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

Qiu Gen Zhou, Xiao Jing Fu, Guo Yu Xu, Wei Cao, Hong Fa Liu, Jing Nie, Min Liang, and Fan Fan Hou
Division of Nephrology, Nanfang Hospital, Southern Medical University, Key Lab for Organ Failure Research, Ministry of Education, Guangzhou, China

Submitted 24 May 2012; accepted in final form 20 August 2012

Zhou QG, Fu XJ, Xu GY, Cao W, Liu HF, Nie J, Liang M, Hou FF. Vascular insulin resistance related to endoplasmic reticulum (ER) 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 stress 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.

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 transectaneous 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 enzyme-linked immunosorbent assay (ELISA) (coated with mouse monoclonal anti-rat insulin antibodies and with rat insulin standard; Millipore). Insulin in the plasma from the patients was determined with human insulin ELISA (Coated with Mouse Monoclonal anti-Human Insulin Antibodies and with human insulin standard).

Systolic and diastolic blood pressure was measured by the tail cuff method using the Softron BP system (SoftroBP-98A, Tokyo, Japan).

Determination of insulin resistance and ER stress. The essential makers of insulin signaling activation such as tyrosine phosphorylation of IR-β and IRS-1, and phosphorylation of Akt, as well as the marker of insulin resistance, serine phosphorylation of IRS-1, were determined with Western blot and immunoprecipitation. The markers of ER stress, such as phosphorylation of PKR-like eukaryotic initiation factor 2a kinase (PERK), inositol-requiring enzyme-1 (IRE-1), 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-l-arginine methyl ester (l-NNAME)] 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 homogenizing buffer (10 mmol/l Tris·HCl, 0.1 mmol/l EDTA, 10 mmol/l sucrose, and 0.8% NaCl, pH 7.4). The nitrite level was estimated in the supernatant with a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI). The amount of nitrite formed was normalized to the protein content of the respective aorta.

Cell culture and treatment. The human endothelial cell hybridoma line EA.hy926, purchased from American Type Culture Collection was cultured and maintained in Dulbecco’s modified Eagle’s high-glucose (4,000 mg/l) medium (Invitrogen, Carlsbad, CA) containing 10% FBS.

Table 1. Biochemical and physical parameters in the experimental rats

<table>
<thead>
<tr>
<th></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>
</tr>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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, mmol/l</td>
<td>6.7 ± 1.1</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>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<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.

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00407.2012 • www.ajpheart.org
A1

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 and phosphorylation of extracellular signal-regulated 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 basal state. **P < 0.01 vs. sham under insulin stimulation.

A2

B1

B2
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 markers 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 (Emax 48.5 ± 4.9% vs. 13.3 ± 1.5%, P < 0.01). The impaired insulin-mediated relaxation was significantly improved by treatment
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
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 IRE1α and PERK, and the expression of GRP78, which are markers of ER stress. Meanwhile, neither control serum nor reintroduction 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 IRE1α and PERK, and the expression of GRP78, which are markers of ER stress. Meanwhile, neither control serum nor reintroduction of

Fig. 5. Uremic serum induced ER stress in cultured vascular endothelial cells. A1: representative products of Western blot analysis for the ER stress markers in cultured human umbilical vein endothelial cells. The cells were incubated with 10% of control or uremic serum for the indicated time. Incubation with uremic serum induced phosphorylation of IRE1α and PERK and increased expression of GRP78 (A2). Data are expressed as means ± SD of three independent experiments. ANOVA, P < 0.05, *P < 0.01 vs. control.

Fig. 6. Involvement of ER stress in 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 or without tunicamycin (Tm, 1 μM) for 8 h followed by insulin stimulation. Treatment with tunicamycin inhibited tyrosine phosphorylation of IRS-1 and phosphorylation of Akt and eNOS in response to insulin stimulation (A2). In another separate experiment, the cells were preincubated with or without PBA (10 mM) for 1 h followed by treatment with 10% of uremic serum for another 3 h. PBA abolished the inhibitory effect of uremic serum on insulin-mediated phosphorylation of eNOS (B) and Akt (C). Treatment with PBA also inhibited uremic serum-induced phosphorylation of JNK (C). Data are expressed as means ± SD of three independent experiments. #P < 0.01 vs. control under insulin stimulation. *P < 0.01 vs. vehicle-treated cells.
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 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
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), its 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. Insulin-stimulated aortic relaxation was also decreased in the aortas. Second, insulin-stimulated activation of eNOS and NO generation were reduced in aortas of rats with RRM. Finally, uremic serum impaired 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-

**Fig. 8.** Effect of JNK inhibitor on uremic serum-induced insulin resistance in cultured vascular endothelial cells. The overnight serum-deprived cells were pretreated with PBA (10 mM) or SP-600125 (JNK inhibitor, 10 μM) for 1 h followed by treatment with 10% of uremic serum for another 3 h. PBA completely abolished the inhibitory effect of uremic serum on insulin-mediated phosphorylation of Akt and eNOS, whereas the JNK inhibitor partially did. Data are expressed as means ± SD of three independent experiments. *P < 0.01 vs. control cells. #P < 0.01 vs. vehicle-treated cells.

**Fig. 9.** The activity of eNOS and release of NO in cultured vascular endothelial cells. The overnight serum-deprived cells were pretreated with PBA, TUDCA, or SP-600125 for 1 h followed by treatment with 10% of uremic serum for another 3 h. PBA or TUDCA completely abolished the inhibitory effect of uremic serum on insulin-mediated activation of eNOS (A) and release of NO (B), whereas the JNK inhibitor partially did (A and B). Data are expressed as means ± SD of six independent experiments. *P < 0.01 vs. control cells under the basal state. #P < 0.01 vs. control cells under insulin stimulation. ×P < 0.01 vs. vehicle-treated cells under insulin stimulation.
tance in aorta needs to be further strictly established by specific induction of ER stress in aorta in control rats. The result of present study is consistent with early reports that endothelial dysfunction and atherosclerosis are associated with ER stress (7, 8, 12). Considering the fact that resistance and conduit arteries have different responses under a pathophysiology condition (28), it is presently unclear whether these findings in a conduit artery also pertain to resistance vessels.

The molecular mechanisms for ER stress and its effect on vascular insulin resistance remain unclear. ER stress in adipocytes induced metabolic insulin resistance mediated by JNK (15). Interestingly, activation of JNK occurred in aorta of rats with CKD. Inhibition of ER stress was associated with inactivation of JNK as well as improvement of insulin resistance in vivo and in vitro. However, the JNK inhibitor could only partially alleviate uremic serum-induced insulin resistance. This suggested that, besides JNK, other signaling pathway were involved in ER stress-induced insulin resistance in vascular endothelial cells. ER stress in human umbilical vein endothelial cells activated endogenous RhoA and Ras, two important small guanosine triphosphatases expressed on the ER surface (45). Thus, the ER surface can provide a platform to organize several signaling events (50). The molecular mechanism linking ER stress to insulin resistance in vascular endothelial cells warrants further study.

The underlying mechanisms for induction of ER stress in vascular endothelial cells under the condition of CKD need to be 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 redox status (9, 34, 39). In consistence 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.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**GRANTS**

This work was supported by the 973 key program (No. 2012CB517703) and National Nature and Science Grant Nos. 30830056 and U0932002 to F. F. Hou and National Nature and Science Grant No. 81070613 to Q. G. Zhou.
REFERENCES


