Vascular insulin resistance related to endoplasmic reticulum stress in aortas from a rat model of chronic kidney disease

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Zhou QG, Fu XJ, Xu GY, Cao W, Liu HF, Nie J, Liang M, Hou FF. Vascular insulin resistance related to endoplasmic reticulum stress in aortas from a rat model of chronic kidney disease. Am J Physiol Heart Circ Physiol 303: H1154–H1165, 2012. First published August 31, 2012; doi:10.1152/ajpheart.00407.2012.—Metabolic insulin resistance has been demonstrated in patients with nondiabetic chronic kidney disease (CKD), yet their vascular insulin signaling remains poorly understood. Here we tested the hypothesis that vascular insulin signaling was impaired and related with endoplasmic reticulum (ER) stress in aortas from the reduced renal mass (RRM) model of CKD. The activity of insulin signaling and markers of ER were determined in aortas from rats with RRM and cultured human umbilical vein endothelial cells. Tyrosine phosphorylation of insulin receptor-β and insulin receptor substrate (IRS)-1 and phosphorylation of protein kinase B and endothelial nitric oxide synthase were all decreased in aorta from RRM rats, whereas serine phosphorylation of IRS-1, a marker of insulin resistance, was increased. In addition, nitric oxide generation and insulin-mediated vasorelaxation were decreased in aortas from RRM rats. Insulin signaling in cultured vascular endothelial cells was impaired by induction of ER stress and was restored in aortas of RRM rats by inhibition of ER stress. Taken together, rats with RRM had vascular insulin resistance that was linked to ER stress. This identified vascular insulin resistance and ER stress as a potential therapeutic target for cardiovascular complications in patients with CKD.

vascular insulin resistance; endoplasmic reticulum stress; aorta; chronic kidney disease; endothelial dysfunction

CARDIOVASCULAR DISEASE (CVD) is the leading cause of death in patients with chronic kidney disease (CKD). Although the mechanisms have not been well-defined, these patients harbor many traditional and nontraditional cardiovascular risk factors (3). Endothelial dysfunction is recognized as an important initial step in the development of CVD (42, 47). Prospective studies showed that endothelial dysfunction predicts cardiovascular events in patients with or without established vascular disease (24). Luminal endothelium is the first target of pathogens and damaging toxic compounds, including risk factors for atherosclerosis. The injured endothelial cells significantly impair vascular function and trigger thrombogenic and atherogenic reaction cascades. Thus, endothelial dysfunction plays a key role in the pathogenesis of CVD (51). The association of progressive impairment in renal function with a parallel endothelial dysfunction has been described in patients with CKD (23, 25, 33). However, the detailed mechanisms underlying the endothelial dysfunction remain elusive.

It has recently emerged that the endothelium is a target tissue of insulin, and insulin resistance can therefore exist at the level of the endothelial cell (13, 43). The vascular function of insulin is related mainly to production of nitric oxide (NO) in endothelium (31, 49). The insufficient insulin-mediated activation of endothelial nitric oxide synthase (eNOS), with subsequent absolute decrease of NO bioavailability, may be one mechanism by which endothelial dysfunction occurred under the condition of insulin resistance (13, 36). Experimental evidence has shown that defects upstream or downstream of insulin signaling pathways result in impairment of insulin-mediated endothelial effects (19, 21). Mice lacking insulin receptor (IR) specifically in endothelium have reduced expression of eNOS (43). Similarly, transgenic mice with a dysfunctional IR in endothelium show a significant reduction in NO bioavailability even in the absence of a metabolic phenotype. Subjects with a genetic polymorphism of insulin receptor substrate-1 (IRS-1) that has been implicated in metabolic insulin resistance is also associated with genetically based endothelial dysfunction (13, 14). Moreover, activation of protein kinase B (Akt) and eNOS is attenuated in vessels from patients with insulin resistance (32). With the loss of NO bioactivity, endothelium is biased toward a vasoconstrictor, prothrombotic, and proinflammatory state. Indeed, the atherosclerotic lesion size was more than twofold higher in mice lacking endothelial insulin signaling (37). Accordingly, insulin resistance in endothelial cells can be causally related with their dysfunctions and involved in the development of CVD.

Even though the metabolic insulin resistance, revealed by homeostasis model assessment and increased levels of plasma insulin, has been demonstrated in patients with nondiabetic CKD (6, 11), the activity of vascular insulin signaling in this setting remains poorly understood. Endoplasmic reticulum (ER) stress has been shown to be linked with vascular dysfunction (5, 7, 9, 12, 52, 52). Thus, we aimed to test the hypothesis that the insulin signaling in aorta might be impaired and linked with ER stress under conditions of CKD. The data showed that insulin resistance occurred in the aortas of rats with CKD. In addition, uremic serum was sufficient to induce insulin resistance in cultured vascular endothelial cells. Furthermore, we found that inhibition of ER stress could significantly improve the vascular insulin resistance in vivo and in vitro. Our results implied that the prevention of vascular insulin resistance might be a potential target against the development of hypertension and CVD in patients with CKD.

MATERIALS AND METHODS

Animal and tissue preparation. Male Sprague-Dawley rats initially weighing 180–200 g (Southern Medical University Animal Experiment Center) were used in this study and maintained under standard-
ized conditions with a standard rodent diet. Rats were housed in temperature-controlled, light-cycled quarters with ad libitum access to food and water. The rats were subjected either to five-sixths nephrectomy by performing a right nephrectomy with surgical resection of two-thirds of the left kidney or to sham operation under anaesthesia (10). After 14–16 wk of the surgery, rats were subjected to analysis. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication no. 85–23, revised 1996). All animal procedures were approved by the Animal Experiment Committee of Southern Medical University.

Rats were fasted overnight (12 h), and blood was drawn by transcutaneous cardiac puncture after anesthesia. The aorta was dissected and placed in ice-cold PBS solution where adhering fat removal and blood cleansing were conducted. Special care was taken not to stretch or damage the intimal layers of the vessel. Isolated aortas were kept in Dulbecco’s modified Eagle’s medium containing 0.1% BSA at room temperature for 2 h and then stimulated with insulin (100 nM; Sigma-Aldrich, St. Louis, MO) or vehicle (PBS) for 30 min at 37°C (18). Next, the aortas were cut and frozen with liquid N2 and kept at −80°C for subsequent analysis.

Measurement of biochemical parameters and blood pressure. Plasma creatinine levels were determined using commercial kits (Maker, Sichuan, China) according to the manufacturer’s instruction. Plasma glucose concentrations were measured according to the standard enzymatic assays. Insulin was measured in plasma with rat insulin (Maker, Sichuan, China) according to the manufacturer’s instruction. Plasma creatinine levels were determined using commercial kits (Maker, Sichuan, China) according to the manufacturer’s instruction.

Table 1. Biochemical and physical parameters in the experimental rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>RRM</th>
<th>RRM + Vehicle</th>
<th>RRM + PBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>513 ± 11</td>
<td>435 ± 26*</td>
<td>433 ± 23</td>
<td>475 ± 28#</td>
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<tr>
<td>Renal function</td>
<td></td>
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<tr>
<td>Plasma creatinine, μmol/l</td>
<td>60 ± 3.8</td>
<td>133 ± 10.2*</td>
<td>132 ± 10.3</td>
<td>125 ± 10.7</td>
</tr>
<tr>
<td>BUN, μmol/l</td>
<td>6.7 ± 1.0</td>
<td>14.2 ± 2.4*</td>
<td>14.1 ± 2.4</td>
<td>12.6 ± 1.1</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.5 ± 1.2</td>
<td>5.3 ± 1.1</td>
<td>5.4 ± 1.1</td>
<td>5.6 ± 1.3</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>129 ± 13</td>
<td>171 ± 14*</td>
<td>168 ± 15</td>
<td>166 ± 14</td>
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<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Systolic BP</td>
<td>121.8 ± 4.4</td>
<td>149.6 ± 8.4*</td>
<td>147.3 ± 7.9</td>
<td>139.5 ± 4.8#</td>
</tr>
<tr>
<td>Mean BP</td>
<td>102.9 ± 3.2</td>
<td>123.5 ± 5.3*</td>
<td>122.6 ± 5.5</td>
<td>117.5 ± 5.0#</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD; n = 6 rats in each group. RRM, reduced renal mass; PBA, 4-phenyl butyric acid; BUN, blood urea nitrogen; BP, blood pressure. *P < 0.01 vs. sham. #P < 0.05 vs. RRM + vehicle.

Immunoprecipitation was performed to observe the tyrosine phosphorylation. Phosphotyrosine-containing proteins from the aortas and cells were immunoprecipitated from equal amounts of cellular protein (500 μg) with anti-phosphotyrosine antibody (10 μg; Cell Signaling) at 4°C overnight. Around 500 μl of protein A-Sepharose were then added and incubated at 4°C for 2 h followed by centrifugation at 10,000 g. Immune-complex was resuspended, washed thoroughly, and subjected to SDS-PAGE followed by immunoblotting with anti-IRβ or IRS-1 antibody.

Vascular reactivity studies. Vasomotor function was assessed ex vivo in aortic rings as previously reported (22, 53). Briefly, the aortas were carefully dissected and placed in ice-cold Krebs-Henseleit solution (KHS) where adhering fat removal and blood cleansing were conducted. Sequential 4-mm segments were cut and immersed in an organ bath containing 37°C KHS bubbled with 95% O2 and 5% CO2 with a pH of 7.3–7.4. Aortic rings were mounted between two stirrups, and isometric tension was recorded. After a stabilization period, the cumulative dose response to phenylephrine (1 nM to 10 μM) was first assessed in the aorta rings. After washing and reequilibration, relaxation responses to acetylcholine (1 nM to 10 μM) and insulin (1 nM to 3 μM) were assessed in the rings preconstricted to 70% of their maximal phenylephrine-induced tension. Relaxation was expressed as the percentage of preconstricted tension. Cumulative dose responses to sodium nitroprusside (SNP, 0.1 nM to 1 μM) also were performed in endothelium-denuded aortic rings where the endothelium was removed mechanically by rubbing the lumen. Each experimental protocol was separated by at least 30 min. In another experiment, aortic rings were incubated for 30 min with an inhibitor of nitric oxide synthase (NOS) [Nω-nitro-arginine methyl ester (l-NAME)] before stimulation with insulin.

Aortic NO generation. Nitrite levels were determined as an index of NO generation in aortic homogenates by the Griess reaction as described previously (4). The aortas were homogenized in ice-cold homogenization buffer (10 mmol/l Tris-HCl, pH 7.4). The homogenate was centrifuged at 1,000 g for(277,806),(722,816)
VASCULAR INSULIN RESISTANCE AND ER STRESS IN CKD

(17). All cultures were grown at 37°C in a humidified atmosphere containing 5% CO2. All of the experiments reported in this paper were carried out on cells between the third and fifth passage. For all experiments, EAhy926 cells were cultured at about 80–90% confluence and starved in serum-free medium for 6–12 h before stimulation. Primary human umbilical vein endothelial cells were cultured and maintained as previously described (16). The quiescent endothelial cells were pretreated with uremic sera or control sera (10% each) for 3 h followed by stimulation with or without insulin (10 nM) in fresh medium for 15 min, and then the cells were subject to analysis.

Uremic serum was collected from 10 patients preparing for initiation dialysis who were diagnosed with nondiabetic kidney disease and free of diabetes mellitus (6 males, age 30 ± 10 yr, glomerular filtration rate 12 ± 4 ml/min, fasting glucose 5.3 ± 0.4, insulin levels 21.3 ± 6.7 mU/l). Control serum was obtained from 10 age-matched healthy populations (fasting glucose 5.4 ± 0.8, insulin levels 15.1 ± 4.7 mU/l). After an overnight fast, 6 ml of venous blood were drawn from each donor and cold centrifuged to obtain serum. Serum was then pooled, separated into aliquots, and stored at −80°C until use.

The investigation conforms to the principles outlined in the Declaration of Helsinki for use of human tissue or subjects. The study was approved by the ethics Review Board of Nanfang Hospital.

The activity of eNOS and NO release in cultured vascular endothelial cells. eNOS activity was determined in whole cell lysates of cultured vascular endothelial cells using a NOS detection system (Cayman Chemical) that measures the ability of NOS to convert L-[^14]C]arginine to L-[^14]C]citrulline, according to a previous report (2). Data were normalized by the amount of protein and the reaction time.

NO released in the medium by the cultured vascular endothelial cells was spectrophotometrically assessed by measuring nitrite, the stable metabolite, with a Nitrate/Nitrite Colorimetric Assay Kit (LDH method) according to the manufacturer’s instructions.

Statistical analysis. All experiments were performed in triplicate. Data were presented as means ± SD of three independent experi-

**Fig. 1.** Insulin resistance occurred in aortas of rats with reduced renal mass (RRM). Representative products of Western blot analysis for the activity of insulin signaling (A1) and activation of mitogen-activated protein kinase (B1) in aortas of rats with RRM. The rats were killed after 14–16 wk of five-sixths nephrectomy. Isolated aortas were kept in Dulbecco’s modified Eagle’s medium containing 0.1% BSA at room temperature for 2 h and stimulated with or without insulin for 30 min at 37°C. Serine phosphorylation (p) of insulin receptor substrate (IRS)-1 was increased in aortas of rats with RRM (A2). Tyrosine phosphorylation of insulin receptor (IR)-β and IRS-1 and phosphorylation of protein kinase B (Akt) and endothelial nitric oxide synthase (eNOS) were decreased in aortas of rats with RRM (A2). Phosphorylation of extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38 was increased in the aortas of rats with RRM (B2). Data are expressed as means ± SD of three independent experiments. *P < 0.01 vs. sham under the basal state. #P < 0.01 vs. sham under insulin stimulation.
ments. All data were analyzed with SPSS 11.0 for Windows. Difference in mean values between groups was tested using one-way ANOVA. To identify significant difference between two groups, comparisons were made using Student’s t-test. P values < 0.05 were considered significant.

RESULTS

Insulin resistance and ER stress in aortas of rats with RRM. Aortic insulin signaling and ER stress markers were measured to assess vascular insulin resistance and ER stress.

The biochemical and physical parameters in the experimental rats were listed in Table 1. As shown in Fig. 1, A1 and A2, tyrosine phosphorylation of IR-β and IRS-1, and phosphorylation of Akt, the essential makers of insulin signaling activation, were all significantly decreased in aortas from RRM rats than that from control rats in both basal and insulin-stimulated groups. Meanwhile, serine phosphorylation of IRS-1, an important marker of insulin resistance (38, 44), was significantly increased in aorta of rats with RRM. In line with these results, the phosphorylation of eNOS was significantly decreased in both basal and insulin-stimulated aorta from rats with RRM. These data suggested that insulin signaling and insulin-mediated phosphorylation of eNOS in aorta were inhibited in the setting of CKD. Mitogen-activated protein kinase (MAPK) is another branch of insulin signaling pathway and is overactivated under the condition of insulin resistance (30). As shown in Fig. 1, B1 and B2, the phosphorylation of ERK, JNK, and p38 MAPK was significantly increased in the basal state and after insulin stimulation in aortas from RRM rats compared with that of sham rats. These data implied that aortas from rats with CKD displayed vascular insulin resistance.

ER stress has been demonstrated to be associated with vascular dysfunction (5, 7, 12). ER stress has also been linked to metabolic insulin resistance (15). To explore whether vascular insulin resistance was linked to ER stress, we first...
measured the expression of ER stress markers in aortas of rats with RRM. As shown in Fig. 2, A1 and A2, the phosphorylation of IRE1α, PERK, the ER stress transducers, and eIF2α and expression of 78-kDa GRP78 were significantly increased in aorta of RRM rats compared with that of sham rats, indicating ER stress occurred in aorta in the setting of CKD. To investigate the link between ER stress and vascular insulin resistance, rats were treated with 4-phenyl butyric acid (PBA), a chemical chaperone that is known to reduce ER stress in vitro and in vivo (46). After 14 wk of surgery, the rats with RRM were divided into the following groups: RRM, RRM + vehicle (PBS), and RRM + PBA (400 μg/kg daily, ig administration) for a further 4 wk. As expected, the expression of ER stress markers in aorta was completely inhibited by the treatment with PBA (Fig. 2, A1 and A2). In addition, activation of JNK, a probable link between ER stress and insulin resistance (15), was also blocked. As shown in Fig. 2, B1 and B2, treatment with PBA significantly reduced serine phosphorylation of IRS-1 in aortas of RRM rats and increased the phosphorylation of Akt and eNOS. Aortic NO generation was used to assess eNOS activity. As shown in Fig. 3E, basal and insulin-stimulated aortic NO generation were reduced in rats with RRM, but the generation was enhanced significantly by PBA.

To determine whether the reduced insulin signaling led to a reduction in insulin-mediated vascular relaxation, we performed functional studies in aortic rings. As expected, a reduced endothelium-dependent response to acetylcholine, a marker of endothelial dysfunction, was observed in aortas of RRM rats (Fig. 3A). Meanwhile, the endothelium-independent response to SNP was comparable between RRM and sham groups (Fig. 3B). Consistent with an early report (22), insulin triggered an aortic relaxation that was blocked by L-NAME (Fig. 3C). Insulin-mediated relaxation was significantly reduced in RRM rats compared with sham rats (E$_{max}$ 48.5 ± 4.9% vs. 13.3 ± 1.5%, $P < 0.01$). The impaired insulin-mediated relaxation was significantly improved by treatment

![Fig. 3. Vasomotor functions and nitric oxide (NO) generation in aortas of rats with RRM. Concentration-response curves for acetylcholine (A), sodium nitroprusside (SNP, B), and insulin (C and D)-induced relaxations, together with data for NO production (E), were shown. The vascular reactivity was assessed in aorta rings from rats with sham, RRM, RRM treated with vehicle, or PBA, respectively. Nitrite levels were determined as an index of NO generation in aortic homogenates. Data are expressed as means ± SD of 8 independent experiments. *$P < 0.01$ vs. sham. §$P < 0.01$ vs. vehicle-treated RRM rats. #P < 0.01 vs. sham under insulin stimulation. †P < 0.01 vs. vehicle-treated RRM rats under insulin stimulation.](http://ajpheart.physiology.org/doi/abs/10.1152/ajpheart.00407.2012)
with PBA (Fig. 3D). Blood pressure in RRM rats treated with PBA was mildly reduced, but the plasma insulin remained unchanged (Table 1).

This implicated ER stress was involved in insulin resistance in aorta in the setting of CKD, and inhibition of ER stress was associated with improved insulin resistance.

Uremic serum induced insulin resistance and ER stress in cultured vascular endothelial cells. Cultured vascular endothelial cells were used to investigate the effects of ER stress on insulin signaling.

First, we studied the effects of uremic serum on the activity of insulin signaling in cultured vascular endothelial cells. The phosphorylation of several signal transducers of the insulin signaling pathway was determined in EA.hy926 cells, a human umbilical vein endothelial cell line, and primary human umbilical vein endothelial cells. As shown in Fig. 4, A1 and A2, serine phosphorylation of IRS-1, an important marker of insulin resistance, was significantly increased in the cells treated with uremic serum compared with control serum. Meanwhile, tyrosine phosphorylation of IR-β and IRS-1, and phosphorylation of Akt, the essential makers of insulin signaling activation, were all significantly decreased in response to insulin stimulation in uremic serum-treated cells compared with control serum-treated cells, suggesting insulin signaling was impaired in uremic serum-treated cells. In line with these results, the phosphorylation of eNOS was also significantly decreased in both the basal and insulin-stimulated state in the cells treated with uremic serum. The inhibitory effect of uremic serum on insulin-mediated phosphorylation of eNOS was time dependent (Fig. 4B). The reduced insulin signaling triggered by

![Fig. 4. Uremic serum induced insulin resistance in cultured vascular endothelial cells. A1: representative products of Western blot analysis for the activity of insulin signaling in cultured human umbilical vein endothelial cells. The overnight serum-deprived cells were incubated with 10% of control or uremic serum for 3 h followed by insulin stimulation. Treatment with uremic serum increased serine phosphorylation of IRS-1 and decrease tyrosine phosphorylation of IR-β and IRS-1 and phosphorylation of Akt and eNOS (A2). In another separate experiment, the cells were incubated with 10% of uremic serum for the indicated time. Uremic serum inhibited insulin-mediated phosphorylation of eNOS in a time-dependent manner (B). The activity of eNOS and NO release in the medium was significantly reduced in uremic serum-treated cells (C). Data are expressed as means ± SD of three or six independent experiments. Ctrl, control. *P < 0.01 vs. control under the basal state. #P < 0.01 vs. control under insulin stimulation. ANOVA, P < 0.05 in B.](http://ajpheart.physiology.org/)

A1

![Western blot analysis of insulin signaling in cultured human umbilical vein endothelial cells](http://ajpheart.physiology.org/)

A2

![Phosphorylation of IRS-1, IR-β, Akt, eNOS](http://ajpheart.physiology.org/)

B

![NO release and eNOS activity](http://ajpheart.physiology.org/)
uremic serum led to a reduction in insulin-mediated activation of eNOS and generation of NO (Fig. 4C)

The effect of uremic serum on ER stress was then investigated in vascular endothelial cells. As shown in Fig. 5, A1 and A2, cultures of endothelial cells with uremic serum led to time-dependent increases in the phosphorylation of IRE-1α and PERK, and the expression of GRP78, which are markers of ER stress. Meanwhile, neither control serum nor reintroduction...
of control serum after serum deprivation affected the expression of the ER stress markers. This suggested that insulin resistance and ER stress were induced in vascular endothelial cells by uremic serum.

To test the hypothesis that induction of ER stress triggered insulin resistance in vascular endothelial cells, these cells were cultured with tunicamycin, a inducer of ER stress in vascular endothelial cells (27). The cells were incubated with tunicamycin (1 μM) for 8 h followed by insulin stimulation. As shown in Fig. 6, A1 and A2, treatment with tunicamycin significantly inhibited tyrosine phosphorylation of IRS-1 and phosphorylation of Akt and eNOS in response to insulin stimulation, indicating that induction of ER stress is sufficient to induce insulin resistance in vascular endothelial cells. Next, the effect of PBA, a chemical chaperone that is known to reduce ER stress in vitro and in vivo, on insulin resistance was tested in cultured endothelial cells. The cells were pretreated with PBA (10 mM) for 1 h and then incubated with or without uremic serum for a further 3 h. Finally, the cells were stimulated with insulin and subjected to analysis. As shown in Fig. 6, B and C, treatment with PBA nearly completely abolished the inhibitory effect of uremic serum on insulin-mediated phosphorylation of eNOS and Akt. PBA alone had no effect on phosphorylation of eNOS. In addition, uremic serum-induced activation of JNK was also blocked (Fig. 6C). Similarly, the inhibitory effect of uremic serum on insulin-mediated phosphorylation of eNOS and Akt could also be abolished by tauroursodeoxycholic acid, another chemical chaperone that has been demonstrated to suppress the ER stress (35) (Fig. 7A). Furthermore, we tested the effect of overexpression of GRP78, which has been demonstrated to blunt ER stress (9), on uremic serum-induced ER stress and insulin resistance in cultured vascular endothelial cells. As shown in Fig. 7, B, C, and D, overexpression of GRP78 completely inhibited uremic serum-induced ER stress and insulin resistance. Thus, blocking ER stress can restore insulin signaling in the cells. We also used SP-600125 (a specific JNK inhibitor) to block the JNK signaling pathway to examine whether ER stress-related insulin resistance was mediated by JNK signaling. As presented in Fig. 8, the JNK inhibitor significantly, but not completely, alleviated the inhibitory effect of uremic serum on insulin-mediated phosphorylation of Akt and eNOS. This suggested that activation of JNK partially contributed to ER stress-related insulin resistance in uremic serum-cultured vascular endothelial cells. In line with these results, insulin-mediated activation of eNOS and production of NO were restored by inhibition of ER stress and partially restored by inhibition of JNK (Fig. 9, A and B).

Oxidative stress, a characteristic feature of CKD (20, 29), has been demonstrated to be related with ER stress (26, 48). To explore whether oxidative stress was responsible for uremic serum-induced ER stress, the cells were pretreated with the antioxidant N-acetylcysteine (NAC) for 1 h before adding uremic serum and incubated for another 3 h. As shown in Fig. 10, treatment with NAC completely abolished uremic serum-induced ER stress (Fig. 10, A1 and A2) and insulin resistance (Fig. 10, A3, B, and C). This implicated that oxidative stress in uremic serum contributed to induction of ER stress.

We concluded that ER stress exerted a pivotal role in the development of vascular insulin resistance.

**DISCUSSION**

The importance of insulin in the regulation of the cardiovascular system has been increasingly recognized (31). The data suggest that ER stress is a key mediator of insulin resistance in vascular endothelial cells.

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**Fig. 7.** Tauroursodeoxycholic acid (TUDCA) and overexpression of GRP78 blocked the inhibitory effect of uremic serum on insulin signaling in cultured vascular endothelial cells. The overnight serum-deprived cells were pretreated with or without TUDCA (500 μM) for 1 h followed by treatment with 10% of uremic serum for another 3 h. Treatment with TUDCA blocked the inhibitory effect of uremic serum on insulin-mediated phosphorylation of eNOS and Akt. In another experiment, the effect of overexpression of GRP78 on uremic serum-induced ER stress and insulin resistance was tested. Human GRP78 was induced to overexpress via infection with the recombinant adenoviral vector containing human GRP78 cDNA in the cells. β-Galactosidase (β-gal) infection was used as a viral control. The cells with or without overexpression of GRP78 were cultured with uremic serum for 3 h and subjected to analysis. Overexpression of GRP78 was achieved in the cells (B). Overexpression of GRP78 completely inhibited uremic serum-induced ER stress and insulin resistance (C and D). Data are expressed as means ± SD of three independent experiments. *P < 0.01 vs. control cells. #P < 0.01 vs. vehicle-treated cells.
Insulin plays a central role in the regulation of glucose and lipid homeostasis in target tissues such as muscle, liver, and adipose tissue. In addition to this metabolic action, insulin also exerts an important effect on vascular functions. Although metabolic insulin resistance has been reported in animal models and in patients with CKD (1, 6, 11), it's vascular action remained poorly understood. Several lines of evidence implied that insulin signaling was impaired in the aortas of rats with RRM. First, serine phosphorylation of IRS-1, which is a key inhibitor of tyrosine phosphorylation of IRS-1 that impairs downstream effectors of insulin, was significantly increased in aortas of rats with RRM, whereas insulin-mediated tyrosine phosphorylation of IR-β and IRS-1, and phosphorylation of Akt, the three important molecules involved in insulin signaling, was decreased in the aortas. Second, insulin-stimulated activation of eNOS and NO generation were reduced in aortas of rats with RRM. Insulin-stimulated aortic relaxation was also reduced. Third, insulin-stimulated activation of MAPK, which implies insulin resistance (30, 47), was significantly increased in aortas of rats with RRM. Finally, uremic serum impaired insulin signaling and activation of eNOS in cultured vascular endothelial cells. Because decreased NO availability has been a central factor in the pathogenesis of endothelial dysfunction, vascular insulin resistance could be an important contributor to endothelial dysfunction in CKD.

The mechanisms underlying the vascular insulin resistance in the setting of CKD remain to be studied. Several lines of evidence implied ER stress in vascular insulin resistance in rats with RRM. First, the ER stress markers were increased in aortas of rats with RRM. Second, exposure of vascular endothelial cells to uremic serum induced ER stress. Third, inducing ER stress caused insulin resistance in cultured vascular endothelial cells. Fourth, inhibition of ER stress alleviated the insulin resistance caused by culturing endothelial cells in uremic serum and in aortas from rats with RRM. Finally, insulin-mediated aortic relaxation as well as the activation of eNOS and generation of NO was significantly improved by inhibition of ER stress. These results suggested that vascular ER stress in rats with RRM led to vascular insulin resistance. The causal relationship between ER stress and insulin resis-
Insulin levels are elevated under the condition of CKD, have been further investigated. Peroxynitrite (9), homocysteine (34), and oxidized low-density lipoproteins (39), whose plasma levels are elevated under the condition of CKD, have been demonstrated to induce ER stress in cultured vascular endothelial cells. Furthermore, their induction of ER stress is probably related with reduct status (9, 34, 39). In consistency with these results, the present study found that the antioxidant NAC completely abolished uremic serum-induced ER stress (A2), insulin resistance revealed by phosphorylation of eNOS and Akt (A3), and activity of eNOS (B) as well as release of NO (C). Data are expressed as means ± SD of three or six independent experiments. #P < 0.01 vs. vehicle-treated cells. *P < 0.01 vs. control cells under the basal state. **P < 0.01 vs. control cells under insulin stimulation.

In summary, aortas of rats with CKD had insulin resistance secondary to ER stress. Because insulin is important in maintaining normal vascular function, and vascular insulin resistance may accelerate CVD, this may be an important target for prevention and treatment of CVD in patients with CKD.
REFERENCES


