Effect of aldosterone on renal transforming growth factor-β

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Juknevicius, Irmanitas, Yoav Segal, Stefan Kren, Rutha Lee, and Thomas H. Hostetter. Effect of aldosterone on renal transforming growth factor-β. Am J Physiol Renal Physiol 286: F1059–F1062, 2004.—Aldosterone participates in the pathophysiology of several models of progressive chronic renal disease. Because of the causal connection between transforming growth factor-β1 (TGF-β) and scarring in many such models, we hypothesized that aldosterone could evoke TGF-β in the kidney. Aldosterone infusion for 3 days in otherwise normal rats caused a more than twofold increase in TGF-β excretion without changes in systolic pressure or evidence of kidney damage. Concurrent treatment with amiloride did not alter this effect, indicating that aldosterone’s stimulation of TGF-β was independent of its regulation of sodium or potassium transport. However, concurrent treatment with spironolactone did block the increase in TGF-β, indicating that the effect depends on the mineralocorticoid receptor. Renal mRNA for serum glucocorticoid kinase rose, but no change in TGF-β message occurred, suggesting posttranscriptional enhancement of renal TGF-β. In summary, aldosterone provokes renal TGF-β, and this action may contribute to aldosterone’s fibrotic propensity.

ALDOSTERONE AND ANG II perpetuate injury in many chronic renal diseases (6). Aldosterone also generates fibrosis in the heart, and in vitro studies have confirmed that this pathology derives at least in part from nonhemodynamic actions of the steroid (20, 22, 26). Several plausible pathways for tissue injury and remodeling by aldosterone have been proposed in addition to its potential toxicity as a hypertensive agent (4, 20, 22). Transforming growth factor-β1 (TGF-β1) has a well-established role in a wide range of chronic renal injuries (1, 3, 12, 18, 23, 27). ANG II likely effects some of its deleterious actions through the agency of TGF-β because ANG II stimulates TGF-β synthesis in vitro (8, 27). Because of the increasingly recognized connection between aldosterone and progressive kidney disease, we questioned whether the mineralocorticoid might, like ANG II, induce TGF-β. Renal TGF-β expression is elevated in mineralocorticoid-salt hypertension (9). Also, its expression rises in the remnant kidney model, a phenomenon that also appears to depend at least in part on aldosterone (5). However, in these two models, the rats had substantial renal injury, and the TGF-β may have represented a late or nonspecific response only very indirectly related to the mineralocorticoid. For this reason, we examined the renal TGF-β response to aldosterone in normal rats before injury or hypertension supervened.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 250 and 300 g were used for these studies. They had free access to standard rat chow (24% protein and 29% sodium; Teklad Premier Laboratory Diets, Madison, WI) and tap water. Under methohexitol anesthesia, osmotically driven minipumps (Alzet) were implanted subcutaneously. Aldosterone was infused at 80 μg·kg⁻¹·day⁻¹. Some of the controls had implantation of a pump with saline added to the infusion chamber. Other controls underwent only anesthesia and a sham incision. One group of rats received amiloride dissolved in DMSO and further diluted in olive oil at 3 mg·kg⁻¹·day⁻¹ subcutaneously. Another group of rats received spironolactone in powdered chow of the same composition mixed to deliver ~400 mg·kg⁻¹·day⁻¹. Between the 2nd and 3rd day after the procedure, urine was collected for 24 h. On the 3rd day, systolic blood pressures were measured in the awake state by tail cuff. Later on the 3rd day, the animals were killed, and trunk blood and kidneys were obtained for analyses. The kidneys were divided into cortex and medulla. The studies were approved by Research Animal Resources of the University of Minnesota. The National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed.

Aldosterone and plasma renin activity were determined by RIA. For renin activity, the generation of ANG I was measured using a kit from New England Nuclear (Boston, MA). For aldosterone, a kit manufactured by Diagnostic Products (Los Angeles, CA) was used. TGF-β was measured by immunobiosorbence using a kit from Promega (Madison, WI). Sodium and potassium were measured by flame photometry. Urinary protein was assayed by the Coomasie dye method (Bio-Rad Laboratories, Hercules, CA). For the RNase protection assays, the kidney pieces were quickly divided into cortex and medulla. The studies were approved by the Animal Resources of the University of Minnesota. The National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed.

Most of the data were analyzed using a two-tailed Student’s t-test, and a significance level of P < 0.05 was accepted. Where appropriate, data were also analyzed using analysis of variance (ANOVA) with post hoc analysis by Bonferroni correction.

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1 Three major isoforms of TGF-β exist and are denoted by Arabic numerals. In this paper, TGF-β refers to the type 1 isofrom.
Table 1. Effects of aldosterone infusion

<table>
<thead>
<tr>
<th></th>
<th>Initial Body Wt, g</th>
<th>3-Day Body Wt, g</th>
<th>Systolic Blood Pressure, mmHg</th>
<th>U$_{juxta}-V$, ng/day</th>
<th>P$_{aldosterone}$, pg/ml</th>
<th>U$_{aldosterone}$, ng/day</th>
<th>UTGF-β1, ng/ml</th>
<th>UTGF-β2, V, ng/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>264±7</td>
<td>275±4</td>
<td>113±8</td>
<td>21±9</td>
<td>50±5.4</td>
<td>13±3</td>
<td>0.42±0.13</td>
<td>6.1±1.9</td>
</tr>
<tr>
<td>Aldosterone infused</td>
<td>270±8</td>
<td>284±9</td>
<td>105±5</td>
<td>21±2</td>
<td>913±150*</td>
<td>184±26*</td>
<td>0.77±0.36†</td>
<td>16.3±6.9*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 rats/group. U$_{juxta}-V$, urinary protein excretion rate; P$_{aldosterone}$, plasma aldosterone concentration; U$_{aldosterone}$, urinary aldosterone excretion rate; UTGF-β1, urinary transforming growth factor (TGF)-β1, excretion rate. *P < 0.05 and †P = 0.05, initial and 3-day body weight, rat weight initially, and 3 days after insertion of aldosterone containing minipump or sham procedure.

Table 2. Effects of aldosterone infusion on plasma electrolytes and renin activity

<table>
<thead>
<tr>
<th></th>
<th>P$_{Na}$, meq/l</th>
<th>P$_{K}$, meq/l</th>
<th>PRA, ng/ml/hr</th>
<th>PRA, ng/ml/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>145±1</td>
<td>4.2±0.3</td>
<td>1.76±0.58</td>
<td>1.56±0.58</td>
</tr>
<tr>
<td>Aldosterone infused</td>
<td>147±1</td>
<td>3.4±0.3*</td>
<td>≤0.30±0.10*</td>
<td>1.62±0.10*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 rats/group. P$_{Na}$ and P$_{K}$, plasma sodium and potassium, respectively; PRA, plasma renin activity. Electrolytes and PRA were measured in separate rats. *P < 0.05.

Table 3. Effects of amiloride on aldosterone infusion

<table>
<thead>
<tr>
<th></th>
<th>Initial Body Wt, g</th>
<th>3-Day Body Wt, g</th>
<th>UTGF-β1, V, ng/day</th>
<th>UTGF-β2, V, ng/day</th>
<th>P$_{K}$, meq/l</th>
<th>PRA, ng/ml/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>276±16</td>
<td>281±12</td>
<td>9.7±4.4</td>
<td>4.0±0.2</td>
<td>2.01±0.55</td>
<td></td>
</tr>
<tr>
<td>Aldosterone infused and amiloride treated</td>
<td>274±10</td>
<td>284±13</td>
<td>18.5±6.7*</td>
<td>4.2±0.4</td>
<td>3.42±2.70</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 5 sham rats and 6 aldosterone-infused and amiloride-treated rats. *P < 0.05, initial and 3-day body weight, rat weight initially, and 3 days after insertion of aldosterone containing minipump or sham procedure.
(12, 13, 15, 18, 24). Moreover, the elevated renal levels of TGF-β observed in models of progressive kidney disease fall with angiotensin-converting enzyme (ACE) inhibition or angiotensin receptor blockade (1, 5, 7). Finally, ANG II stimulates TGF-β production in vivo and in vitro (2, 27). Thus one path of injury may proceed through generation of ANG II with the resultant TGF-β production responsible for fibrotic consequences. However, aldosterone and ANG II mediate some of the ill effects of the RAAS in renal injury. The present results argue for an effect of aldosterone on TGF-β separate from that of ANG II. That is, the augmentation of TGF-β occurred despite suppression of renin activity and a likely parallel reduction in ANG II. Thus ANG II and aldosterone may each exert their injurious actions through TGF-β.

The absence of an increase in TGF-β message does not preclude an increase in the protein’s renal production. Increased production is likely, because an increase in release of preformed TGF-β without ongoing synthesis seems unlikely to persist for even the short interval of the present protocol. Also, a nonspecific increase in urinary TGF-β, perhaps resulting from a change in protein reabsorption, is inconsistent with the similar rates of total protein excretion in the infused and uninfused rats. A posttranscriptional increase in its rate of synthesis and secretion is the most probable mechanism. Indeed, such an event has been described for TGF-β in several cell lines. Moreover, this mode of regulation of TGF-β secretion has been best documented for retinoid and other steroid secretagogues of TGF-β (10, 11, 25). Nontranscriptional control of production seems the most likely explanation for the elevation in urinary TGF-β with aldosterone.

TGF-β manifests an extraordinarily wide range of actions (3, 16, 19). Renal research has largely focused on its pathological effects (2, 3, 12, 18, 23, 27). However, levels are detectable in the normal kidney, and the medulla contains a higher concentration than the kidney as a whole and comparable to that of the glomeruli (15). Regulation of renal cell proliferation may be a primary role, but the cytokine may perform other homeostatic functions. Stokes (21) discovered that TGF-β antagonizes the action of mineralocorticoids on inner medullary collecting duct cells. Specifically, when such cells are grown in culture and a mineralocorticoid is applied, they develop a sodium current indicative of the stimulation of sodium transport. Addition of TGF-β, at levels comparable to those in the normal medulla, blocks this mineralocorticoid-induced sodium transport. Taken with our present results, these data raise the possibility that TGF-β may act as a local counterregulator to the sodium retentive actions of aldosterone. Precedents exist for such a pairing (between hormonal and paracrine/autocrine effectors in the renal medulla). For example, the opposing actions of circulating antidiuretic hormone and locally produced prostaglandin exert fine control of water reabsorption in the medulla (14). Further studies will be required to assess this hypothesized counterregulatory role of TGF-β.

In summary, aldosterone increases urinary TGF-β within 3 days and without hypertension or renal injury. Aldosterone may in pathological states exert profibrotic effects through this pathway.

ACKNOWLEDGMENTS
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GRANTS
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REFERENCES
8. Kagami S, Border WA, Miller DE, and Noble NA. Angiotensin II stimulates extracellular matrix protein synthesis through induction of

Table 4. Effects of aldosterone infusion on gene expression

<table>
<thead>
<tr>
<th>Riboprobe</th>
<th>Size (bases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGK</td>
<td>333</td>
</tr>
<tr>
<td>Collagen IV</td>
<td>289</td>
</tr>
<tr>
<td>Collagen I</td>
<td>234</td>
</tr>
<tr>
<td>Collagen III</td>
<td>201</td>
</tr>
<tr>
<td>TGF-β</td>
<td>171</td>
</tr>
<tr>
<td>βActin</td>
<td>115</td>
</tr>
</tbody>
</table>

Fig. 1. Representative image of gel from RNase protection assay. SGK, serum glucocorticoid kinase; TGF-β, transforming growth factor-β.


