Effect of aldosterone on renal transforming growth factor-β

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Juknevicius, Irmanantas, 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-β1) 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 methohexital 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 immunobosorbence using a kit from Promega (Madison, WI). Sodium and potassium were measured by flame photometry. Urinary protein was assayed by the Coomassie dye method (Bio-Rad Laboratories, Hercules, CA). For the RNase protection assays, the kidney pieces were quickly divided into cortex and papilla and frozen in liquid nitrogen. RNA was extracted from the tissues using Ultraspec RNA (Biotec Laboratories, Houston, TX). Templates for riboprobe synthesis were amplified by PCR from a rat kidney cDNA library (BD Biosciences Clontech, Palo Alto, CA) or isolated as restriction fragments from EST clones (Research Genetics, Huntsville, AL), cloned into the vector pBC KS(+) (Stratagene, La Jolla, CA) and verified by direct sequencing. With corresponding GenBank accessions, nucleotide (nt) positions, and sizes, these templates provided riboprobes for β-actin (NM_031144.1, nt 1002–1116, 115 bases), TGF-β (NM_021578.1, nt 941–1111, 171 bases), collagen-α1(I) (XM_213440.2, nt 4430–4663, 234 bases), and serum glucocorticoid kinase (SGK; NM_019232.1, nt 2422–2090, 333 bases). Riboprobes were synthesized from linearized templates using T7 polymerase in the presence of [³²P]UTP. RNase protection assays were carried out using the RPA III kit from Ambion (Austin, TX). The kidney RNA and the riboprobes were hybridized at 60°C overnight and then exposed to RNase. After precipitation, the hybridization products were reconstituted, loaded onto a 6% denaturing poly-

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

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Acrlylamide gel, and electrophoresed. The phosphorimaging of the gel was analyzed by densitometry. The densities were normalized to the β-actin value for that sample. Yeast tRNA samples were used as negative controls.

Statistical analysis was performed using Student’s t-test for unpaired samples. The results are presented as means ± SD.

RESULTS

Infusion of aldosterone for 3 days more than doubled the urinary excretion of TGF-β compared with the sham controls and increased the urinary TGF-β concentration (Table 1). As expected, both the urinary excretion rate and the plasma level of aldosterone were higher in the rats receiving the mineralocorticoid infusion. Over this period, we observed no detectable effects of the hormone on body weight, systolic blood pressure, electrolytes and renin activity (Table 2).

We questioned the roles of potassium depletion and extracellular volume expansion with consequent renin inhibition in the observed stimulation of urinary TGF-β. To separate these effects, we studied separate rats simultaneously infused with aldosterone as before but also treated with the diuretic amiloride. These rats were compared with control rats receiving neither aldosterone nor amiloride (Table 3). The diuretic abrogated the hypokalemia and the apparent volume expansion, as indicated by plasma renin activity. However, a similar augmentation of urinary TGF-β occurred. Thus aldosterone enhances urinary TGF-β independent of its actions on cation transport. In another two groups of rats, we tested the effects of the mineralocorticoid receptor blocker spironolactone over the same 3-day time period. Compared with animals receiving aldosterone alone (n = 8), those receiving concurrent spironolactone (n = 6) did not show reductions in serum potassium (3.4 ± 0.2 vs. 4.2 ± 0.3 meq/l, respectively, P < 0.0001) but also did not increase urinary TGF-β (9.6 ± 2.0 vs. 6.2 ± 3.7 ng/day, respectively, P < 0.05). Thus the action of aldosterone on TGF-β works through the mineralocorticoid receptor.

To further assess the mechanism of the increased TGF-β, we measured its level of gene expression along with those of type 1 collagen, SGK, and β-actin. The last was used as a normalizing measure in the calculations, and indeed its level did not differ between aldosterone- and nonaldosterone-infused rats (Fig. 1). RNA samples extracted from cortex and medulla were analyzed separately. The only detectable change was an aldosterone-induced increase of approximately twofold in the ratio of SGK message to that of β-actin in the cortex (Table 4). This increase in SGK transcripts was predictable in view of the central role played by this kinase in the signal transduction pathway for aldosterone (17). The lack of an increase in TGF-β message suggests that its elevation occurs through a nontranscriptional route.

DISCUSSION

Aldosterone stimulated urinary TGF-β excretion by a route that is independent of the steroid’s effects on potassium and extracellular volume expansion and without augmentation of TGF-β gene transcription. The absence of hypertension or renal injury was by design. Production of the standard mineralocorticoid-salt model of hypertension requires longer-term steroid administration, a high salt intake, and unilateral nephrectomy. In the present study, the lack of injury as gauged by proteinuria, hypertension, or collagen I gene expression indicates that the action of aldosterone on TGF-β is a relatively direct one and not simply a late, nonspecific response to hypertensive renal injury.

Both TGF-β and the renin-angiotensin-aldosterone system (RAAS) participate in progressive renal injury (1, 6). Blockade of the RAAS at multiple sites mitigates kidney damage in experimental and clinical settings (6). TGF-β causes kidney damage when overexpressed in transgenic mice, and in vitro its application to cells provokes extracellular matrix production.
(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 uninjured 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.

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GRANTS

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