Enhanced Aldosterone Signaling in the Early Nephropathy of Rats with Metabolic Syndrome: Possible Contribution of Fat-Derived Factors

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Metabolic syndrome is an important risk factor for proteinuria and chronic kidney disease independent of diabetes and hypertension; however, the underlying mechanisms have not been elucidated. Aldosterone is implicated in target organ injury of obesity-related disorders. This study investigated the role of aldosterone in the early nephropathy of 17-wk-old SHR/NDmcr-cp, a rat model of metabolic syndrome. Proteinuria was prominent in SHR/NDmcr-cp compared with nonobese SHR, which was accompanied by podocyte injury as evidenced by foot process effacement, induction of desmin and attenuation of nephrin. Serum aldosterone level, renal and glomerular expressions of aldosterone effector kinase Sgk1, and oxidative stress markers all were elevated in SHR/NDmcr-cp. Mineralocorticoid receptors were expressed in glomerular podocytes. Eplerenone, a selective aldosterone blocker, effectively improved podocyte damage, proteinuria, Sgk1, and oxidant stress. An antioxidant tempol also alleviated podocyte impairment and proteinuria, along with inhibition of Sgk1. As for the mechanisms of aldosterone excess, visceral adipocytes that were isolated from SHR/NDmcr-cp secreted substances that stimulate aldosterone production in adrenocortical cells. The aldosterone-releasing activity of adipocytes was not inhibited by candesartan. Adipocytes from nonobese SHR did not show such activity. In conclusion, SHR/NDmcr-cp exhibit enhanced aldosterone signaling, podocyte injury, and proteinuria, which are ameliorated by eplerenone or tempol. The data also suggest that adipocyte-derived factors other than angiotensin II might contribute to the aldosterone excess of this model.

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s is (23,24), putatively mediating aldosterone excess in obesity-related disorders. On the basis of these findings, we postulated a hypothesis that circulating aldosterone level and its signaling in the kidney are enhanced in metabolic syndrome, which causes podocyte injury via oxidative stress. In this study, we analyzed proteinuria, podocyte injury, and aldosterone and its effector Sgk1 in SHR/cp and nonobese SHR and examined the effects of eplerenone and the antioxidant tempol. We also explored the possible role of fat-derived aldosterone-releasing factors in SHR/cp.

Materials and Methods

Animals

Male SHR/cp (n = 44) and SHR (n = 32) were purchased from Japan SLC (Shizuoka, Japan). All animal procedures were in accordance with the guidelines for the care and use of laboratory animals approved by University of Tokyo Graduate School of Medicine. SHR/cp and SHR at 13 wk of age were fed a normal rat diet for 4 wk. Some SHR/cp were pretreated with eplerenone (1.25 g/kg food) or tempol (6 mM in tap water; Sigma, St. Louis, MO). For time-course analysis, SHR/cp and SHR at the indicated ages were used. The final number of rats for each group was four to eight.

Systolic BP was measured by the tail-cuff method (9). Rats were placed in metabolic cages for 24-h urine collection. After fasting for 16 h, rats were anesthetized with ether, and kidneys, adrenals, and epididymal fats were harvested. Glomerular fraction was isolated by the graded sieving method (25). Biochemical and hormonal data in epididymal fats were harvested. Glomerular fraction was isolated by the guidelines for the care and use of laboratory animals approved by University of Tokyo Graduate School of Medicine. SHR/cp and SHR at 13 wk of age were fed a normal rat diet for 4 wk. Some SHR/cp were treated with eplerenone (1.25 g/kg food) or tempol (6 mM in tap water; Sigma, St. Louis, MO). For time-course analysis, SHR/cp and SHR at the indicated ages were used. The final number of rats for each group was four to eight.

Real-Time PCR

Total RNA was extracted using an RNeasy kit or RNeasy lipid tissue kit (Qiagen, Hilden, Germany). Gene expression was determined by real-time quantitative reverse transcription PCR using ABI PRISM 7000, TaqMan chemistry, and assay-on-demand primers and probe sets, as described previously (Applied Biosystems, Foster City, CA) (9).

Western Blotting

Western blotting was performed as described previously (9). The membrane was immunoblotted with rabbit anti-rat nephrin (1:10000; a gift from Dr. Kawachi, Niigata University, Niigata, Japan), rabbit anti-human Sgk1 (1:1000; Cell Signaling Technology, Danvers, MA), or rabbit anti-actin (1:500; Sigma).

Immunohistochemistry and Periodic Acid-Schiff Staining

Immunohistochemistry and semiquantitative analysis of desmin were performed as described (9). Immunofluorescence double staining was carried out as follows: For mineralocorticoid receptor (MR) and synaptopodin double staining, cryosections (4 μm) were boiled for antigen retrieval, incubated with rabbit anti-rat MR (1:1000; antibody raised against amino acids 103 to 507 of rat MR; a gift from Dr. Kawata, Kyoto Prefectural University of Medicine, Kyoto, Japan) (26), peroxidase-conjugated anti-rabbit IgG, and Cy3-tyramide (PerkinElmer Life Sciences, Boston, MA). Samples then were immunolabeled with mouse anti-rat synaptopodin (1:10; Research Diagnostics, Flanders, NJ) and FITC-conjugated anti-mouse IgG. For MR and WT-1 immunostaining, pretreated sections were incubated with mouse anti-human MR (1:200; antibody against amino acids 2 to 99 of human MR; Perseus Proteomics, Tokyo, Japan), peroxidase-conjugated anti-mouse IgG, biotinyl-tyramide, and streptavidin-FITC. Subsequently, samples were immunolabeled with rabbit anti-human WT-1 (1:500; Santa Cruz Biototechnology, Santa Cruz, CA) and Cy3-conjugated anti-rabbit IgG. The specificity of anti-rat MR was described in detail previously (26). We confirmed the specificity of these two MR antibodies as follows: Western blotting using the antibodies revealed a band with molecular mass of >100 kD (compatible with the size of 106 kD), and immunohistochemistry without primary antibody resulted in negative staining. Periodic acid-Schiff staining was performed as described (9).

Electron Microscopy

Ultrastructure of glomerular podocytes was analyzed using Hitachi transmission electron microscope H-7000 (Tokyo, Japan), as described previously (25). Morphology of epididymal fats was examined using Hitachi scanning electron microscope S-3500N, as described previously (27).

Urinary 8-OHdG Excretion

Urine was ultrafiltrated using Microcon YM-10 (Millipore, Billerica, MA) to separate interfering substances. Then urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration was measured using the 8-OHdG ELISA Kit (Japan Institute for the Control of Aging, Shizuoka, Japan).

Aldosterone Releasing Activity of Adipocytes

Isolation of adipocytes and preparation of fat cell–conditioned medium (FCCM) were performed according to the method by Ehrrhart-Bornstein et al. (23). Briefly, fresh adipose tissues were minced immediately into small pieces in Krebs Ringer Bicarbonate buffer that contained 3% BSA and 4 mM glucose, digested with 0.05% collagenase type II (Sigma) for 60 min at 37°C, filtered through a nylon mesh (425 μm), and washed. Isolated adipocytes were cultured in 4 vol of DMEM/F12 supplemented with antibiotics and 10% FBS at 37°C for 24 h. The FCCM was collected and kept frozen at −20°C until use.

NCI-H295R human adrenocortical cells were purchased from American Type Culture Collection (Manassas, VA). Cells were maintained in DMEM/F12 supplemented with insulin (66 nM), hydrocortisone (10 nM), 17β-estradiol (10 nM), transferrin (10 μg/ml), selenite (30 nM), antibiotics, and 2% FBS at 37°C. For aldosterone secretion assay, cells were plated at a density of 70,000 cells/cm² and precultured for 96 h. Then the medium was replaced with FCCM that contained previously mentioned supplements, and cells were incubated for 36 h. Aldosterone concentration in the medium was determined by RIA using SPAC-S aldosterone kit (TFB, Tokyo, Japan). In some experiments, aldosterone-releasing activity was evaluated after pretreatment with AngII type 1 receptor antagonist candesartan (1 × 10⁻⁵ mol/L).

Statistical Analyses

Data are expressed as mean ± SEM. Statistical analyses were performed by unpaired t test, ANOVA and subsequent Tukey post hoc test, or Mann-Whitney test. P < 0.05 was considered to be statistically significant.

Results

Metabolic Parameters

Table 1 summarizes the metabolic parameters of SHR and SHR/cp at 17 wk of age. SHR/cp were obese, and serum insulin, triglycerides, and free fatty acids were markedly higher in SHR/cp than SHR (P < 0.01), whereas there was no difference in fasting blood glucose. Systolic BP was comparably elevated in both strains. Therefore, SHR/cp rats can be considered as a model of metabolic syndrome. Table 2 shows the
Table 1. Metabolic parameters of SHR and SHR/cp at 17 wk of agea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR (n = 8)</th>
<th>SHR/cp (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>359 ± 7</td>
<td>530 ± 8b</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>198 ± 8</td>
<td>179 ± 7</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>162 ± 17</td>
<td>178 ± 14</td>
</tr>
<tr>
<td>Serum insulin (ng/ml)</td>
<td>0.3 ± 0.1</td>
<td>21.3 ± 3.1b</td>
</tr>
<tr>
<td>triglycerides (mg/dl)</td>
<td>53 ± 7</td>
<td>592 ± 48b</td>
</tr>
<tr>
<td>free fatty acids (mg/dl)</td>
<td>310 ± 31</td>
<td>1275 ± 147b</td>
</tr>
</tbody>
</table>

aData are mean ± SEM.

bP < 0.01 by unpaired t test.

temporal changes in BP and fasting glucose concentration in SHR/cp.

Urinary Protein Excretion Is Exaggerated in SHR/cp

We first compared the profile of proteinuria between SHR and SHR/cp (Figure 1). Urinary protein excretion remained low in nonobese SHR. By contrast, the metabolic syndrome model SHR/cp had exaggerated proteinuria despite similar BP elevation. Urinary protein was already increased at 12 wk of age (P < 0.01), which was increased progressively thereafter.

Podocyte Injury Is an Early Event in the Nephropathy of SHR/cp

We assessed podocyte damage and tubulointerstitial injury as possible causes of proteinuria in young SHR/cp. Renal histologic changes were not apparent under periodic acid-Schiff–stained light micrograph in 17-wk-old SHR/cp (Figure 2A). Gene expressions of osteopontin and macrophage chemotactic protein-1 were not increased in the whole kidney samples of SHR/cp, suggesting the absence of tubulointerstitial proinflammatory responses (Figure 2B).

Conversely, the glomerular expression of nephrin, a slit diaphragm–associated protein in the podocytes, was significantly reduced in SHR/cp compared with SHR (Figure 2C). Nephrin expression also was diminished at the mRNA level, which was decreased in parallel with the temporal profile of proteinuria (Figure 2D). Expression of desmin, an injured podocyte marker, was induced in some glomeruli of SHR/cp but not of SHR (Figure 2E). Electron microscopy revealed podocyte foot process effacement, vacuolization, and accumulation of dense deposits in 17-wk-old SHR/cp. SHR had intact ultrastructure of podocytes (Figure 2F). These findings suggest that podocyte injury underlies the cause of early proteinuria in SHR/cp.

Circulating Aldosterone Level and Expression of Its Effector Sgk1 Are Elevated in SHR/cp

Recent studies suggested that aldosterone is a potential mediator of proteinuria and that it often is overproduced in obesity hypertension. Therefore, we evaluated aldosterone and its effectors in SHR/cp. Serum aldosterone concentration was elevated significantly in obese SHR/cp compared with nonobese SHR at 17 wk of age (P < 0.05; Figure 3A). Time-course analysis revealed that serum aldosterone increased in an age-dependent manner (P < 0.05; Figure 3B). There was a positive correlation between circulating aldosterone concentration and proteinuria (r² = 0.44; Figure 3C).

Sgk1 is a widely known effector of aldosterone and an index of MR activation. The Sgk1 mRNA expression was increased in the kidneys as well as glomerular fraction of SHR/cp compared with those of SHR (Figure 3D). Sgk1 upregulation also was observed at the protein level (Figure 3E). These results suggest that aldosterone and its signaling in the kidney and glomeruli are enhanced in SHR/cp.

MR Is Expressed in Podocytes

Several reports demonstrate the presence of MR in cultured renal cells. However, the precise in vivo localization of MR within the kidney has not been shown clearly. Therefore, we performed immunohistochemical analysis of MR in the kidney of SHR/cp (Figure 4). As previously reported, MR was localized predominantly in the nuclei of SHR/cp (Figure 4A). Double immunostaining of MR and WT-1, which is expressed in the podocyte nuclei, yielded a considerable number of double-positive cells (Figure 4B). Double immunostaining of MR and synaptopodin,

Table 2. Temporal profile of BP and fasting blood glucose concentration in SHR/cp

<table>
<thead>
<tr>
<th>Parameter</th>
<th>6 Wk</th>
<th>12 Wk</th>
<th>18 Wk</th>
<th>24 Wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 ± 8</td>
<td>179 ± 13b</td>
<td>179 ± 9b</td>
<td>189 ± 12b</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>168 ± 12</td>
<td>168 ± 5</td>
<td>198 ± 30</td>
<td>203 ± 24</td>
</tr>
</tbody>
</table>

aData are mean ± SEM; n = 4 per group.

bP < 0.01 versus 6 wk by ANOVA and subsequent Tukey post hoc test.
Figure 2. (A) Representative photomicrograph of periodic acid-Schiff–stained renal section from 17-wk-old SHR/cp. Bar = 100 μm. (B) Quantitative analysis of osteopontin and macrophage chemotactic protein-1 (MCP-1) mRNA expressions in the kidneys of SHR and SHR/cp determined by real-time PCR (n = 8 per group). (C) Western blotting of nephrin in the glomeruli of 17-wk-old SHR and SHR/cp. (Top) Representative bands for nephrin and control actin. (Bottom) Result of densitometric analysis (n = 3 per group). (D) Glomerular mRNA expression of nephrin determined by real-time PCR. Comparison between 17-wk-old SHR and SHR/cp (left; n = 8 per group) and time-course analysis in SHR/cp (right; n = 4 per group). (E) Representative immunostaining for desmin in the kidneys of SHR (left) and SHR/cp (right). Bars = 100 μm. (F) Representative transmission electron micrographs of glomeruli from SHR (left) and SHR/cp (right). Bars = 1 μm. *P < 0.05, **P < 0.01 versus SHR; #P < 0.05, ##P < 0.01 versus 6 wk.

Figure 3. (A) Serum aldosterone concentration in 17-wk-old SHR and SHR/cp (n = 8 per group). (B) Time-course analysis of serum aldosterone in SHR/cp (n = 3 to 4 per group). (C) Relationship between serum aldosterone level and proteinuria in SHR/cp (n = 23). (D) Gene expression of Sgk1 in the whole kidney (left) and glomerular fraction (right) of 17-wk-old SHR and SHR/cp (n = 8 per group). (E) Western blotting of Sgk1 in the kidneys of SHR and SHR/cp (n = 3 per group). **P < 0.01 versus SHR.
which is present in the podocyte cytoplasms, indicated that many MR-positive cells are located just outside the synaptopodin-positive podocyte cytoplasms (Figure 4C). These results suggest that podocytes constitute substantial portions of the MR-expressing cells within the glomeruli.

**Eplerenone Ameliorates Proteinuria and Podocyte Damage in SHR/cp: Possible Role of Oxidative Stress**

The above findings suggest that excessive aldosterone signaling may contribute to the increased susceptibility to proteinuria and podocyte injury in SHR/cp. Therefore, we examined the effect of selective aldosterone blocker eplerenone. After 4 wk of treatment, eplerenone significantly reduced proteinuria in SHR/cp ($P < 0.05$; Figure 5A). In association with the decrease in proteinuria, eplerenone prevented the reduction of nephrin protein and mRNA and alleviated the induction of desmin (Figure 5, B through D). Eplerenone also inhibited the upregulated expression of Sgk1 in the kidneys and glomeruli of SHR/cp (Figure 5E). Eplerenone tended to reduce BP (167 ± 4 mmHg; 0.05 < $P < 0.1$) but did not change fasting blood glucose (203 ± 25 mg/dl) or cause hyperkalemia (4.6 ± 0.1 mEq/L).

We tested the role of oxidative stress in this process, because induction of reactive oxygen species (ROS) is proposed to be a mediator of injurious effects of aldosterone (14). Oxidative stress markers such as urinary 8-OHdG and glomerular expressions of NADPH oxidase components p22phox and gp91phox were increased in SHR/cp, which was completely inhibited by eplerenone (Figure 5, F and G). These results suggest that endogenous aldosterone excess, via induction of oxidative stress, causes podocyte injury and proteinuria in SHR/cp.

**Effect of Tempol on Proteinuria, Podocyte Injury, and Sgk1 Expression in SHR/cp**

To confirm the contribution of oxidative stress to the nephropathy of SHR/cp, we treated these rats with the anti-
dant tempol. Administration of tempol for 4 wk significantly reduced proteinuria (P < 0.05; Figure 6A). Reduced expression of nephrin and upregulation of desmin also were alleviated by tempol (Figure 6, B through D). It is interesting that the upregulated expression of Sgk1 in SHR/cp also was abrogated by tempol (Figure 6, E and F). Tempol did not affect BP (184 ± 13 mmHg) and fasting glucose (186 ± 22 mg/dl).

Fat-Derived Factors May Contribute to Hyperaldosteronism in SHR/cp

Finally, we explored the mechanisms of high aldosterone state in SHR/cp. Expression of aldosterone synthase was enhanced in the adrenal glands from SHR/cp but was below the detection level in the kidney (Figure 7A), suggesting that aldosterone production in the adrenals is responsible for high circulating aldosterone in SHR/cp. SHR/cp displayed lower plasma renin activity (Figure 7B), suggesting that this is not the responsible factor. Ehrhart-Bornstein et al. (23) reported adipocyte-derived substances that stimulate adrenal aldosterone production. Scanning electron micrographs showed markedly enlarged visceral adipocytes in SHR/cp (Figure 7C), implicating the pathogenetic role for adipocytes.

Indeed, aldosterone production in H295R adrenocortical cells was increased markedly by FCCM from SHR/cp but not that from nonobese SHR (Figure 7D). Aldosterone-releasing activity of FCCM from SHR/cp was not mediated by AngII, because it was not inhibited by candesartan (Figure 7E), and angiotensinogen mRNA expression in the adipose tissues was lower in SHR/cp (Figure 7F). The activity was not recapitulated by the known adipocytokines (data not shown). FCCM from SHR/cp but not from SHR stimulated the expression of aldosterone synthase in H295R cells (Figure 7G). FCCM from SHR/cp upregulated mRNA expression of steroidogenic acute regulatory protein (StAR), another key factor in aldosterone synthesis that mediates transfer of cholesterol to mitochondria (Figure 7H). Adrenal steroidogenic acute regulatory protein (StAR) expression also was enhanced in SHR/cp compared with SHR (Figure 7I). These findings suggest the involvement of adipocyte-derived substances other than AngII in hyperaldosteronism in SHR/cp.

Discussion

Our study demonstrates that SHR/cp, a rat model of metabolic syndrome, exhibit enhanced proteinuria as well as glomerular podocyte injury. Circulating aldosterone level, glomerular expression of Sgk1, and oxidative stress markers were increased in SHR/cp compared with SHR. Eplerenone effectively ameliorated podocyte damage and inhibited elevated oxidative stress and Sgk1 expression. Tempol also alleviated podocyte impairment along with inhibition of Sgk1 expression. Furthermore, adipocytes from obese SHR/cp secreted substances that stimulate aldosterone production in the adrenocortical cells. Our findings suggest that enhanced aldosterone signaling plays a key role in podocyte injury in SHR/cp via induction of oxidative stress and that adipocyte-derived factors might contribute to the aldosterone excess in this model.

Although metabolic syndrome is associated with proteinuria independent of diabetes and hypertension (2,3), the underlying mechanisms have not been elucidated. In this study, we examined proteinuria in SHR/cp, a derivative of SHR with spontaneous nonsense mutation in the leptin receptor gene (17,18). This strain manifested a clustering of abdominal obesity, hypertension, hyperinsulinemia, hypertriglyceridemia, and elevated free fatty acids at 17 wk of age, fulfilling the criteria of metabolic syndrome. Podocyte injury was reported previously in this animal model, but the analysis was made at a later stage, when other abnormalities such as tubulointerstitial changes are evident (10,29). Our findings indicate that podocyte injury should be an early key manifestation in the nephropathy of this model, because at this phase, we did not detect apparent renal morphologic changes or tubulointerstitial inflammatory alterations.

Multiple factors are proposed to be involved in the initiation of renal injury in metabolic syndrome: Enhanced renin-angiotensin-aldosterone system, insulin resistance, sympathetic nerve overactivation, and hyper-hemodynamics (30). We consider that enhanced aldosterone signaling plays a critical role in the proteinuria and podocyte injury of this model for the following reasons. First, circulating aldosterone level was elevated in SHR/cp along with aldosterone effectors such as oxidant stress and Sgk1, and aldosterone levels were correlated with the degree of proteinuria. Second, eplerenone could reverse the proteinuria and podocyte damage of this model, together with suppression of oxidative stress and Sgk1. Finally, we found that aldosterone/salt rats develop marked podocyte injury and massive proteinuria (Shibata et al. unpublished observation, Figure 6. Effects of tempol (Temp) on proteinuria, podocyte injury, and Sgk1 expression in SHR/cp. SHR/cp at 13 wk of age were treated with Temp for 4 wk (SHR/cp + Temp). (A) Proteinuria in SHR/cp and SHR/cp + Temp (n = 4 per group). (B) Western blotting of nephrin in the glomeruli (n = 3 per group). (C) Glomerular nephrin mRNA expression (n = 4 per group). (D) Immunostaining score of desmin in the glomeruli (n = 4 per group). (E) Western blotting of Sgk1 (n = 3 per group). (F) Real-time PCR of Sgk1 (n = 4 per group). *P < 0.05, **P < 0.01 versus SHR/cp.
Aldosterone has been implicated as an important mediator of proteinuria and glomerular damage in CKD or diabetic or hypertensive nephropathy (9, 31–33). Our study would be the first report to show the involvement of aldosterone in the early nephropathy of the metabolic syndrome model.

To date, few studies have demonstrated clearly the precise localization of MR within the kidney. Our data showed immunolocalization of MR on glomerular cells, including podocytes. Glomerular expression of MR also was described by Gomez-Sanchez et al. (28). We confirmed the presence of MR as well as activation of MR signaling by exposure to aldosterone using a cultured podocyte cell line (Shibata et al., unpublished observation, 2006). These findings suggest that the proteinuric effects of aldosterone should be mediated at least in part through direct action on podocytes, although hemodynamic alteration by aldosterone also might be involved (34).

We observed that Sgk1 expression is enhanced in the kidneys of SHR/cp. Sgk1 is a transcriptionally regulated serine threonine kinase and considered as one of the main effectors of aldosterone (35–37). Sgk1 is induced by aldosterone not only in distal tubular cells but also in vascular cells (37). Aldosterone infusion increased Sgk1 expression in the whole kidney and glomeruli (38). Studies have demonstrated the presence of Sgk1 in both mesangial cells and podocytes (39, 40). Quinkler et al. (16) demonstrated increased expression of aldosterone effectors, including Sgk1, in kidney biopsies of patients with heavy proteinuria, implicating the close relationship between Sgk1 and proteinuria. The pivotal role of Sgk1 in the pathogenesis of proteinuria also is suggested by the report that gene targeting of Sgk1 protects against DOCA/salt-induced albuminuria (41).

Oxidative stress is postulated to be an important mediator of aldosterone actions (14). Our data indicated that oxidative stress markers are elevated in SHR/cp and that tempol ameliorated proteinuria and podocyte injury, along with inhibition of the enhanced Sgk1 expression. Importantly, eplerenone suppressed the elevated oxidative stress markers in SHR/cp. Sgk1 regulation by oxidative stress was demonstrated previously in other cells (42). These results suggest that aldosterone increases ROS generation, which causes Sgk1 upregulation and podocyte injury. Nishiyama et al. (14, 43) demonstrated that aldosterone increases ROS, which in turn activate extracellular signal–regulated kinase 1/2, c-Jun N-terminal kinase, and big mitogen-activated protein kinase (BMK1) but not p38 mitogen-activated protein kinase in rat renal cortex and cultured mesangial cells.
Thus, multiple kinases seem to be involved in the actions of aldosterone. Further studies are necessary to elucidate the mechanisms by which Sgk1 causes podocyte injury, including the cross-talk between Sgk1 and mitogen-activated protein kinases.

Adipose tissue now is recognized as a dynamic endocrine organ that secretes a number of adipocytokines, not just an inert storage depot (44). Although AngII is a major regulator of adrenal aldosterone production, aldosterone excess in SHR/cp was not accompanied by increased renin activity in this study. On the basis of the comparison between obese SHR/cp and nonobese SHR, we assumed that factors that are responsible for aldosterone excess in SHR/cp are likely to reside in adipocytes. Ehrhart-Bornstein et al. (23) showed that some adipocytokines, although as yet unidentified, stimulate aldosterone secretion from adrenocortical cells. In this study, we first demonstrated a possible pathogenic role for these fat-derived products. We found that this aldosterone-releasing activity of adipocytes was upregulated in the adipocytes from SHR/cp compared with those from nonobese SHR. It should be noted that hyperaldosteronism that is caused by these adipocyte-derived factors is not inhabitable by angiotensin-converting enzyme inhibitors or AngII receptor antagonists. Thus, eplerenone should have benefit over AngII blockade in situations in which such factors are overproduced. Goodfriend et al. (24) reported that epoxy-keto derivative of linoleic acid, one of the oxidized products of fatty acids, not native linoleic acid, stimulates aldosterone secretion in rat adrenal cells. Although they originally hypothesized that the site of oxidative modification might be the liver and showed that incubation of linoleic acid with hepatocytes gave rise to compounds that enhanced aldosterone production in adrenal cells, adipocytes also might contribute to the epoxy-keto modification of linoleic acid in our model. We expect that these adipocyte-derived aldosterone-releasing factors, if identified, can be a novel target of therapy in metabolic syndrome.

Conclusion
We demonstrated that enhanced aldosterone signaling, such as increased oxidative stress and Sgk1 upregulation, plays a crucial role in podocyte injury and proteinuria in metabolic syndrome model SHR/cp and that adipocyte-derived aldosterone secretagogues might be involved in the aldosterone excess. We also showed that eplerenone effectively ameliorates podocyte injury and proteinuria in this model without causing hyperkalemia. Recent animal studies revealed that aldosterone blockers are effective in treating diabetic and hypertensive nephropathy, atherosclerosis, and balloon-induced vascular injury (9,45–48). Although future studies should be awaited, we believe that aldosterone blockade would be a clinically promising strategy toward nephropathy in metabolic syndrome as well.

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