</td>
<td>0.205±0.019</td>
<td>0.199±0.012</td>
</tr>
<tr>
<td>Elastin density, %</td>
<td>37.7±1.5</td>
<td>37.0±1.2</td>
<td>37.6±0.9</td>
<td>38.4±0.7</td>
<td>39.5±0.7</td>
</tr>
<tr>
<td>Collagen density, %</td>
<td>13.4±0.6</td>
<td>12.5±0.5</td>
<td>13.5±0.4</td>
<td>13.3±0.5</td>
<td>13.8±0.5</td>
</tr>
<tr>
<td>Collagen/elastin ratio</td>
<td>0.36±0.02</td>
<td>0.34±0.02</td>
<td>0.36±0.01</td>
<td>0.35±0.01</td>
<td>0.35±0.01</td>
</tr>
<tr>
<td>Thoracic aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCSA, mm²</td>
<td>0.697±0.014</td>
<td>0.831±0.028*</td>
<td>0.746±0.015§</td>
<td>0.725±0.017‡</td>
<td>0.743±0.018†</td>
</tr>
<tr>
<td>Elastin density, %</td>
<td>44.7±1.2</td>
<td>43.3±1.2</td>
<td>43.0±0.8</td>
<td>42.7±0.5</td>
<td>42.9±1.2</td>
</tr>
<tr>
<td>Collagen density, %</td>
<td>15.9±0.6</td>
<td>15.4±0.6</td>
<td>16.9±0.6</td>
<td>18.1±0.9</td>
<td>16.0±0.5</td>
</tr>
<tr>
<td>Collagen/elastin ratio</td>
<td>0.36±0.02</td>
<td>0.36±0.01</td>
<td>0.40±0.02</td>
<td>0.43±0.02</td>
<td>0.38±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*P<0.01 Aldo-salt vs Control; †P<0.05; ‡P<0.01 Aldo-salt-Epl vs Aldo-salt.
arteries. Aldosterone administration produced a significant increase in pulse pressure, carotid arterial stiffness, MCSA, and medial EIIIA fibronectin with no change in wall stress and collagen density. The effects of aldosterone are dose-dependently prevented by administration of eplerenone.

**Model of Uninephrectomized Aldo-Salt Rats**

Previous studies have demonstrated that chronic administration of aldosterone plus high salt for 6 weeks produced a significant arterial wall hypertrophy without accumulation of collagen within the media of great vessels. These structural changes were independent of AP changes. We examine the effects of aldosterone in the absence of major increase in diastolic and mean AP. It has been reported that 4 weeks of Aldo administration increased SAP but had no significant effect on MAP.

Using invasive central AP measurements, we confirmed the significant elevation in SAP, as previously reported by the tail cuff method. The SAP elevation was more important than the increase in DAP, leading to a significant increase in PP with minimal change in MAP. Both cardiac (ventricular ejection volume) and arterial (aortic stiffness or wave reflections) factors might potentially influence central PP. In animal models involving increased sodium intake or aldosterone, previous investigations have shown that cardiac output is either normal or even increased at an early phase. Thus, it seems likely that an increased ventricular ejection volume contributes to the increase in PP. One major question was to determine whether arterial stiffness could also participate in the increase in PP in this model.

**Figure 2.** Mean carotid Einc-wall stress curves in control, Aldo-salt, and Aldo-salt-Epl–treated rats (1, 10, or 30 mg/kg·d⁻¹). Einc-wall stress curve of the Aldo-salt rats was shifted upward compared with that of control rats (497-kPa mean shift). The curves in Aldo-salt-Epl rats were shifted downward in a dose-dependent manner compared with those of Aldo-salt rats.

**Figure 3.** EIIIA Fn immunohistological staining of the abdominal aorta in control (A), Aldo-salt–treated (B), and Aldo-salt-Epl–treated rats (30 mg/kg·d⁻¹) (C). EIIIA Fn was significantly increased in Aldo-salt rats vs control rats and Aldo-salt-Epl rats; *P<0.05.
Arterial Stiffness
This is the first study to show that chronic Aldo treatment increased carotid arterial stiffness. Einc/wall stress curve evaluates the intrinsic mechanical behavior of the wall material, whereas arterial distensibility/pressure curve evaluates the global elasticity of the artery. Despite the lack of distensibility changes, Einc for a given level of wall stress was increased in Aldo-treated animals. This clearly indicates that intrinsic stiffness of the arterial wall is increased in Aldo-treated rats.

One could suggest that the increase in arterial stiffness was only attributable to the high-salt diet. We have previously shown that SHR receiving a sodium loading developed a higher level of wall stress with no upward shift of the Einc-wall stress curve. In addition, administration of a high-salt diet alone for 4 weeks in uninephrectomized SD rats did not produce any significant increase in wall stress nor Einc (unpublished data). These findings indicate that in both normotensive rats and SHR, a high-salt diet does not modify the mechanical behavior of the arterial wall. Our study shows that an increase in arterial stiffness does not necessarily require a sustained elevation of MAP or mean circumferential wall stress. This implies that after Aldo treatment, early modifications of arterial thickness as well as modifications in structural components occurred to produce these mechanical changes.

Arterial Wall Hypertrophy and Composition
An important structural abnormality was a significant arterial wall hypertrophy in Aldo-treated rats, thus confirming the work of Garwitz et al. Our results indicate that the vascular wall hypertrophy did not involve quantitative changes in elastin and collagen densities. The effects of Aldo-salt treatment on collagen expression have only been studied in elastin and collagen densities. The effects of Aldo-salt treatment on collagen expression have only been studied in elastin and collagen densities. The effects of Aldo-salt treatment on collagen expression have only been studied in elastin and collagen densities.

Arterial stiffness and accumulation of Fn in this model with no changes in heart weight. In liquorice-induced hypertension, which allows cortisol to activate mineralocorticoid receptors, eplerenone normalizes both blood pressure and vascular function. Eplerenone has recently been shown to attenuate constrictive remodeling and collagen accumulation in angio-plastied porcine coronary arteries, effects that are not attributed to AP changes. Our results show that eplerenone at 30 mg/kg per day blunted the increase in PP in Aldo-treated rats, normalized the intrinsic stiffness of large arteries, and reduced medial hypertrophy and EIIIA Fn density. We suggest that the antagonism of Aldo receptors has no effect on collagen density, because we did not observe any significant reduction of AP nor collagen accumulation compared with other models. On the other hand, eplerenone was able to decrease arterial stiffness in a dose-dependent manner and reduced EIIIA Fn. The preventive effects of eplerenone confirm the implication of aldosterone in mediating the increase in FN and arterial stiffness. We can suggest that in eplerenone aldosterone-treated rats, maintenance of cardiac hypertrophy associated with normalization of arterial elasticity did not result in mechanical heart-vascular uncoupling because of the concomitant suppression of cardiac interstitial collagen fibrosis.

The presence of mineralocorticoid receptors in smooth muscle cells and in endothelial cells provides strong evidence for a local action of Aldo in large vessels. Potential mechanisms including activation of endothelin, angiotensin II, plasminogen activator inhibitor (PAI-1), transforming growth factor-β, as well as inhibition of NO and norepinephrine uptake have been described. Whereas aldosterone is able to increase ACE mRNA levels in neonatal rat cardiocyte cultures, a reduction in plasma levels of Ang II has been
reported in this model. Although the exact mechanisms remain unknown, our results suggest a potential role of Aldo receptor antagonists to prevent increase in large artery stiffness associated with increased plasma levels of aldosterone or aldosterone synthase gene polymorphism in essential hypertension. Aldo receptor antagonists may also produce beneficial effects in heart failure patients treated chronically with ACE inhibitors, in whom aldosterone escape associated with reduced arterial compliance has been demonstrated.

In conclusion, this study demonstrates that aldosterone-salt administration in rat is able to increase large artery stiffness associated with Fv accumulation independently of wall stress. These arterial modifications represent an early step in the development of hypertension and cardiac fibrosis. All of these changes were reversed if rats were treated with eplerenone. These results suggest a direct role for mineralocorticoid receptors in mechanical and structural alterations of large vessels in rat hyperaldosteronism.

Acknowledgments
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References
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