Chronic Activation of Glomerular Mitogen-Activated Protein Kinases in Dahl Salt-Sensitive Rats

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Abstract. The in vivo role of mitogen-activated protein kinases (MAPK) in the development of glomerular injury is poorly understood. In the present study, glomerular MAPK activities, including extracellular signal-regulated kinases (ERK), c-Jun NH₂-terminal kinases (JNK), and transcriptional factor, activator protein-1 (AP-1) were examined in glomerular injury of salt-induced hypertensive rats. Six-week-old Dahl salt-sensitive (Dahl-S) and salt-resistant (Dahl-R) rats were maintained on a high-salt (8.0% NaCl) diet for 1, 5, and 10 wk. In Dahl-S rats, as shown by in-gel kinase assay, an increase in BP by a high-salt diet was followed by chronic activation of glomerular ERK and JNK, which continued until 10 wk after a high-salt diet. Western blot analysis demonstrated a significant increase in the protein expression of glomerular ERK and JNK in Dahl-S rats fed a high-salt diet. As determined by gel-mobility shift assay, ERK and JNK activations were associated with an increase in glomerular AP-1 DNA binding activity. On the other hand, in Dahl-R rats fed a high-salt diet, BP remained normal throughout the experiments. However, glomerular ERK and JNK activities and AP-1 DNA binding activity in Dahl-R rats were not affected by 1 or 5 wk of a high-salt diet, but significantly increased by 10 wk of treatment with a high-salt diet, indicating that chronic sodium overload itself stimulated glomerular ERK and JNK and AP-1 activities. These kinase activations in both Dahl-S and Dahl-R rats were accompanied by an increase in urinary protein excretion and renal growth. These observations provide the first evidence that salt-sensitive hypertension causes chronic activation of glomerular ERK and JNK, probably leading to the activation of AP-1. Thus, glomerular MAPK may be responsible for the development of salt-induced glomerular injury.

Dietary salt intake plays an important role not only in determining BP, but also in the progression of glomerular injury (1–3). The Dahl salt-sensitive (Dahl-S) rats have been regarded as the most popular model of human salt-sensitive hypertension (4). Furthermore, a high-salt diet significantly accelerates glomerular hypertrophy and sclerosis in Dahl-S rats (4,5). However, the molecular mechanism of glomerular injury by salt-induced hypertension remains to be elucidated.

Extracellular signal-regulated kinase (ERK) and c-Jun NH₂-terminal kinase (JNK), belonging to the subgroup of mitogen-activated protein kinases (MAPK), are protein serine/threonine kinases (6,7). Accumulating in vitro evidence indicates that ERK is activated mainly by mitogenic stimuli such as growth factors and vasoactive hormones (7,8). JNK is activated mainly by stress signal or inflammatory cytokines (9,10). Activated ERK is known to be involved in induction of c-fos via the phosphorylation of TCF/Elk-1 (11). Furthermore, JNK is known to increase c-Jun transactivation activity by phosphorylation of the N-terminal-activating domain (11). Fos and Jun proteins form transcriptional factor, activator protein-1 (AP-1) (11), which is known to regulate the expression of various genes such as transforming growth factor-β1 (TGF-β1) (12,13). However, previous reports on MAPK were almost limited to in vitro studies, and the activation and role of MAPK in vivo are poorly understood. Recently, we have provided in vivo evidence that acute hypertension by angiotensin II (AngII) infusion causes the rapid and transient activation of glomerular ERK and JNK, followed by the activation of AP-1 (14). However, the chronic effect of hypertension on glomerular ERK and JNK remains to be examined. Moreover, the role of ERK and JNK in development of glomerular injury has not yet been determined.

In the present study, using Dahl-S rats, we examined the effects of salt-induced hypertension on glomerular MAPK. We have obtained in vivo evidence that glomerular injury induced by salt-sensitive hypertension was associated with the chronic enhancement of glomerular ERK and JNK activities.

Materials and Methods

Materials

Myelin basic protein was purchased from Sigma Chemical Co. (St. Louis, MO). Rabbit anti-polyclonal IgG raised against p44 ERK (C-16), p42 ERK (C-14), JNK (FL), c-Fos (K-25), and c-Jun (N) were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-polyclonal phospho-specific ERK antibody was purchased from New England Biolabs (Beverly, MA). Anti-monoclonal α-tubulin antibody (Ab-1) was purchased from Oncogene Research Products (Cambridge, MA).

Animals and Experimental Design

All procedures were in accordance with institutional guidelines for the care and use of laboratory animals. Male Dahl-S and Dahl salt-resistant (Dahl-R) rats were purchased from Japan SLC (Shizuoka,
Japan) and were maintained on 0.3% NaCl (a low-salt diet) until the start of the experiments (6 wk of age).

Six-week-old Dahl-S and Dahl-R rats were maintained on 8% NaCl (a high-salt diet) or a low-salt diet for 1, 5, and 10 wk. These high-salt and low-salt diets were identical in composition except for NaCl content and were purchased from Oriental Yeast Co. (Tokyo, Japan). The diets and tap water were given ad libitum throughout the experiments. Systolic BP of conscious rats was measured by the tail-cuff method. Twenty-four-hour urine samples were collected in metabolic cages for measurement of urinary protein excretion. At 1, 5, or 10 wk of treatment, rats were decapitated and bilateral kidneys were immediately removed. Then, glomeruli were isolated from bilateral kidneys of each animal by the sieving method in ice-cold phosphate-buffered saline containing 2.5 mmol/L ethylenediamine tetra-acetic acid (EDTA), 10 mmol/L NaF, 1 mmol/L Na3 VO4, and 25 mmol/L β-glycerophosphate (14). For each animal, half of the isolated glomeruli were immediately frozen in liquid nitrogen and stored at −80°C until protein kinase assay or Western blot analysis. Half were rapidly homogenized to extract nuclear proteins for gel-mobility shift assay, as described below.

**Preparation of Glomerular Protein**

For protein kinase assay and Western blot analysis, frozen glomeruli from each rat were homogenized on ice with a Polytron homogenizer (PCU-11; Kinematica, Littau/Luzern, Switzerland) in 0.4 ml of buffer A (20 mmol/L Hepes, pH 7.2, 25 mmol/L NaCl, 2 mmol/L ethyleneglycol-bis-(β-aminoethyl ether)-N,N′-tetra-acetic acid [EGTA], 50 mmol/L NaF, 1 mmol/L Na3 VO4, 25 mmol/L β-glycerophosphate, 0.2 mmol/L dithiothreitol [DTT], 1 mmol/L phenylmethylsulfonyl fluoride [PMSF], 60 μg/ml aprotinin, and 2 μg/ml leupeptin) and incubated on ice for 30 min. After the homogenates were sonicated on ice for 15 s, the supernatants were obtained by centrifugation for 30 min at 15,000 rpm at 4°C and stored at −80°C until use.

**In-Gel Protein Kinase Assay**

The activities of glomerular ERK and JNK were determined by in-gel kinase assay as described previously (14). In brief, protein extracts (each 10 and 20 μg for ERK and JNK assay, respectively) were subjected to electrophoresis on 12% sodium dodecyl sulfate-polyacrylamide gels containing myelin basic protein (0.5 mg/ml) or glutathione S-transferase-c-Jun (1-79) fusion protein (0.1 mg/ml) as a substrate of ERK or JNK, respectively. After electrophoresis, gels were washed with 20% isopropanol in 50 mmol/L Tris-HCl, pH 8.0, and then washed with 5 mmol/L β-mercaptoethanol in 50 mmol/L Tris-HCl, pH 8.0. Proteins in the gels were denatured by incubation for 1 h in 6 mol/L guanidine-HCl, 5 mmol/L β-mercaptoethanol, and 50 mmol/L Tris-HCl, pH 8.0, and renatured in 0.04% Tween 40, 5 mmol/L β-mercaptoethanol, and 50 mmol/L Tris-HCl, pH 8.0, at 4°C. Kinase reaction was performed by incubation in kinase buffer (40 mmol/L Hepes, pH 7.5, 0.1 mmol/L EGTA, 20 mmol/L MgCl2, and 2 mmol/L DTT) with 25 μmol/L ATP and 25 μCi [γ-32P]ATP at 25°C for 1 h. After kinase reaction, gels were washed extensively with 5% TCA and 1% sodium pyrophosphate, dried, and subjected to autoradiography. To evaluate kinase activities, we digitized autoradiograms and measured their density using the public domain National Institutes of Health IMAGE program.

**Electrophoretic Gel Mobility Shift Assay**

For electrophoretic gel mobility shift assay, glomerular nuclear proteins were extracted, as described in detail (14). In brief, isolated glomeruli from each rat were rapidly suspended in 0.4 ml of 10 mmol/L Hepes, pH 7.9, 10 mmol/L KCl, 0.1 mmol/L EDTA, 0.1 mmol/L EGTA, 1.5 mmol/L MgCl2, 10 mmol/L NaF, 1 mmol/L Na3 VO4, 1 mmol/L DTT, 20 mmol/L β-glycerophosphate, 0.5 mmol/L PMSF, 60 μg/ml aprotinin, and 2 μg/ml leupeptin. The suspension was gently homogenized with Dounce homogenizer and incubated on ice for 15 s. Twenty-five microliters of 10% Nonidet

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**Figure 1. Effects of a high-salt diet on BP (A) and urinary protein excretion (UprotV) (B) in Dahl salt-sensitive and salt-resistant rats.** Dahl-S (high salt), Dahl salt-sensitive rats fed 8% NaCl diet; Dahl-S (low salt), Dahl salt-sensitive rats fed 0.3% NaCl diet; Dahl-R (high salt), Dahl salt-resistant rats fed 8% NaCl diet; Dahl-R (low salt), Dahl salt-resistant rats fed 0.3% NaCl diet. Each value represents the mean ± SEM. *P < 0.01 compared with Dahl-S (low salt); †P < 0.05 compared with Dahl-R (low salt).
P-40 was then added, and the homogenate was vigorously vortex-mixed for 10 s. The nuclear fraction was precipitated by centrifugation at 5000 rpm for 10 min at 4 °C and then resuspended in 50 μl of 20 mmol/L Hepes, pH 7.9, 0.4 mol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1.5 mmol/L MgCl₂, 20% glycerol, 10 mmol/L NaF, 1 mmol/L Na₃VO₄, 0.2 mmol/L DTT, 20 mmol/L β-glycerophosphate, 0.5 mmol/L PMSF, 60 μg/ml aprotinin, and 2 μg/ml leupeptin. The mixture was incubated on ice for 15 min and centrifuged at 15,000 rpm at 4 °C for 10 min, and the resulting supernatants were stored at −80°C until use.

Electrophoretic gel mobility shift assays of nuclear protein extracts were performed, as described in detail (14). Briefly, the sequences of double-stranded oligonucleotide used for gel-mobility shift assays were as follows: consensus AP-1, 5′-CGCTTGA TGACTCA GCCGGAA-3′; mutant AP-1, 5′-CGCTTGA TGACTTG GCCGGAA-3′. These consensus probes were end-labeled with [γ-32P]ATP using T4 polynucleotide kinase and purified by chromatography on a Bio-Spin column (Bio-Rad, Richmond, CA). For DNA-protein binding reaction, the samples of nuclear extracts (5 μg of protein) were incubated with 32P-labeled consensus oligonucleotide for 20 min at room temperature in 20 mmol/L Hepes, pH 7.9, 0.3 mmol/L EDTA, 0.2 mmol/L EGTA, 80 mmol/L NaCl, 1 mmol/L DTT, 0.2 mmol/L PMSF, 6% glycerol, and 2 μg of poly(deoxyinosinic-deoxyctydyllic) (Pharmacia, Uppsala, Sweden). The DNA-protein complexes were separated from free DNA probe by electrophoresis on 4% nondenaturing acrylamide gels in 6.7 mmol/L Tris-HCl, pH 7.5, 3.3 mmol/L sodium acetate, 0.1 mmol/L EDTA, and 2.5% glycerol. Gels were run at 200 V at 4 °C for 3 h, dried, and subjected to autoradiography.

To demonstrate the specificity of DNA-protein binding, the reactions were performed in the presence of nonlabeled consensus oligonucleotide competitor or nonlabeled mutant oligonucleotide competitor. Furthermore, supershift assay was carried out by using rabbit

Table 1. Effects of 10 wk of a high-salt diet on body weight and kidney weight

<table>
<thead>
<tr>
<th>Category</th>
<th>n</th>
<th>BW (g)</th>
<th>KW (g)</th>
<th>KW/BW (g/100 g BW)</th>
</tr>
</thead>
<tbody>
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<td>Dahl-S (low-salt)</td>
<td>10</td>
<td>406.4 ± 11.7</td>
<td>2.44 ± 0.09</td>
<td>0.60 ± 0.02</td>
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<tr>
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<td>320.8 ± 13.2</td>
<td>3.30 ± 0.14</td>
<td>1.03 ± 0.02</td>
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<tr>
<td>Dahl-R (low-salt)</td>
<td>9</td>
<td>457.6 ± 13.1</td>
<td>2.40 ± 0.07</td>
<td>0.52 ± 0.01</td>
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<tr>
<td>Dahl-R (high-salt)</td>
<td>10</td>
<td>463.6 ± 7.0</td>
<td>3.29 ± 0.07</td>
<td>0.71 ± 0.01</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM. BW, body weight; KW, kidney weight; Dahl-S, Dahl salt-sensitive rats; Dahl-R, Dahl salt-resistant rats.

b P < 0.01 versus Dahl-S (low-salt).

c P < 0.01 versus Dahl-R (low-salt).

Figure 2. Effects of a high-salt diet on glomerular extracellular signal-regulated kinases (ERK) (A) and c-Jun NH₂-terminal kinases (JNK) (B) activities in Dahl salt-sensitive (Dahl-S) rats. The top panels show representative autoradiograms of glomerular ERK (A) and JNK (B) activities from Dahl-S rats fed a low-salt (0.3% NaCl) or a high-salt (8% NaCl) diet for 5 wk, as determined by in-gel kinase assay. Bar graphs show the density of each autoradiogram, quantified by using an imaging analyzer as described in Materials and Methods. The mean value of kinase activities from Dahl-S rats fed a low-salt diet at each time point is represented as 1. Each value represents the mean ± SEM (n = 6).
polyclonal IgG against c-Fos or c-Jun. The specific antibodies were added to samples after the initial binding reaction between nuclear protein extracts and 32P-labeled consensus oligonucleotide, and the incubation was performed at room temperature for 1 h.

**Statistical Analyses**

Data are expressed as mean ± SEM. Statistical significance was determined by unpaired t test. Differences were considered statistically significant at \( P < 0.05 \).

**Results**

**BP, Urinary Protein Excretion, and Kidney Weight in Dahl-S and Dahl-R Rats**

As shown in Figure 1A, both Dahl-R and Dahl-S rats fed a low-salt diet were normotensive throughout the experiments. In Dahl-S rats, a high-salt diet progressively increased BP (156 ± 3, 209 ± 5, and 202 ± 6 mmHg at 1, 5, and 10 wk, respectively). On the other hand, BP of Dahl-R rats was hardly affected by a high-salt diet and remained normotensive throughout the treatment with a high-salt diet (115 ± 3, 112 ± 3, and 117 ± 2 mmHg at 1, 5, and 10 wk, respectively).

As shown in Figure 1B, a high-salt diet significantly and progressively increased urinary protein excretion of Dahl-S rats, compared with a low-salt diet (4.4-, 4.3-, and 9.0-fold increase after 1, 5, and 10 wk of a high-salt diet, respectively). After 10 wk of treatment with a high-salt diet, the kidney weight of Dahl-S rats was also significantly increased compared with that of Dahl-S rats fed a low-salt diet (Table 1). As shown in Figure 1B and Table 1, although Dahl-R rats were normotensive throughout 10 wk of a high-salt diet, urinary protein excretion and kidney weight of Dahl-R rats significantly increased after 10 wk of a high-salt diet, compared with a low-salt diet. Body weight of Dahl-S rats was decreased by a high-salt diet, consistent with a previous report (15). Decreased body weight in Dahl-S rats fed a high-salt diet may be partly due to an increase in urinary excretion.

**Glomerular ERK and JNK Activities in Dahl-S Rats**

As shown by in-gel kinase assay of ERK in Figure 2A, glomerular p42 ERK and p44 ERK activities of Dahl-S rats were increased by 1.8- and 2.0-fold, respectively, after 5 wk of a high-salt diet treatment. A 10-wk treatment with a high-salt diet further increased glomerular p42 ERK and p44 ERK activities of Dahl-S rats, compared with treatment with a low-salt diet.

As shown in Figure 2B, glomerular p55 JNK and p46 JNK activities in Dahl-S rats were already significantly increased by 2.4- and 1.8-fold, respectively, by a high-salt diet for 1 wk. The increase in p55 JNK and p46 JNK activities peaked at 5 wk of treatment with a high-salt diet, and continued until 10 wk of the high-salt diet.

**Glomerular ERK and JNK Protein Levels in Dahl-S Rats**

We performed Western blot analysis using specific antibodies against ERK or JNK to determine protein levels of glomerular ERK and JNK. As shown in Figure 3, protein levels of glomerular p44 ERK and p42 ERK in Dahl-S rats fed a high-salt diet for 5 and 10 wk were significantly higher than Dahl-S rats fed a low-salt diet for the same period.

Glomerular p46 JNK protein levels of Dahl-S rats were increased by 1.7-, 2.6-, and 1.5-fold by a high-salt diet for 1, 5, and 10 wk, respectively (Figure 3). Sensitivity of Western blot analysis did not permit us to detect glomerular p55 JNK protein band from Dahl-S rats fed a low-salt diet for 1, 5, and 10 wk. However, by Western blot analysis, we could detect glomerular p55 JNK protein band only in Dahl-S rats fed a high-salt diet for 1, 5, or 10 wk, indicating that like p46 JNK protein, p55 JNK protein levels were higher in Dahl-S rats fed a high-salt diet.

**Glomerular ERK and JNK Activities and Protein Levels in Dahl-R Rats**

Figures 4 and 5 showed glomerular activities and protein levels of ERK and JNK in Dahl-R rats, respectively. As shown in Figure 4, a high-salt diet for 5 wk did not affect glomerular activities of p42 ERK, p44 ERK, p46 JNK, or p55 JNK. However, 10 wk of treatment with a high-salt diet significantly increased both ERK and JNK activities in Dahl-R rats. As shown by Western blot analysis in Figure 5, glomerular protein...
levels of p44 ERK, p42 ERK, or p46 JNK were not changed by a high-salt diet for 5 wk, but were significantly increased by a high-salt diet for 10 wk. Glomerular p55 JNK protein band was detected only in Dahl-S rats fed a high-salt diet for 10 wk, but not detected in those fed a low-salt for the same period, indicating that a high-salt diet for 10 wk increased glomerular p55 JNK protein in Dahl-R rats, like p46 JNK.

**Glomerular AP-1 DNA Binding Activity in Dahl-S and Dahl-R Rats**

Gel mobility shift assay in Figure 6A showed a broad shifted band of glomerular AP-1 complexes in Dahl-S rats receiving a high-salt diet. The density of this shifted band was decreased by addition of unlabeled AP-1 consensus oligonucleotide in a dose-dependent manner, indicating that this band represented specific binding for AP-1. The AP-1 binding complex was supershifted by addition of either anti-c-Fos or anti-c-Jun antibodies. As shown in Figure 6, B and C, glomerular AP-1 binding activity in Dahl-S rats was increased by 1.7- and 3.2-fold at 5 and 10 wk, respectively, by a high-salt diet. In Dahl-R rats, glomerular AP-1 binding activity was not affected by a high-salt diet for 5 wk but was increased by 1.5-fold by a high-salt diet for 10 wk.

**Phosphorylation of Glomerular ERK in Dahl-S Rats**

As shown in Figure 7, an increase in protein expression of ERK1 and ERK2 in Dahl-S rats fed a high-salt diet was associated with the increase in tyrosine phosphorylation of ERK1 and ERK2. Furthermore, a-tubulin, which is a housekeeping protein and commonly used as internal control protein, was not significantly different between high-salt and low-salt groups. To detect tyrosine phosphorylation of JNK, we used several kinds of commercially available antibodies against phospho-specific JNK. Unfortunately, the present study did not allow us to detect the bands of tyrosine phosphorylation of JNK, thereby suggesting that the amount of tyrosine phosphorylation form of JNK may be small. Further study is needed to conclude our assumption.

**Discussion**

It has been well established that ERK and JNK are important mediators of the intracellular signal transduction pathway, which are responsible for cell growth, cell differentiation, cell apoptosis, or the regulation of transcriptional factors and gene expression (7,8,10). In cultured mesangial cells, ERK and JNK are activated by numerous agonists implicated in the pathogenesis of glomerular injury, such as AngII, endothelin, platelet-derived growth factor, and interleukin-1 (7,16–19). However, these previous findings on the regulation and function of MAPK have come from in vitro studies using cultured cells. Their regulation and role in glomerular diseases in vivo remain unknown. In the present study, we demonstrated that salt-sensitive hypertension in Dahl-S rats induced chronic activa-

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**Figure 4.** Effects of a high-salt diet on glomerular ERK and JNK activities in Dahl salt-resistant (Dahl-R) rats. The top panels show representative autoradiograms of glomerular ERK (A) and JNK (B) activities from Dahl-R rats fed a low-salt (0.3% NaCl) or a high-salt (8% NaCl) diet for 10 wk, as determined by in-gel kinase assay. Bar graphs show the density of each autoradiogram of glomerular ERK and JNK activities from Dahl-R rats fed a low-salt or a high-salt diet for 5 and 10 wk. The mean value of these kinase activities from Dahl-R rats fed a low-salt diet at each time point is represented as 1. Each value represents the mean ± SEM (n = 6).
tion of glomerular ERK and JNK in vivo, and suggested that the prolonged MAPK activation might be involved in the development of salt-induced glomerular injury. Thus, the present study provided new insight into the molecular mechanism of salt-induced glomerular disease.

We have recently investigated the effects of AngII-induced acute hypertension on glomerular ERK and JNK in vivo and found that acute hypertension induces rapid and transient activation of glomerular ERK and JNK (14). Very recently, we (20) and Bokemeyer et al. (21) have also shown the activation of MAPK in glomerulonephritis in rats. However, glomerular MAPK in chronic hypertension has not yet been examined. Because Dahl-S rats are well known to be the salt-sensitive hypertensive model with severe glomerular injury, Dahl-S rats are a useful model to study the mechanism of glomerular injury by salt-sensitive hypertension (4,5). These findings encouraged us to examine glomerular ERK and JNK in Dahl-S rats. The present study demonstrated that proteinuria and renal growth in Dahl-S rats by salt overload were associated with the progressive increase in glomerular ERK activities. Unlike the ERK activities, JNK activities rose by 1 wk and tended to drop off by 10 wk, indicating that the increase in glomerular JNK activity was before glomerular injury. Glomerular JNK may play a role in the early phase of glomerular injury rather than the late phase. Interestingly, glomerular ERK and JNK activity in Dahl-S rats remained elevated until 10 wk of salt overload. To examine whether the sustained increase in glomerular ERK and JNK activity in Dahl-S rats was solely due to hypertension, we examined glomerular MAPK in spontaneously hypertensive rats (SHR) with similar hypertension and found no increase in glomerular ERK or JNK activity in SHR compared with normotensive control Wistar-Kyoto rats (unpublished data). These findings support the notion that the sustained activation of glomerular ERK and JNK in Dahl-S rats may be due to salt sensitivity. Of note, in the present study, a chronic increase in glomerular ERK and JNK activities in Dahl-S rats...
Supershift analysis with anti-c-Fos and anti-c-Jun antibodies indicated that glomerular AP-1 binding complexes contained both c-Fos and c-Jun proteins. These observations suggested that the chronic activation of glomerular ERK and JNK might lead to the activation of AP-1 in vivo.

In vitro studies have shown that AP-1 regulates various gene expressions, such as TGF-β1 (23), by binding to consensus sequences present in their promoter region (24). Indeed, AP-1 activation is demonstrated to increase the promoter activity of TGF-β1 (12,13). In cultured vascular smooth muscle cells, the inhibition of AP-1 by using the decoy approach limits AngII-induced TGF-β1 expression (25). Furthermore, glomerular TGF-β1 gene expression is elevated in Dahl-S rats by a high-salt diet (26). Thus, these findings suggest that the activation of AP-1 may be involved in enhanced TGF-β1 expression in Dahl-S rats. However, further study is needed to elucidate this theory.

In conclusion, the present study provided the first in vivo evidence that salt-sensitive hypertension leads to the chronic increase in glomerular ERK and JNK activities and glomerular AP-1 activity. Moreover, long-term high-salt intake itself also induces the activation of glomerular ERK, JNK, and AP-1. Thus, we propose that chronic activation of ERK and JNK may be responsible for the development of salt-induced glomerular injury.

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