Involvements of Rho-Kinase and TGF-β Pathways in Aldosterone-Induced Renal Injury

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Recent studies have suggested a role for aldosterone in the pathogenesis of renal injury. This study investigated the potential contributions of Rho-kinase and TGF-β pathways to aldosterone-induced renal injury. Rats were uninephrectomized and then treated for 5 wk with 1% NaCl in a drinking solution and one of the following: Vehicle (2% ethanol, subcutaneously; n = 9); aldosterone (0.75 μg/h, subcutaneously; n = 9); or aldosterone + fasudil, a specific Rho-kinase inhibitor (10 mg/kg per d, subcutaneously; n = 8). Phosphorylation of myosin phosphate target subunit-1 (MYPT1) and Smad2/3 in renal cortical tissue was measured by Western blotting with anti-phospho MYPT1 and Smad2/3 antibodies, respectively. Rats that received aldosterone infusion exhibited hypertension and severe renal injury characterized by proteinuria, glomerular sclerosis, and tubulointerstitial fibrosis with increases in α-smooth muscle actin staining and numbers of monocytes/macrophages in the interstitium. Renal cortical mRNA levels of types I and III collagen, TGF-β, connective tissue growth factor, and monocyte chemoattractant protein-1 as well as Smad2/3 phosphorylation were significantly increased in rats that received aldosterone infusion. All of these changes were associated with an increase in renal tissue MYPT1 phosphorylation. Treatment with fasudil did not alter BP but significantly ameliorated proteinuria and renal injury in rats that received aldosterone infusion. Furthermore, fasudil prevented MYPT1 phosphorylation and markedly decreased α-smooth muscle actin staining, numbers of monocytes/macrophages, mRNA levels of types I and III collagen, TGF-β, connective tissue growth factor and monocyte chemoattractant protein-1, and Smad2/3 activity in renal cortical tissues. These results provide evidence, for the first time, that Rho-kinase is substantially involved in aldosterone-induced renal injury through activation of a TGF-β–dependent pathway.


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that the authors also showed that treatment with an MR antagonist prevented myosin light-chain phosphorylation and improved renal injury. These data suggest that the aldosterone/Rho-kinase pathway plays a role in mediating renal injury in Dahl salt-sensitive hypertensive rats. However, to the best of our knowledge, there is no convincing evidence to indicate the potential contribution of Rho-kinase to aldosterone-induced renal injury.

Our study was designed to test the hypothesis that Rho-kinase is substantially involved in the progression of aldosterone-induced renal injury. To test this hypothesis, we investigated whether renal injury that is induced by chronic treatment with aldosterone is associated with activation of Rho-kinase. Because Rho-kinase inhibits myosin phosphatase by phosphorylating its myosin-binding subunit, myosin phosphatase target subunit-1 (MYPT1) (18), we measured phosphorylated levels of MYPT1 in renal tissues as a marker of Rho-kinase activity (18,23,26). Further studies also were performed to examine the effects of a specific Rho-kinase inhibitor, fasudil (27,28), on renal injury that is induced by long-term treatment with aldosterone. Several studies using kinase assays have demonstrated that fasudil selectively inhibits Rho-kinase activity (27). It also has been shown that the specificity of fasudil for Rho-kinase is 100-fold higher than that for protein kinase C and 1000-fold higher than that for myosin light-chain kinase (28). Because both aldosterone and Rho-kinase have been suggested to induce tissue fibrosis through activation of TGF-β-dependent mechanism (22,24,29–31), we also examined the effects of fasudil on renal TGF-β, connective tissue growth factor (CTGF), and Smad2/3 in aldosterone-treated rats.

Materials and Methods

Animal Preparation

All experimental procedures were performed according to the guidelines for the care and use of animals established by Kagawa University Medical School. Five-week-old male Sprague-Dawley rats (CLEA Japan, Inc., Shizuoka, Japan) that weighed 182 to 206 g at the beginning of the experiments underwent right uninephrectomy under anesthesia with sodium pentobarbital (50 mg/kg, intraperitoneally). After 2 wk of recovery from surgery, rats were treated for 5 wk with 1% NaCl in a drinking solution and one of the following: (1) Vehicle (2% ethanol, subcutaneously; n = 9), (2) aldosterone (0.75 µg/h, subcutaneously; n = 9; Wako Co., Osaka, Japan), or (3) aldosterone + fasudil (10 mg/kg per d, subcutaneously; n = 8; Asahi Kasei Co., Tokyo, Japan). An osmotic minipump (model 2002; Alza Co, Palo Alto, CA) was implanted subcutaneously to infuse the vehicle and aldosterone and replaced every 2 wk under sodium pentobarbital anesthesia. The doses of aldosterone and fasudil were determined according to the results of previous studies on rats (3,4,21,32). We also performed preliminary experiments in aldosterone/salt-treated hypertensive rats (at week 5) to determine the subpressor dose of fasudil. The results showed that 20 mg/kg fasudil significantly decreased systolic BP (change at 1 h, −18 ± 5 mmHg; at 6 h, −7 ± 3 mmHg; n = 4). However, 10 mg/kg fasudil did not significantly alter SBP (change at 1 h, −5 ± 3 mmHg; at 6 h, −1 ± 3 mmHg; n = 4). On the basis of these data, we adopted 10 mg/kg per d as the subpressor dose of fasudil in these experiments.

SBP was measured in conscious rats by tail-cuff plethysmography (BP-98A; Softron Co., Tokyo, Japan), and 24-h urine samples were collected at 0, 1, 3, and 5 wk. Blood and kidney samples were collected at the end of week 5 under sodium pentobarbital anesthesia. Kidneys were perfused with chilled saline solution, then a piece of cross-sectioned kidney tissue (3 mm long) was fixed in 10% paraformaldehyde (pH 7.4) and embedded in paraffin for histologic examination. Remaining renal tissues were snap-frozen in liquid nitrogen and stored at −80°C until processing for RNA and protein extractions.

Histologic Examination

Kidneys were sectioned into 4-µm slices, then stained with periodic acid-Schiff (PAS) and Masson’s trichrome reagents. The severity of glomerular proliferation, interstitial fibrosis, and tubular proteinaceous cast scores were evaluated using light microscopy according to previously described methods (4,25,33–37). Briefly, glomerular proliferative lesions were scored into five grades as follows: 0, no proliferation; 1, minor (segmental lesion <25%) but <50%; 2, mild (segmental lesion >25% but <50%); 3, moderate (diffuse proliferation without severe sclerotic change); and 4, severe (diffuse proliferation with nearly complete sclerosis) (4,33,34,36). A minimum of 40 glomeruli were examined in each PAS-stained specimen. The severity of tubulointerstitial fibrosis was assessed by counting the percentage of injured areas per field and then scored as follows: 0, normal interstitium; 0.5, <5% of areas injured; 1, 5 to 15%; 1.5, 16 to 25%; 2, 26 to 35%; 2.5, 36 to 45%; and 3, >45% (35,36). A minimum of 20 fields were evaluated for each Masson’s trichrome–stained specimen. Tubular proteinaceous cast scores were scored into five grades as follows: 0, no damage; 1, mild (patchy isolated damage); 2, moderate (damage <25%); 3, severe (damage >25% but <50%); and 4, very severe (damage >50%). A minimum of 10 fields were evaluated for each Masson’s trichrome–stained specimen. Proteinaceous cast–positive areas in tubuli (strong red) were calculated using Image-Pro plus software (Media Cybernetics, Silver Spring, MD), and these affected areas in turn were divided by the total tubular area of the microscopic field (25,34,37). The above histologic analyses were performed using a color image analyzer (DP 70 and DP controller; Olympus, Tokyo, Japan) in a blind manner to avoid bias.

Immunohistochemistry

Commercially available antibodies were used for immunohistochemistry of α-smooth muscle actin (α-SMA; Sigma Chemical Co., St. Louis, MO) and monocytes/macrophages (ED1; Serotec, Oxford, UK). α-SMA is an actin isofrom that is expressed in smooth muscle cells, fibroblasts, and mesangial cells that undergo myofibroblastic transformation and thus is associated with matrix synthesis and therefore acts as a marker of future fibrosis (36,38). Sections were immunostained following standard procedures using a robotic system (Dako Autostainer; Dako, Carpinteria, CA), as described previously in detail (36).

Western Blot Analysis

As described previously (23,26,39), we used immunoblotting with antibodies against phospho-MYPT1 (at Thr-696; 1:2000 dilution; Upstate Biochemistry, Lake Placid, NY) and phospho-Smad2/3 (at Ser-433/435; 1:2000 dilution; Santa Cruz Biotechnology Inc., Santa Cruz, CA) to evaluate Rho-kinase and Smad2/3 activation, respectively. To check for equal loading, membranes were reprobed with an antibody against β-actin (1:10,000 dilution; Sigma Chemical). Data are expressed as the relative differences between vehicle-treated rats and aldosterone- or aldosterone + fasudil–treated rats after normalization to β-actin expression.

Real-Time PCR

The mRNA expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fiber-forming collagens (types I and III collagen), TGF-
β₁, CTGF, and monocyte chemotactic protein-1 (MCP-1) were analyzed by real-time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) (17,33,34). The oligonucleotide primer sequences of GAPDH and types I and III collagen were reported previously (17,33). TGF-β₁, CTGF, and MCP-1 mRNA expression were measured using TaqMan Gene Expression Assay kits (Applied Biosystems) (33,34). Data are expressed as the relative differences in aldosterone- or aldosterone + fasudil-treated rats compared with vehicle-treated rats after normalization to the expression of GAPDH.

Other Analytical Procedures
Protein concentrations in urine were determined using assay kits (microTP-test, Wako Co.). Creatinine concentrations in plasma and urine also were measured using assay kits (Creatinine-test; Wako Co.).

Statistical Analyses
Values are presented as means ± SEM. Statistical comparisons of differences were performed using one- or two-way ANOVA combined with Newman-Keuls post hoc test. P < 0.05 was considered statistically significant.

Results

Body and Kidney Weights and BP
As shown in Table 1, rats that received aldosterone infusion showed a reduced body weight compared with animals that receive vehicle infusion, but treatment with fasudil did not alter the body weight of rats that received aldosterone infusion. However, kidney weight and the kidney weight/body weight ratio were much higher in rats that received aldosterone infusion than in rats that received vehicle infusion. Treatment with fasudil significantly decreased the kidney weight and kidney weight/body weight ratio of rats that received aldosterone infusion (Table 1). The temporal profile of SBP is depicted in Figure 1A. SBP was almost identical in the three groups at the beginning of the protocol (2 wk after uninephrectomy). It remained unaltered in rats that received vehicle infusion, whereas rats that received aldosterone infusion progressively developed hypertension (193 ± 3 mmHg at week 5). Treatment with fasudil did not significantly alter SBP in rats that received aldosterone infusion (191 ± 9 mmHg at week 5).

Figure 1. The profiles of systemic BP (SBP; A) and urinary excretion rate of protein (Uprotein/V; B). Rats that received aldosterone infusion showed hypertension and severe proteinuria. Treatment with fasudil did not alter SBP but markedly ameliorated proteinuria in rats that received aldosterone infusion. *P < 0.05 versus vehicle; †P < 0.05, aldosterone versus aldosterone + fasudil.

Urinary Excretion Rate of Protein, Plasma Creatinine, andCreatinine Clearance
The temporal profile of urinary excretion rate of protein is depicted in Figure 1B. Aldosterone infusion resulted in severe proteinuria (362 ± 93 mg/d at week 5), and treatment with fasudil markedly reduced urinary excretion rate of protein in rats that received aldosterone infusion (96 ± 22 mg/d at week 5). As shown in Table 1, rats that received aldosterone infusion showed increased plasma creatinine levels and decreased creatinine clearance compared with rats that received vehicle infusion. Treatment with fasudil prevented aldosterone-induced changes in plasma creatinine levels and creatinine clearance (Table 1).

Histologic Findings
The glomerular histologic findings with PAS staining at week 5 are shown in Figure 2, A through E, and renal cortical and medullary histologic findings with Masson’s trichrome staining are illustrated in Figure 3, A through C and D through F, respectively. Rats that received vehicle infusion showed almost

Table 1. Effects of aldosterone and fasudil on body weight, left kidney weight, the kidney weight/body weight ratio, plasma creatinine, and creatinine clearance in uninephrectomized rats that treated with 1% NaCl for 5 wk

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (2% Ethanol; n = 9)</th>
<th>Aldosterone (0.75 μg/h; n = 9)</th>
<th>Aldosterone + Fasudil (10 mg/kg per d; n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>522 ± 8</td>
<td>430 ± 10b</td>
<td>450 ± 7b</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>2.97 ± 0.14</td>
<td>5.74 ± 0.23b</td>
<td>4.75 ± 0.34b,c</td>
</tr>
<tr>
<td>Kidney weight/body weight (%)</td>
<td>0.52 ± 0.03</td>
<td>1.32 ± 0.08b</td>
<td>0.95 ± 0.07b,c</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>0.59 ± 0.01</td>
<td>0.73 ± 0.09b</td>
<td>0.60 ± 0.02c</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per g kidney wt)</td>
<td>0.58 ± 0.04</td>
<td>0.26 ± 0.01b</td>
<td>0.35 ± 0.02b,c</td>
</tr>
</tbody>
</table>

aData are means ± SEM.

bP < 0.05 versus 1% NaCl + vehicle.

cP < 0.05: 1% NaCl + aldosterone versus 1% NaCl + aldosterone + fasudil.
normal glomeruli (Figure 2, A and F) and tubulointerstitium (Figure 3, A, D, G, and H); however, rats that received aldosterone infusion exhibited injured glomeruli characterized by cell proliferation and sclerosis (Figure 2, B, C, and F). In rats that received aldosterone infusion, severe tubulointerstitial fibrosis and proteinaceous casts also were observed (Figure 3, B, E, G, and H). Treatment with fasudil significantly attenuated aldosterone-induced renal injuries (Figures 2 and 3).

Renal cortical and medullary immunohistochemistry with α-SMA antibody at week 5 are illustrated in Figure 4, A through C and D through F, respectively. Renal cortical immunohistochemistry with ED-1 antibody are illustrated in Figure 4, G through I. In rats that received vehicle infusion, α-SMA was observed only on the blood vessel walls (Figure 4, A and D). Furthermore, ED-1–positive cells rarely were observed in renal interstitial spaces in rats that received vehicle infusion (Figure 4G). However, in rats that received aldosterone infusion, renal injury was associated with increases in the numbers of α-SMA–positive (Figure 4, B and E) and ED-1–positive (Figure 4H) cells in the renal interstitium. The numbers of α-SMA– and ED-1–positive cells in the renal interstitium seemed to be much less in aldosterone + fasudil–treated rats compared with rats that were treated with aldosterone alone (Figure 4, C, F, and I).

**MYPT1 and Smad2/3 Phosphorylation**

Western blot analysis revealed that the levels of MYPT1 phosphorylation in renal cortical tissues were markedly increased in rats that received aldosterone infusion (5.5 ± 0.7-fold increase versus rats that received vehicle infusion; Figure 5A), indicating that Rho-kinase was activated in kidneys of rats that received aldosterone infusion. The aldosterone-induced increase in renal cortical MYPT1 phosphorylation was prevented by treatment with fasudil (Figure 5A).

As shown in Figure 5B, renal cortical phosphorylated levels of Smad2/3, which are intracellular mediators of TGF-β signaling pathways (39), were significantly increased in rats that received aldosterone infusion (4.9 ± 0.2-fold increase versus rats that received vehicle infusion; Figure 5B). Treatment with fasudil attenuated aldosterone-induced renal cortical Smad2/3 phosphorylation (Figure 5B).

**Gene Expression of Collagen, TGF-β1, CTGF, and MCP-1**

Compared with rats that received vehicle infusion, rats that received aldosterone infusion showed increased renal cortical mRNA levels of types I and III collagen, TGF-β1, CTGF, and MCP-1 by 3.8 ± 0.2-, 3.0 ± 0.1-, 2.9 ± 0.1-, 2.8 ± 0.2-, and 3.2 ± 0.2-fold, respectively (Figure 6). Treatment with fasudil significantly decreased these mRNA levels in rats that received aldosterone infusion (Figure 6).

**Discussion**

In agreement with previous studies (5,34), our study showed that long-term administration of aldosterone and salt to uninephrectomized rats induces hypertension and severe renal injury characterized by proteinuria, glomerular sclerosis, and interstitial fibrosis. Our study also provides evidence, for the first time, that aldosterone-induced renal injury is associated
with increases in phosphorylated levels of MYPT1, a marker of Rho-kinase activity (18,23,26). In addition, treatment with fasudil, a specific Rho-kinase inhibitor (27,28), did not alter BP but markedly attenuated the progression of renal injury in these rats. These data support the hypothesis that Rho-kinase is substantially involved in aldosterone-induced renal injury.

Renal fibrosis is characterized mainly by an excessive synthesis and accumulation of extracellular matrix components, including collagen (40). TGF-β has been shown to play roles in the process of fibrogenesis and collagen synthesis through activation of its downstream effectors, including Smad and CTGF (39). This study showed that in rats that received aldosterone infusion, renal fibrosis is associated with increases in types I and III collagen expression, accompanied by increases in TGF-β1 and CTGF expression as well as Smad2/3 activity. These data suggest a possible contribution of TGF-β pathway to aldosterone-induced renal fibrosis. Of interest, recent studies also have indicated that Rho-kinase plays a role in mediating renal fibrosis through a TGF-β–dependent mechanism (22,24,29,30). Nishikimi et al. (24) showed that increased TGF-β and collagen expression in injured kidneys of Dahl salt-sensitive hypertensive rats was associated with augmented gene expression of several Rho-kinase families. Furthermore, treatment with a subdepressor dose of fasudil attenuated augmentation of TGF-β and collagen expression and improved renal injury in these animals (24). Similarly, another specific Rho-kinase inhibitor, Y-27632, suppressed interstitial fibrosis and augmentation of TGF-β and collagen expression in mouse kidneys with unilateral ureteral obstruction (22). Recent in vitro studies also demonstrated that Rho-kinase is an essential factor for mechanical stretch-induced TGF-β synthesis in hepatic stellate cells (29). Furthermore, the inhibition of Rho-kinase activity prevents TGF-β–induced increases in CTGF accumulation in cultured human renal fibroblasts (30). In our study, Rho-kinase inhibition with fasudil significantly attenuated aldosterone-induced renal fibrosis and augmentation of collagen, TGF-β1, and CTGF expression as well as Smad2/3 activity in the kidney. These data suggest that Rho-kinase plays an important role in mediating aldosterone-induced activation of TGF-β–dependent pathway, possible leading to collagen accumulation and the progression of renal fibrosis.

Renal fibrosis also is induced by cell differentiation (36,38,40). Campbell et al. (41) showed that long-term treatment with aldosterone results in increases in α-SMA expression in

Figure 3. Photomicrographs of cortex (A through C) and medulla (D through F). Tubulointerstitial fibrosis (G) and proteinaceous casts in tubuli (H) were evaluated as described in Materials and Methods. Rats that received aldosterone infusion exhibited interstitial fibrosis (B, E, and G) and proteinaceous casts in tubuli (B, E, and H). Treatment with fasudil significantly attenuated these aldosterone-induced renal injuries (C, F, G, and H). *P < 0.05 versus vehicle; †P < 0.05, aldosterone versus aldosterone + fasudil. Magnification, ×100, Masson’s trichrome.

Figure 4. Representative photographs of immunohistochemistry for α-smooth muscle actin (α-SMA; A through F) and ED-1 (G through I). In rats that received vehicle infusion, α-SMA was observed only on the blood vessel walls (A and D), whereas ED-1–positive cells rarely were observed (G). However, in rats that received aldosterone infusion, renal injury was associated with increases in the numbers of α-SMA–positive (B and E) and ED-1–positive (H) cells in the renal interstitium. The numbers of α-SMA– and ED-1–positive cells in the renal interstitium seemed to be much less in aldosterone + fasudil–treated rats compared with rats that were treated with aldosterone alone (C, F, and I). Magnifications: ×100 in A through F; ×200 in A through I.
the myocardium. Enhanced expression of α-SMA is a marker of interstitial phenotypic changes in renal fibrosis, and these activated interstitial cells are known as myofibroblasts (36,38). It is interesting that it also has been revealed that the Rho-kinase signaling pathway plays a key role in the regulation of myofibroblastic transformation (22,42–45). In mouse kidneys with unilateral ureteral obstruction, treatment with a Rho-kinase inhibitor attenuated renal interstitial α-SMA expression and fibrosis (22). Mack et al. (42) reported that smooth muscle differentiation marker gene expression is regulated by Rho-kinase–mediated actin polymerization and that Rho-kinase inhibition blocks the synthesis of the α-SMA isoform. It also was shown that Rho-kinase regulates α-SMA promoter activity in glomerular mesangial cells (43) and induces mesenchymal transdifferentiation of proximal tubular cells (44). Further in vitro studies demonstrated that TGF-β1 induces mesenchymal transdifferentiation through RhoA-dependent pathway (45). In our study, aldosterone-induced renal fibrosis was associated with increases in Rho-kinase activity and α-SMA expression. Moreover, Rho-kinase inhibition with fasudil did not alter BP but markedly attenuated aldosterone-induced augmentation of α-SMA expression and renal fibrosis. These data suggest that aldosterone-induced Rho-kinase activation contributes to myofibroblastic transformation in renal cells, possibly contributing to the progression of renal fibrosis.

Monocyte/macrophage infiltration is one of the key mechanisms of the progression of renal fibrosis (36,38). Consistent with a previous study (5), chronic treatment with aldosterone/salt resulted in enhancement of ED-1–positive monocyte/macrophage infiltration with an increase in expression of MCP-1 as a potent chemoattractant of monocytes/macrophages (36,38). We also revealed that the inhibition of Rho-kinase with fasudil strongly suppressed the expression of MCP-1 and monocyte/macrophage infiltration. These findings are in agreement with those of previous studies indicating the protective effects of Rho-kinase inhibitors on monocyte/macrophage infiltration and interstitial fibrosis in different renal disease models (21–23). Furthermore, in vitro studies using chemoattractants have demonstrated that neutrophil chemotaxis is significantly inhibited by fasudil (46). Collectively, these data suggest that at least some of the renoprotective effects of Rho-kinase inhibitor on aldosterone-induced renal injury are mediated by inhibition of monocytes/macrophage infiltration.

In our study, treatment with a Rho-kinase inhibitor did not alter BP but markedly attenuated the progression of renal injury in rats that received aldosterone infusion, suggesting the potential contribution of aldosterone-induced Rho-kinase activation to renal injury, independent of BP changes. These results are in agreement with previous studies indicating that aldosterone has BP-independent deleterious effects on the kidney (6–17). However, it has been reported that deoxycorticosterone-salt hypertensive rats elicit significant diurnal variation (47).

Figure 5. (A) Phosphorylated levels of myosin phosphate target subunit-1 (MYPT1) in renal cortical tissues. MYPT1 phosphorylation in renal cortical tissues was markedly increased in rats that received aldosterone infusion, indicating Rho-kinase activation in the kidney. The aldosterone-induced increase in renal cortical MYPT1 phosphorylation was prevented by treatment with fasudil. (B) Phosphorylated levels of Smad2/3 in renal cortical tissues. Smad2/3 phosphorylation in renal cortical tissues was markedly increased in rats that received aldosterone infusion, suggesting activation of the TGF-β–dependent signaling pathway in the kidney. The aldosterone-induced increase in renal cortical Smad2/3 phosphorylation was attenuated by treatment with fasudil. Data are expressed as the relative differences between vehicle-treated rats and aldosterone- or aldosterone + fasudil–treated rats after normalization to β-actin expression. *P < 0.05 versus vehicle; †P < 0.05, aldosterone versus aldosterone + fasudil.
Furthermore, because it is difficult to detect small changes in BP using tail-cuff plethysmography, a telemetry system for continuous monitoring of actual BP changes would be necessary to address these issues.

**Conclusion**

Our study provides evidence, for the first time, that aldosterone-induced renal injury is associated with activations of Rho-kinase and TGF-β/β1 pathways. In addition, treatment with fasudil, a specific Rho-kinase inhibitor, attenuates aldosterone-induced TGF-β activation and ameliorates the progression of renal injury. These data support the hypothesis that Rho-kinase is substantially involved in aldosterone-induced renal injury via activation of TGF-β/β1-dependent pathway. Inhibition of the Rho-kinase pathway therefore might be a useful therapeutic strategy for preventing aldosterone-dependent renal injury.

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