**Original Article**

**Hypertension Accelerates Diabetic Nephropathy in Wistar Fatty Rats, a Model of Type 2 Diabetes Mellitus, via Mitogen-Activated Protein Kinase Cascades and Transforming Growth Factor-β1**

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Although it is known that diabetic nephropathy is accelerated by hypertension, the mechanisms involved in this process are not clear. In this study we aimed to clarify these mechanisms using male Wistar fatty rats (WFR) as a type 2 diabetic model and male Wistar lean rats (WLR) as a control. Each group was fed a normal or high sodium diet from the age of 6 to 14 weeks. We determined the blood pressure and urinary albumin excretion (UAE). At the end of the study, the expressions of mitogen-activated protein kinases (MAPK) and transforming growth factor-β1 (TGF-β1) were examined in the isolated glomeruli by Western blot analysis, and the number of glomerular lesions was determined by conventional histology. High sodium load caused hypertension and a marked increase in UAE in the WFR but not in the WLR. Glomerular volume was increased in the hypertensive WFR. There was no difference among the four groups in the expression of c-Jun-NH₂-terminal kinase (JNK). In contrast, the expressions of extracellular signal-regulated kinase 1/2 (ERK1/2) and its upstream regulator, MAPK/ERK kinase 1 (MEK1), were augmented in the hypertensive WFR. Expression of p38 MAPK was increased in the normotensive WFR, and further enhanced in the hypertensive WFR. Moreover, administration of high sodium load to WFR augmented the expression of TGF-β1. In conclusion, systemic hypertension in WFR accelerates the diabetic nephropathy in type 2 diabetes via MEK-ERK and p38 MAPK cascades. TGF-β1 is also involved in this mechanism.

(Hypertens Res 2003; 26: 339–347)

**Key Words:** diabetic nephropathy, Wistar fatty rats, hypertension, transforming growth factor-β1, mitogen-activated protein kinase

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**Introduction**

Along with the growing prevalence of diabetic nephropathy, it has become increasingly urgent to determine the pathogenetical mechanisms responsible for this disorder. Before the onset of overt proteinuria, there are various functional changes in the kidney, including glomerular hyperfiltration and glomerular hypertension (1). These changes are accompanied by structural changes such as glomerular hypertrophy, basement-membrane thickening or mesangial expansion (2). Accumulating evidence suggests that the metabolic pathway associated with hyperglycemia plays a pivotal role in the pathogenesis of diabetic nephropathy in part via the en-
hanced expression of transforming growth factor-β (TGF-β)
which leads to the accumulation of extracellular matrix pro-
teins (3). Analysis of the mitogen-activated protein kinase
(MAPK) cascade, an intracellular signal transduction path-
way associated with the TGF-β activity, has demonstrated
that extracellular signal-regulated kinase (ERK1/2) (6)
protein expression (4) and p38 MAPK activity (5) are increased
in glomeruli from streptozotocin-induced type 1 diabetes model
rats. Accordingly, high glucose condition has been shown to
activate ERK1/2 (6) and p38 activity (7) in cultured mesan-
gial cells. However, it remains unknown whether TGF-β or
MAPK is involved in the pathogenesis of early stage diabetic
nephropathy in type 2 diabetes.

Glomerular hypertension caused by dilatation of the affer-
ent arterioles (8) has been reported to play an important role
in the hemodynamic pathway, and can be considered another
pathogenetical mechanism of diabetic nephropathy (9).
Glomerular hypertension causes mechanical stretch stimula-
tion of the mesangial cells (10), resulting in the enhance-
ment of TGF-β production (11) or the stimulation of ERK1/2
and p38 activity (12). On the other hand, type 2 diabetes
mellitus is very frequently accompanied by systemic hyper-
tension, and blood pressure elevation has been known to ag-
gravate diabetic nephropathy. This mechanism, however, has
not been proved in vivo because no experimental model of
type 2 diabetes accompanied by hypertension has been made
available.

Wistar fatty rats (WFR), a model of type 2 diabetes, have
been reported to demonstrate proteinuria from the age of 12
weeks, and light microscopic examination of the kidneys
from 26-week-old WFR revealed an expanded glomerular
mesangial area and nodular-like lesions (13). We recently
showed that WFR were normotensive under a normal sodi-
um diet, but that they became extremely hypertensive at 8
weeks of age when administered a high sodium diet from 6
weeks of age (14). Thus WFR fed a high sodium diet may
constitute a single model of both type 2 diabetes and hyper-
tension. We preliminarily found that urinary albumin excre-
tion in WFR was greatly increased in association with blood
pressure elevation. Thus we have postulated that diabetic
nephropathy in WFR could be accelerated by hypertension.

The present study was designed to determine the role of
the hemodynamic pathway activated by blood pressure ele-
vation in the pathogenesis of diabetic nephropathy in type 2
diabetes mellitus using WFR with a sodium load. We inves-
tigated this issue from both a histopathological and a bio-
chemical point of view, with special emphasis on the TGF-β
and MAPK expressions.

Methods

Animals and Experimental Protocols

All experiments were performed according to the Guiding
Principles for the Care and Use of Laboratory Animals of
the Japanese Pharmacological Society. In this study we uti-
лизed WFR, which were established as an obesity-related
type 2 diabetes model in 1981 (15). This strain was derived
by crossing obese Zucker rats with Wistar Kyoto rats that
have relatively poor glucose tolerance. The homozygote
(faa/faa) of WFR develops obesity and displays the character-
istics of type 2 diabetes - including hyperglycemia, hyperin-
sulinemia and insulin-resistance - at 6 weeks of age. We
used male WFR as a model of type 2 diabetes mellitus and
male Wistar lean rats (WLR) as controls. They were main-
tained at constant humidity (55 ± 5%) and temperature (23 ±
1°C) with an electrically controlled 12-h light-dark cycle.
All rats had free access to a normal salt (NS) diet containing
0.7% NaCl and tap water ad libitum until 6 weeks of age.
At 6 weeks of age the rats were divided into the following four
groups: group 1, WLR fed a normal salt diet (WLR-NS);
group 2, WLR fed a high salt diet (WLR-HS); group 3, WFR
ed a normal salt diet (WFR-NS); and group 4, WFR fed a
high salt diet (WFR-HS). The high salt diet contained 7% NaCl.
All rats had free access to their food and tap water un-
til the end of the study.

Measurement of Blood Pressure

Systolic blood pressure (SBP) was first measured at the age
of 6 weeks as a baseline using the tail-cuff method (ECG
PROCESSOR BP-98A; Softron, Saitama, Japan). The SBP
was then measured in all four groups at the ages of 8, 10 and
14 weeks. The median of five successive measurements was
used. At each measurement, the rats were weighed before the
determination of blood pressure.

Measurement of Urinary Albumin Excretion

Rats were housed in individual metabolic cages and daily
urine was collected at baseline and at 8, 10, and 14 weeks of
age and used to calculate urine volume and urinary albumin
excretion (UAEx).

Measurement of Blood Parameters

At 14 weeks of age, rats were intraperitoneally anesthetized
with pentobarbital, and then blood was drawn from the aorta
for the determination of blood chemistry parameters. Plasma
glucose and creatinine concentration were measured with
commercially available kits. Plasma insulin level was deter-
mined by radioimmunoassay.

After drawing the blood, the kidneys were perfused with
ice-cold saline and then rapidly removed. They were then
fixed in 10% buffered formalin for histological examinations
or prepared for isolation of glomeruli followed by Western
blot analysis.
Table 1. Characteristics of Wistar Lean (WLR) and Fatty Rats (WFR) with Normal or High Sodium Load at 14 Weeks of Age

<table>
<thead>
<tr>
<th></th>
<th>Normal sodium (N)</th>
<th>High sodium (N)</th>
<th>Normal sodium (N)</th>
<th>High sodium (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>362±18.4 (7)</td>
<td>326.6±12.9 (8)</td>
<td>511.1±18.5* † (7)</td>
<td>513±7.0* † (9)</td>
</tr>
<tr>
<td>Kidneys weight (g)</td>
<td>2.67±0.06 (7)</td>
<td>2.71±0.12 (8)</td>
<td>3.25±0.13* † (7)</td>
<td>3.38±0.06* † (9)</td>
</tr>
<tr>
<td>(body weight: g/kg)</td>
<td>7.63±0.20</td>
<td>8.25±0.32</td>
<td>6.24±0.11* †</td>
<td>6.42±0.14* †</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.44±0.02</td>
<td>0.48±0.04</td>
<td>0.44±0.02</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>146.1±8.2 (10)</td>
<td>144.1±5.4 (8)</td>
<td>234.7±18.4* † (7)</td>
<td>241.8±17.4* † (9)</td>
</tr>
<tr>
<td>Insulin (µU/dl)</td>
<td>31.7±3.5 (10)</td>
<td>30.1±4.8 (8)</td>
<td>237.5±45.6* † (6)</td>
<td>122.9±8.7* † (10)</td>
</tr>
</tbody>
</table>

Values are means ± SEM. * p < 0.01 vs. WLR-NS. † p < 0.01 vs. WLR-HS. ‡ p < 0.01 vs. WFR-NS.

Histological Studies

The fixed kidneys were cut cross-sectionally including the papilla, and then embedded into paraffin for histopathological examinations. Paraffin sections were cut and stained with periodic acid-methenamine silver (PAS). To assess the glomerular lesions, glomerular tuft volume (Vg) was determined by measuring the mean glomerular cross-sectional area (A0) of all glomerular profiles. We measured the A0 of the five glomeruli that showed maximum diameter using Photoshop 7.0 (Adobe Systems Inc., San Jose, USA). Then the mean glomerular volume was calculated from A0 by the method of Weibel (16).

Isolation of Glomeruli and Western Blot Analysis of Glomerular Protein

The renal cortex was separated from the medulla. Cortical pieces were placed into ice-cold RPMI 1640 medium and cut into 2 mm³ pieces. Glomeruli were isolated by the technique of differential sieving using stainless steel grids of 80, 150 and 200 mesh size, and resuspended in RPMI.

Isolated glomeruli were centrifuged at 25,000 × g for 5 min at 4°C, and then the sediment was homogenized in a Tris-ethylenediamine tetraacetic acid (EDTA) solution containing 0.001% leupeptin. Homogenates were centrifuged at 15,000 × g for 10 min at 4°C. The supernatant was separated, and protein concentrations were determined using the Bradford method (17). After boiling at 100°C for 2 min, samples containing 25 µg of protein were subjected to electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) and then transferred to Hybond-ECL membranes (Amersham Life Sciences Inc., Arlington Heights, USA) for 50 min. The membranes were blocked with Tris buffered saline containing 0.05% Tween 20 (TTBS) with 5% nonfat powdered milk overnight at 4°C. After washing three times for 10 min with TTBS, membranes were incubated for 2 h with primary antibodies in TTBS containing 5% nonfat powdered milk diluted 1:200 at room temperature. Antibodies for ERK1/2, MAPK/ERK kinase 1 (MEK1), p38 MAPK, c-Jun-NH₂-terminal kinase (JNK) and TGF-β1 were purchased from Santa Cruz Biotech Inc. (Santa Cruz, USA). Membranes were then incubated with a horseradish peroxidase-conjugated secondary antibody against rabbit or mouse immunoglobulin G (IgG) at a 1:5000 dilution for 1 h at room temperature after being washed as described above. The Western blot was developed with an ECL Western Blotting Analysis system (Amersham Pharmacia Biotech, Buckinghamshire, UK) using RX-U autoradiography film (Fuji Film Co., Tokyo, Japan). Autoradiograms were quantitated by scanning densitometry, and relative intensities of the bands were assessed using computer densitometry software (AE-6900-M; ATTO, Tokyo, Japan).

Statistical Analysis

Data are expressed as the means ± SEM. Data were analyzed by analysis of variance (ANOVA) followed by Fisher’s PLSD test for multiple comparisons. Values of p < 0.05 were considered to indicate statistical significance.

Results

Blood Pressure and Body and Kidney Weights

At 6 weeks of age - i.e., before the modulation of dietary sodium - the body weight was not statistically different among the four groups (data not shown). At 14 weeks of age, the body weights in the WFR-NS and WFR-HS were significantly heavier than those in the WLR-NS and WLR-HS, respectively. There was no significant difference in body weight between the WLR-NS and the WLR-HS or between the WFR-NS and WFR-HS (Table 1). The kidney weight in the WFR-NS and the WFR-HS was significantly heavier than that in WLR-NS and WLR-HS (Table 1).

Figure 1 shows the SBP in the four experimental groups throughout the 8-week study period. Throughout the study period, the SBP rose with age in all four groups. The SBP in the WFR-NS was similar to those in the WLR-NS and WLR-HS. However, the SBP in the WFR-HS was significantly higher than that in the WLR-NS, WLR-HS or WFR-NS. Similarly, the mean blood pressure in the WFR-HS was...
significantly higher than that in the other three groups (data not shown).

The plasma glucose concentration in the WFR-NS and the WFR-HS was significantly higher than that in the WLR-NS and WLR-HS. There was no difference in plasma glucose level between the WFR-NS and WFR-HS or between the WLR-NS and the WLR-HS (Table 1). Significant hyperinsulinemia was observed in the WFR-NS and the WFR-HS as compared to the WLR-NS and the WLR-HS. Plasma insulin in the WFR-NS was significantly higher than that in the WFR-HS (Table 1). There was no difference in creatinine concentration among the four groups.

Urinary Albumin Excretion

Figure 2 shows the time course of daily UAE. UAE (mg/day) in the WLR-HS showed a slight increase compared to that in the WLR-NS, but the difference was not statistically significant throughout the experimental period. UAE in the WFR-NS tended to be higher than that in the WLR-NS or the WLR-HS. Similar to blood pressure alteration, UAE was markedly elevated in the WFR-HS. At 14 weeks of age, UAE in the WFR-HS was about 10 times higher than that in the WFR-NS and about 20 times higher than in the WLR-NS or the WLR-HS. Even though the value of UAE was corrected for urinary creatinine concentration, the UAE at 14 weeks of age was markedly changed in WFR-HS (Table 2).

Renal Histopathology

Figure 3 shows representative histopathological findings of the kidney by light microscopic examination with PAS staining in the four experimental groups at 14 weeks of age. Glomerulomegaly was observed in the WFR-NS (Fig. 3c) and the WFR-HS (Fig. 3d). Glomerular volume as determined by the tracing method was significantly higher in the WFR-NS and WFR-HS than in the WLR-NS or WLR-HS (Fig. 4), respectively. Moreover, glomerular volume in the WFR-HS was greater than that in the WFR-NS. Glomeruli

### Table 2. Urinary Albumin Excretion (UAE) with Correction by Urinary Creatinine Concentration in Wistar Lean (WLR) and Fatty Rats (WFR)

<table>
<thead>
<tr>
<th>Rat (N)</th>
<th>Before UAE (mg/g Cr)</th>
<th>8-week-old UAE (mg/g Cr)</th>
<th>10-week-old UAE (mg/g Cr)</th>
<th>14-week-old UAE (mg/g Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WLR-NS (8)</td>
<td>30.4 ± 1.6</td>
<td>24.4 ± 2.2</td>
<td>18.7 ± 2.0</td>
<td>18.4 ± 0.2</td>
</tr>
<tr>
<td>WLR-HS (9)</td>
<td>34.0 ± 3.7</td>
<td>31.8 ± 5.4</td>
<td>36.4 ± 0.9</td>
<td>37.6 ± 2.4</td>
</tr>
<tr>
<td>WFR-NS (7)</td>
<td>56.0 ± 5.9**</td>
<td>68.5 ± 4.4*</td>
<td>68.2 ± 2.7</td>
<td>171.4 ± 80.0*</td>
</tr>
<tr>
<td>WFR-HS (9)</td>
<td>52.0 ± 6.5**</td>
<td>194.0 ± 25.2**</td>
<td>276.3 ± 30.2**</td>
<td>582.7 ± 62.0**</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *p < 0.05 vs. WLR-NS, **p < 0.01 vs. WLR-NS, †p < 0.05 vs. WLR-HS, ††p < 0.01 vs. WLR-HS, ‡p < 0.05 vs. WFR-NS. The abbreviations are the same as Table 1.
from the WFR-NS (Fig. 3c) and the WFR-HS (Fig. 3d) revealed focal mesangial matrix accumulation. Hyaline cast formation was also observed in the WFR-NS and the WFR-HS (not shown). Although not scored, these findings seemed to be more prominent in the WFR-HS.

TGF-β1 Expression in Isolated Glomeruli

The isolated glomeruli of the WFR-NS showed a slight but not significant increase of TGF-β1 expression compared to those of the WLR-NS or the WLR-HS. The expression of TGF-β1 in the WFR-HS was significantly enhanced compared to that in the WFR-NS, while TGF-β1 expression in the WLR-HS was similar to that in the WLR-NS (Fig. 5).

MAPK and MEK Expressions in Isolated Glomeruli

Both ERK1 and ERK2 expressions in the WFR-NS were similar to those in the WLR-NS and the WLR-HS. However, these expressions in the WFR-HS were markedly enhanced as compared to those in the WFR-NS (Fig. 6). In contrast to

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**Fig. 3.** The histopathological findings of glomeruli with PAS staining at 14 weeks of age. a: A normal glomerulus from the WLR-NS, original magnification at ×400. b: A glomerulus from the WLR-HS, original magnification at ×400. There was no alteration in the glomerular size or mesangial area as compared with the WLR-NS. c: A glomerulus from the WFR-NS, original magnification at ×400. Mild glomerulomegaly and focal mesangial expansion were observed. d: A glomerulus from the WFR-HS, original magnification at ×400. Glomerulomegaly and mesangial expansion were more prominent than in the WFR-NS. PAS, periodic acid-methenamine silver. The other abbreviations are the same as Fig. 1.

**Fig. 4.** The glomerular volume at 14 weeks of age. The glomerular volume was significantly increased in the WFR-NS compared to that in the WLR-NS, and was further augmented in the WFR-HS. * p < 0.05 and ** p < 0.01 vs. WLR-NS. ♂ p < 0.01 vs. WLR-HS. † p < 0.01 vs. WFR-NS. The abbreviations are the same as Fig. 1.
Fig. 5. The expression of transforming growth factor (TGF)-β1 at 14 weeks of age. The upper panel shows a representative band of TGF-β1 proteins determined by Western blot analysis. The lower panel shows quantitative results determined by densitometric analysis. TGF-β1 expression in WFR-HS was the highest among the four groups. * p < 0.01 vs. WLR-NS, † p < 0.01 vs. WLR-HS, ‡ p < 0.01 vs. WFR-NS. The abbreviations are the same as Fig.1.

Fig. 6. The expression of extracellular signal-regulated kinase1/2 (ERK1/2) at 14 weeks of age. The upper panel shows the bands corresponding to the ERK1/2 proteins. The middle and lower panels show quantitative results of ERK1 or ERK2 determined by densitometric analysis, respectively. The expressions of ERK1/2 in the WFR-HS were significantly higher than those in the other three groups. * p < 0.01 vs. WLR-NS, † p < 0.01 vs. WLR-HS, ‡ p < 0.01 vs. WFR-NS. The abbreviations are the same as Fig.1.

Fig. 7. The expression of p38 mitogen-activated protein kinase (p38 MAPK) at 14 weeks of age. The upper panel shows a representative band of p38 MAPK protein determined by Western blot analysis. The lower panel shows quantitative results determined by densitometric analysis. The expression of p38 MAPK was significantly higher in the WFR-NS than in the WLR-NS, and was further augmented in the WFR-HS. * p < 0.01 vs. WLR-NS, † p < 0.05 and ‡ p < 0.01 vs. WLR-HS, † p < 0.05 vs. WFR-NS. The abbreviations are the same as Fig.1.

Fig. 8. The expression of c-Jun NH2-terminal kinase (JNK) at 14 weeks of age. The upper panel shows a representative JNK protein band determined by Western blot analysis. The lower panel shows quantitative results determined by densitometric analysis. There were no differences in the JNK expression among the four groups. The abbreviations are the same as Fig.1.
ERK1/2, p38 MAPK expression in the WFR-NS was significantly greater than that in the WLR-NS, and was further increased in the WFR-HS (Fig. 7). On the other hand, there was no difference in JNK expression among the four groups (Fig. 8).

Finally, we determined the expression of MEK1, an upstream kinase of ERK1/2, in the isolated glomeruli. Similar to the expressions of ERK1/2, MEK1 expression was not altered in the WFR-NS, while that in the WFR-HS was significantly higher than that in the other three groups (Fig. 9).

**Discussion**

Systemic hypertension has been reported to aggravate diabetic nephropathy through its additive elevation of intraglomerular pressure. However, this association has been investigated only in an experimental model of type 1 diabetes mellitus induced by streptozotocin (18). The purposes of this study were to determine whether this mechanism would be observed in type 2 diabetes mellitus, and if so to elucidate its intracellular signaling mechanism. In this study we used male WFR as a model of type 2 diabetes. We recently reported that WFR were normotensive on a normal sodium diet, but became extremely hypertensive when administered a high sodium diet. In the present study WFR became hypertensive 2 weeks after sodium loading - i.e., at 8 weeks of age - and remained hypertensive throughout the remainder of the experimental period (Fig. 1), as previously reported (14). Based on our preliminary observation that UAE was enormously increased in association with the blood pressure elevation, we have postulated that diabetic nephropathy in the WFR was accelerated by the salt load-induced hypertension. In fact, WFR without salt loading showed normal blood pressure and only a slight increase in UAE. However, UAE in the WFR with salt loading was extremely enhanced as the blood pressure was elevated (Fig. 2). Moreover, glomerulomegaly in the WFR was significantly augmented by the blood pressure elevation with sodium load (Fig. 4). Mesangial expansion and the formation of nodular lesions were also prominent in the hypertensive WFR (Fig. 3). These observations indicate that the diabetic nephropathy in young WFR could be accelerated by systemic hypertension.

WFR showed extreme hyperinsulinemia, which was less pronounced in the presence of hypertension (Table 1). With regard to the relationship between hyperinsulinemia and diabetic nephropathy, glomerular hypertrophy has recently been reported to be closely related to hyperinsulinemia (19). Thus it is unlikely that insulin itself had any effect on the advance of diabetic nephropathy in the hypertensive WFR. However, the pathogenesis and physiological roles of the relatively lower hyperinsulinemia in this condition remain to be determined.

Genetic experimental models of hypertension, such as Dahl salt-sensitive rats (DSSR) (20) or spontaneously hypertensive rats (SHR) (21), do not show such glomerular damage. However, they do show advanced glomerular injury when they became diabetic by streptozotocin injection. The intraglomerular pressure is thought to be elevated in diabetes mellitus since the renal afferent arteriole is enormously dilated in this setting (8). Thus, the intraglomerular pressure was thought to have been elevated in the normotensive WFR in this study, and the systemic hypertension may have accelerated the glomerular hypertension, resulting in the progression of renal damage.

MAPK plays a crucial role in the intracellular signaling cascade conducting the cellular proliferation or the protein synthesis. There are three main MAPK cascades: the p44 MAPK and p42 MAPK, also called ERK1 and ERK2, JNK1 and p38 MAPK (22). The hyperglycemic stimuli to the cultured mesangial cells result in mRNA expressions of TGF-β and α1-collagen or fibronectin, extracellular matrix proteins, via ERK activation (23). Kang et al. (5) also suggested that in cultured mesangial cells, a high glucose condition activated ERK1/2 and p38 activity. The relationship between MAPK cascades and diabetes has been investigated both in vitro and in vivo. ERK1/2 protein expression has been shown to be augmented in the glomeruli from streptozotocin-induced type 1 diabetic rats (4).

The elevation of intraglomerular pressure provokes mechanical strain stimuli to the mesangial cells (24). Glomerular hypertension induces the expressions of various proteins in the mesangial cells, resulting in glomerular proliferation and sclerosis. MAPK cascades, which are activated by high
glucose conditions in vitro (5) or in experimental diabetes models in vivo (4), have also been reported to be activated by mechanical strain stimuli to the glomeruli. In cultured mesangial cells, ERK1/2 were activated by mechanical stretch via MEK activation, which occurs upstream of ERK (25). Moreover, MAPK, especially ERK and JNK, mediate the overproduction of extracellular matrix proteins in the mesangial cells exposed to mechanical stretch in vitro (25).

In this study, we observed that the expressions of ERK1/2 were enhanced in the hypertensive WFR but not in the normotensive WFR (Fig. 6). On the other hand, the expression of p38 MAPK was augmented in the normotensive WFR, and was further increased in the hypertensive WFR (Fig. 7). These observations suggest that only p38 MAPK, which is probably activated by hyperglycemia and in part by glomerular hypertension, is involved in the mechanism of diabetic nephropathy in normotensive WFR. In addition to the p38 MAPK cascade, ERK1/2 contributes to this process when the intraglomerular pressure is possibly further elevated by the systemic hypertension. Moreover, a recent report demonstrated that JNK expression was enhanced when cultured mesangial cells were mechanically stretched under a high glucose condition (26). Thus, in the present model, JNK might not have been involved in the mechanisms of diabetic nephropathy even if the WFR were hypertensive (Fig. 8). This difference in JNK expression under the diabetic condition may have been related to the difference in experimental design between the two studies.

Available data suggest that TGF-β, a prosclerotic cytokine, plays a central role in the processes of diabetic nephropathy. Yamamoto et al. first reported that TGF-β1 expression was increased in experimental and human diabetic nephropathy (3). Moreover, in subsequent studies TGF-β1 appears to have been closely related to the accumulation of extracellular matrix proteins in mesangial cells under a high glucose condition in vitro (27) and in an experimental model of diabetes in vivo (28). In the present study, TGF-β1 expression in isolated glomeruli tended to be increased in normotensive WFR. Sodium load to the WFR caused blood pressure elevation and augmentation of the glomerular TGF-β1 expression, suggesting that the augmentation of the glomerular hypertension induced by the systemic hypertension in addition to the hyperglycemia in the WFR can enhance the TGF-β1 expression in the isolated glomeruli. Interaction between the hemodynamic pathway stimulated by the systemic hypertension and the metabolic pathway seems to be involved in the glomerular TGF-β1 expression in WFR in vivo. Hemodynamic and metabolic interaction in vivo was also reported by Kang et al., who showed that the expression of TGF-β receptors in the glomeruli was increased in SHR when they became diabetic by streptozotocin injection (29).

This study has clearly shown that systemic hypertension augments the glomerular expressions of ERK1/2, p38 MAPK, and TGF-β1 in a rat model of type 2 diabetes. In this study, we did not examine the signaling between MAPK cascades and TGF-β1. It has generally been shown that TGF-β1 induces activation of p38 MAPK (30) or ERK1/2 (31) in various types of cells. On the other hand, recent reports demonstrate that stretch-induced TGF-β1 expression in cultured mesangial cells is mediated by p38 MAPK activation (32). Similarly, activation of the MEK-ERK cascade by high glucose stimuli to the mesangial cells is followed by the augmentation of TGF-β1 expression (23). Moreover, substantial evidence has suggested that angiotensin II (ANG II) contributes to the development of diabetic nephropathy both in humans (33) and in an experimental model (34), probably through its induction of TGF-β expression in the mesangial cells (35). ANG II has also been proved to activate ERK1/2 (36) and p38 MAPK (37) in the mesangial cells. Taken together, these results suggest that ANG II plays a pivotal role in the mechanisms by which systemic hypertension accelerates diabetic nephropathy. The precise mechanisms of the signal transduction between ERK1/2 or p38 MAPK and TGF-β1, including the activity of ANG II, should be further investigated in this model.

This study was the first to demonstrate an increase in UAE and an aggravation of histopathological changes in association with blood pressure elevation in young WFR, a rat model of type 2 diabetes. In conclusion, systemic hypertension in WFR accelerated the diabetic nephropathy via the MEK-ERK and p38 MAPK cascades. TGF-β1 was also involved in this mechanism. Sodium-loaded WFR are thus a suitable experimental model for early diabetic nephropathy in type 2 diabetes mellitus.

Acknowledgements

We are very grateful to Ms. Yasuko Ishii for her excellent technical assistance.

References