Effects of Angiotensin II and Insulin on ERK1/2 Activation in Fibroblasts From Hypertensive Patients

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**Background:** Insulin resistance, a frequent finding in hypertensive patients, leads to accelerated cardiovascular damage. It has been suggested that a crosstalk between angiotensin II and insulin signaling pathways may provoke insulin resistance, and may contribute to the development of cardiovascular damage. To identify a common pathophysiologic pathway between metabolic disorders and cardiovascular remodeling, we investigated the effect of angiotensin II and insulin on extracellular signal regulated kinases 1 and 2 (ERK1/2), isoforms of mitogen-activated protein kinases (MAPK) involved in cellular proliferation and extracellular matrix deposition.

**Methods:** Skin fibroblasts from normotensive subjects, insulin sensitive hypertensive subjects, and insulin resistant hypertensive subjects were cultured and used after four passages. The ERK1/2 expression and phosphorylation were measured by Western blot using specific antibodies, respectively anti-ERK1/2 and anti-pERK1/2. Expression of AT1 receptor for angiotensin II was determined by reverse transcriptase–polymerase chain reaction in real time.

**Results:** The ERK1/2 were similarly expressed in skin fibroblasts from all groups; ERK1/2 phosphorylation evoked by angiotensin II was significantly higher in fibroblasts from hypertensive patients in comparison to normotensive subjects, but the increase was observed only in insulin resistant hypertensive subjects. The effect of insulin on ERK1/2 phosphorylation was not significantly different in the three groups. Treatment with the combination of insulin and angiotensin II increased ERK1/2 phosphorylation to a greater extent in comparison to the single agonists in normotensive subjects and in insulin sensitive but not in insulin resistant hypertensive subjects.

**Conclusions:** Angiotensin II stimulated ERK1/2 activation is increased in insulin resistant hypertensive subjects, and it may play a role in the pathogenesis of insulin resistance and accelerated cardiovascular damage. Am J Hypertens 2004;17:604–610 © 2004 American Journal of Hypertension, Ltd.

**Key Words:** Mitogen-activated protein kinase, insulin, angiotensin II, hypertension, insulin resistance, diabetes, signal transduction.

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The cardiovascular system in the hypertensive patients is remodeled to normalize wall stress. In the heart, the typical response is concentric left ventricle hypertrophy, whereas in the resistance arteries, the wall thickness and the wall/lumen ratio are increased. It has been proposed that such “remodeling” of the cardiovascular system is not only caused by hemodynamic overload but also by growth factor stimulation and increased cellular response. Angiotensin II (Ang II) acts as a growth factor for cardiomyocytes and vascular smooth muscle cells by activating mitogen-activated protein kinases (MAPK), a family of ubiquitous ser/thr kinases involved in proliferation and cellular growth, through AT1 receptor stimulation. Two members of the MAPK family, the extracellular signal regulated kinases 1 (ERK 1) and 2 (ERK 2), which induce early growth response genes when phosphorylated on tyrosine and threonine residues, are activated also by insulin. Insulin promotes a growth response through the Ras/ERKs signaling pathway and regulates the translocation of GLUT4, necessary for insulin stimulated glucose uptake, through the phosphatidylinositols 3-kinase (PI 3-kinase)/AKT signaling pathway. In insulin resistant hyper-
tensive patients, glucose uptake in the skeletal muscles is reduced because insulin signaling via the PI 3-kinase/AKT pathway is impaired, whereas the MAPK pathway is unaffected or even increased.

An interaction between Ang II and insulin on ERK1/2 activation may account for develop accelerated cardiovascular damage in hypertensive patients with insulin resistance and hyperinsulinemia. Therefore, the present study was designed to investigate the interactions between Ang II and insulin on ERK1/2 in normotensive subjects and hypertensive patients, classified by their insulin sensitivity. To this aim, we used cultured skin fibroblasts in vitro, which can be easily obtained via a skin biopsy. These fibroblasts express a variety of different receptors including G-protein coupled AT1 receptors, activating well known pathways of intracellular signal transduction. Furthermore, they are actively involved in the process of renal and cardiovascular fibrosis and in the development of target organ damage in hypertensive and diabetic patients. They can also be cultured for several passages in standardized conditions, offering a useful model for the investigation of intrinsic (possibly genetic) defects of cell function, independently of the environmental abnormalities caused by hypertension and hyperinsulinemia in vivo.

**Methods**

**Patient Selection**

We recruited 12 healthy normotensive volunteers without a family history of hypertension and diabetes, and 21 patients with essential hypertension from the Hypertension Outpatient Clinic of the Department of Clinical and Experimental Medicine, University Hospital of Padua, Italy. All subjects gave informed consent to the study, which had been approved by the local Ethical Committee. Arterial hypertension was diagnosed according to the World Health Organization—International Society of Hypertension guidelines (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg).

Insulin sensitivity was assessed according to the model-based method for assessing insulin sensitivity from the oral glucose tolerance test (OGTT) (75 g) after an overnight fast and is expressed as oral glucose insulin sensitivity (OGIS) index. According to a previous report, OGIS is calculated using a model-derived formula from the OGTT glucose and insulin concentration, and includes six constants optimized to match the clamp results.

Blood samples were obtained in fasting conditions and 30, 60, 120, 180 min after the oral glucose load. Plasma glucose and insulin were performed by a colorimetric enzymatic test and by radioimmunoassay, respectively. The patients were classified as insulin sensitive if the OGIS index was ≥400 mL·min⁻¹·m⁻² (the lowest value of normotensive control subjects) or insulin resistant if it was <400. Ten hypertensive patients were insulin sensitive (OGIS index 465 ± 10) and 11 were insulin resistant (OGIS index 323 ± 26, P < .01).

**Cell Culture**

Human fibroblasts were derived from a skin biopsy taken from the anterior surface of the left forearm by excision under topical anesthesia with ethyl chloride, and cultured in Nutrient Mixture F-10 HAM medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 4 mmol/L glucose. Cells were seeded onto a 25-cm² flask and incubated at 37°C, and the medium was changed every 2 to 3 days. Fibroblasts were obtained from each subject and grown separately, and were used for the experiments at the fourth passage. The fibroblasts were identified morphologically. In particular, they were strictly diploid and, upon morphologic confluence, they appeared oriented with respect to one another, forming a typical parallel array of cells with no dividing nuclei visible by microscope. As previously described, the cells were in the plateau phase of growth, in which there is a steady state condition with an almost completely ceased cell proliferation. There were no morphologic differences in fibroblasts from hypertensive subjects, either insulin sensitive or insulin resistant, and normotensive and frequent observations with phase-contrast optic revealed no differences in granularity and vacuolation that might have a bearing on the health status of the culture.

**Immunoblot Analysis of ERK1/2 Expression and Phosphorylation**

Fibroblasts were grown in 10% FBS F10-Ham medium until confluenced, after which they were switched to serum-free F10-HAM for 24 to 36 h for quiescence. For the experiments, cells were stimulated with Ang II or insulin in serum-free HAM for 24 h. After treatment, cells were collected with a rubber scraper on ice by using sample buffer (12.5 mmol/L Tris, 2 mmol/L EGTA, 25 mmol/L β-glycerophosphate, 2 mmol/L Na3VO4, 10 μmol/L PMSF, 1 μmol/L leupeptin, 5 μmol/L aprotinin), sonicated on ice, and centrifuged at 10,000 g for 10 min. The supernatant was solubilized in Laemmli buffer and then separated by electrophoresis through a 10% polyacrylamide gel. Proteins separated on the gels were electroblotted onto nitrocellulose membrane (Hybond ECL; Amersham Bioscience, Little Cherkfont, Buckinghamshire, United Kingdom) in blotting buffer containing Tris 48 mmol/L, glycine 39 mmol/L, sodium dodecyl sulfate 0.037%, methanol 20% (vol/vol) for 3 h at 100 V in the cold, using a Transblot cell (Elettrofors, Padova, Italy). The membranes were blocked overnight at 4°C in T-PBS containing phosphate buffered saline (PBS) and 0.05% (vol/vol) Tween along with 5% bovine serum albumin. Membranes were exposed to primary antibody (1:4000 dilution) for anti-ERK1/2 (Santa Cruz Biotechnology, Santa Cruz, CA) (1:1500 dilution) for anti-p-ERK1/2 (New England Biolabs, Beverly, MA) overnight at 4°C. Membranes were washed (four times for 20 min) with the same buffer and then incubated with 1:4000 goat anti-
rabbit antibody conjugate to horseradish peroxidase. Detection was made using the enhanced chemiluminescence system (ECL) from Amersham. The blots probed with phosphoantibodies were stripped and reprobed with a non-phospho antibody to assure equal loading. The density of resulting protein bands was analyzed by using a Chemiluminescence Molecular Imaging Systems (VersaDoc Model 1000; Bio-Rad, Hercules, CA), and results were expressed relative to the control(s) on the same blot, set at 100% and by pERK/ERK densitometric ratio. In all experiments, expression and phosphorylation of ERK2 was higher than that of ERK1 but pERK/ERK densitometric ratio was similar. Furthermore, they responded to agonists with the same increase. Therefore, and because no difference in function and regulation between ERK1 and ERK2 has so far been identified to our knowledge, we used their average value.

**RNA Extraction From Human Fibroblasts**

RNA was extracted from human fibroblasts by RNAzol B (Life Technologies, Rockville, MD). Total RNA was isolated according to the manufacturer’s suggested protocol with the addition of a chloroform extraction step and phase separation, and wash of the isolated RNA in 75% ethanol. RNA was resuspended in polymerase chain reaction (PCR) grade water and the purity of RNA was determined from the ratio of absorbance readings at 260 nm and 280 nm (Perkin-Elmer, Foster City, CA).

**Multiplex Real-Time Quantitative Reverse Transcriptase–Polymerase Chain Reaction With iCycler for mRNA AT1 Receptor**

Real-time quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) was performed using specific TaqMan primers and probes by the iCycler iQ system (Bio-Rad, Hercules, CA). Primer and probe sets for AT1 type receptor and for housekeeping gene GAPDH were designed from sequences in the Genbank database using Primer 3 (provided by the Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA) and Operon’s Oligo (Operon Technologies, Alameda, CA) software. The probe of AT1 type receptor was labeled with a reporter 6-carboxy-fluorescein (FAM) dye at its 5'-end and a Quencher dye (DABCYL) at its 3'-end. The probe housekeeping gene was labeled with TEXAS red dye at its 5'-end and DABCYL at its 3'-end. All the reactions were performed with “one-tube RT-PCR assay” in a total volume of 50 μL in 96-well plates. The thermal cycling was carried out as follows: RT at 50°C for 30 min, followed by PCR: 40 thermal cycles of 30 sec at 94°C for denaturation, and for 90 sec at 62°C for annealing and extension. Quantitative analysis of gene expression was done using the standard curve and comparative Ct (ΔΔCt) methods, in which Ct is the threshold cycle number (the minimal number of cycles needed before the product can be detected) and the arithmetic formula is given by $2^{-\Delta\Delta Ct}$.

**Statistical Analysis**

Analysis was carried out using the SPSS software package, version 10.0.1 (SPSS Inc., Chicago, IL). Statistical comparisons were performed with nonparametric tests for paired and unpaired data. A value of $P < .05$ was considered to be statistically significant. Values are expressed as means ± SEM.

**Results**

**Patient Characteristics**

Characteristics of study population are listed in Table 1. Hypertensive patients had an increased age, body mass index, and higher blood pressure levels than normotensive subjects. Gender distribution was similar across groups. When hypertensive patients were divided according to the sensitivity of insulin (based on OGIS index), insulin resistant hypertensive patients had increased body mass index, and insulin and fasting glucose levels in comparison to normotensive subjects and insulin sensitive hypertensive subjects (Table 1).

### Table 1. Clinical characteristics of the study groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>P (NT v HT-IS)</th>
<th>P (NT v HT-IR)</th>
<th>P (HT-IS v HT-IR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects (male)</td>
<td>12 (4)</td>
<td>10 (6)</td>
<td>11 (3)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Age (y)</td>
<td>37 ± 3</td>
<td>44 ± 3</td>
<td>52 ± 3</td>
<td>NS</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>125.6 ± 2.0</td>
<td>160.4 ± 4.8</td>
<td>156.2 ± 4.7</td>
<td>&lt; .01</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>79.1 ± 1.8</td>
<td>100.2 ± 1.4</td>
<td>94.7 ± 1.6</td>
<td>&lt; .01</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.7 ± 0.8</td>
<td>27.6 ± 1.1</td>
<td>31.8 ± 1.3</td>
<td>&lt; .01</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Serum glucose (mg/dL)</td>
<td>88.4 ± 6.2</td>
<td>97.3 ± 5.3</td>
<td>115.5 ± 6.4</td>
<td>NS</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Insulin (μUI/mL)</td>
<td>6.4 ± 2.2</td>
<td>7.1 ± 1.0</td>
<td>19.1 ± 4.1</td>
<td>NS</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>OGIS (mL·min⁻¹·m⁻²)</td>
<td>459 ± 18</td>
<td>465 ± 10</td>
<td>323 ± 26</td>
<td>NS</td>
<td>&lt; .01</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Insulin sensitivity was calculated as OGIS index as reported in the methods.

BMI = body mass index; DBP = diastolic blood pressure; HT = hypertensive; IR = insulin resistant; IS = insulin sensitive; NS = not significant; NT = normotensive; SBP = systolic blood pressure.
Time Course and Dose-Response of Ang II on ERK1/2 Phosphorylation

We first characterized profiles of ERK1/2 phosphorylation in response to Ang II and insulin. We used phosphospecific antibodies that recognized only dually phosphorylated (activated) forms of ERK1/2 and performed Western blot analyses. As shown in Fig. 1A, Ang II activated ERK1/2 in confluent serum-starved human skin fibroblasts in a dose-dependent manner, with a maximal stimulation seen between 100 and 1000 nmol/L. The dose of 250 nmol/L was therefore used in subsequent time-course experiments. A characteristic time course of Ang II–induced increase in ERK1/2 phosphorylation in this cell type is shown in Fig. 1B. Activation was rapid with a maximal response within 2 min after exposure to Ang II. The phosphorylation signal subsequently declined, returning to baseline within 20 min. Inhibition of the AT1 receptor with losartan (500 nmol/L)5 abolished activation of ERK1/2, whereas the inhibitor of AT2 receptor, PD123319 (500 nmol/L),5 had no statistically significant effect. Furthermore, incubation with 1 μmol/L/L PD 98059, an inhibitor of the MAPK pathway,16 significantly reduced Ang II–induced ERK1/2 activation (Fig. 1C).

We then determined the time course of insulin-induced ERK1/2 phosphorylation in human fibroblasts. Insulin (100 nmol/L) stimulated ERK1/2 phosphorylation with a peak at 10 minutes and later declined after 1 h (data not shown).

ERK1/2 Expression and Phosphorylation Induced by Ang II and Insulin in Normotensive and Hypertensive Patients

ERK1/2 expression was not significantly different between normotensive subjects and hypertensive subjects. Ang II (250 nmol/L for 2 min) stimulated phosphorylation of ERK1/2 to a larger extent in fibroblasts from hypertensive subjects in comparison to normotensive subjects (by 68% ± 5% and 46% ± 4%, respectively, P < .01). On the contrary, the insulin induced increase of ERK1/2 phosphorylation was similar in normotensive subjects and hypertensive subjects (54% ± 6% and 71% ± 6%, respectively).
respectively, $P = \text{ns}$). Basal ERK1/2 phosphorylation was similar in the two groups (data not shown).

Next, we examined the effects of the association of insulin (100 nmol/L for 10 min) and Ang II (250 nmol/L for 2 min) exposure on ERK1/2 phosphorylation. In normotensive subjects the stimulation of ERK1/2 phosphorylation was larger in cells treated with both agonists in comparison with single agents ($108 \pm 22\%$ vs $54 \pm 6\% \pm 4\%$, $P < .05$). Similarly, in fibroblasts from hypertensive subjects ERK1/2 phosphorylation induced by the combination was significantly higher in comparison with either insulin or Ang II alone ($121 \pm 16\%$ vs $71 \pm 6\% \pm 8\%$, $P < .05$).

**ERK1/2 Expression and Phosphorylation Induced by Ang II and Insulin in Insulin-Sensitive and Insulin-Resistant Hypertensive Patients**

Hypertensive patients were divided into insulin-sensitive and insulin-resistant groups, according to their insulin sensitivity, as assessed by OGIS and as described in Methods. All of the normotensive subjects were insulin sensitive. Basal ERK1/2 expression and phosphorylation were similar in insulin-sensitive and insulin-resistant hypertensive subjects (data not shown).

As shown in Fig. 2, treatment with the combination of insulin (100 nmol/L for 10 min) and Ang II (250 nmol/L for 2 min) increased ERK1/2 phosphorylation to a greater extent in comparison to the single agents in cells from insulin sensitive, but not from insulin-resistant hypertensive subjects ($149 \pm 26\%$ vs $78 \pm 10\% \pm 58 \pm 6\%$, $P < .05$, and $92 \pm 13\%$, $64 \pm 8\%$, and $77 \pm 6\%$, $P = \text{NS}$, respectively).

Ang II stimulated ERK1/2 phosphorylation, was higher in insulin-resistant than in insulin-sensitive hypertensive subjects and normotensive subjects ($77% \pm 6% \pm 8% \pm 6\%$, and $43% \pm 4\%$, respectively, $P < .001$), whereas ERK1/2 phosphorylation induced by insulin exposure was not significantly different ($64% \pm 8\%$, $78% \pm 10\%$, and $54% \pm 6\%$, respectively, $P = \text{NS}$; Fig. 2).

**FIG. 2.** Phosphorylation of extracellular signal regulated kinase 1 (ERK 1) (44 kDa) and ERK 2 (42 kDa) in cultured fibroblasts from normotensive and hypertensive patients classified as insulin sensitive (IS) and insulin resistant (IR). The ERK1/2 activation was determined by Western blot analysis, with a polyclonal antibody that specifically recognizes the phosphorylated forms of ERK1/2, and by densitometric analysis. Angiotensin II (Ang II; 250 nmol/L, 2 min) stimulated the phosphorylation of ERK1/2 to a larger extent in insulin resistant hypertensive patients (IR-Hypertensives) in comparison to insulin sensitive hypertensive patients (IS-Hypertensives) and in comparison to normotensive subjects. The effect of insulin (100 nmol/L, 10 min) was similar among the three groups. Treatment with the combination of insulin and angiotensin II increased ERK1/2 phosphorylation to a larger extent than the single agents in normotensive subjects and insulin sensitive hypertensive patients (IS-Hypertensives), but not in insulin resistant hypertensive patients (IR-Hypertensives). Representative immunoblot of phosphorylated ERK-1 and ERK-2 are shown in the upper panel. The lower panel shows quantitative comparisons of blots by densitometric analysis (mean of ERK1 and ERK2 bands). Each value is expressed as a percent increase of phosphorylation from baseline (mean $\pm$ SE). $^*P < .01$ v normotensive group, $^\dagger P < .05$ v Ang II in the same group, $^\ddagger P < .05$ v INS in the same group. INS = insulin.
**AT1 Receptor Expression in Human Fibroblasts**

The effects of Ang II on ERK1/2 phosphorylation are mediated by AT1 and not by AT2 receptors; therefore we assessed the expression of mRNA AT1 receptor in fibroblasts from normotensive subjects, insulin-sensitive subjects, and insulin-resistant hypertensive subjects. We found that AT1 receptor mRNA expression was not different in fibroblasts from normotensive subjects, insulin-sensitive subjects, and insulin-resistant hypertensive subjects (1.10 ± 0.06 v 1.07 ± 0.05 v 1.08 ± 0.04 ΔΔCt respectively, P = NS).

**Discussion**

The present study shows that Ang II stimulated ERK1/2 activation is increased in fibroblasts from insulin-resistant hypertensive patients in comparison with normotensive subjects and insulin-sensitive hypertensive subjects. On the contrary, insulin had a similar effect in hypertensive and normotensive subjects. The simultaneous exposure to the association of insulin and Ang II activated ERK1/2 to a larger extent than either insulin or Ang II alone in normotensive and in insulin-sensitive hypertensive subjects, but not in insulin-resistant hypertensive subjects, suggesting an abnormal crosstalk between Ang II and insulin signaling on ERK1/2 activation in the insulin-resistant syndrome. This indicates that Ang II may reduce the insulin sensitivity of the ERK1/2 pathway. This study therefore provides new evidence for the role of Ang II in accelerating cardiovascular damage in insulin-resistant hypertensive patients.

Several studies have reported an abnormal ERK1/2 activation induced by Ang II in animal models and in humans. In aorta from spontaneously hypertensive rats (SHR) and from salt-induced hypertension (DOCA-salt hypertensive rats), MAPK activation was enhanced in comparison to Wistar-Kyoto (WKY) rats. Furthermore, in vivo it has been shown that in Sprague-Dawley rats Ang II infusion induced hypertension and a marked increase in the ERK1/2 phosphorylation, whereas the pretreatment with PD98059, a specific inhibitor of ERK1/2 pathway, blunted the pressor effect, and ERK1/2 activation induced by Ang II. In lymphoblasts from hypertensive patients, basal ERK1/2 activity was increased, in comparison to normotensive subjects, whereas ERK1/2 expression was similar. In small arteries from hypertensive patients, Ang II induced a higher amount of ERK1/2 phosphorylation in comparison to that in normotensive subjects.

Our data confirm the presence of an abnormal activation of ERK1/2 in fibroblasts from hypertensive subjects but they demonstrate, for the first time, that Ang II dependent ERK1/2 phosphorylation is higher only in hypertensive patients with insulin resistance. This suggests that ERK1/2 phosphorylation and activation is dependent on insulin sensitivity, and that an abnormal regulation of the molecular mechanisms involved in the ERK1/2 dependent pathway is present in insulin resistance.

With regard to the molecular mechanisms contributing to enhanced ERK1/2 signaling, our study confirms that activation of ERK1/2 by Ang II is due to AT1 and not to AT2 receptor binding, and that the hyperresponsiveness of ERK1/2 in fibroblasts from hypertensive patients cannot be accounted for by a different expression of AT1 receptors. Therefore, it has to be regarded as a post-receptor phenomenon. Recent studies suggest that an altered regulation of mediators upstream of ERK1/2, such as c-Src, or a higher transactivation of the epidermal growth factor receptor (EGFR), may be responsible for increased Ang II–dependent ERK1/2 activation.

The activity of ERK1/2 is tightly controlled not only by the rate of phosphorylation but also by dephosphorylation. Serine/threonine protein phosphatases, protein tyrosine phosphatases, and dual specificity protein phosphatases, which include protein phosphatases-1 (MKP-1), inactivate ERK signaling. Increased ERK1/2 activation may be due to reduced MKP-1 expression. In vascular smooth muscle cells from SHR, MKP-1 is reduced in comparison to that in WKY, and high glucose exposure decreases MKP-1 expression in rat vascular smooth muscle cells. It can therefore be hypothesized that insulin resistance decreases MKP-1, leading to a poor inhibitory feedback loop on ERK1/2 signaling.

Unlike the effect of Ang II, ERK1/2 activation induced by insulin was not influenced by hypertension or by insulin sensitivity. This is in agreement with a previous study indicating that ERK1/2 activation induced by insulin is similar, and that PI3-kinase pathway is impaired in human skeletal muscle cells and in adipocytes from insulin resistant obese nondiabetic patients but not in lean control subjects.

Insulin resistance is frequently associated with compensatory hyperinsulinemia, and it has been proposed that overstimulation of the insulin receptor can lead to cardiovascular remodeling through ERK1/2 activation. According to our data, insulin and Ang II do have an additive effect on ERK1/2 phosphorylation in fibroblasts from both normotensive and insulin sensitive subjects, but not in fibroblasts from insulin resistant hypertensive subjects. These results suggest that, in vitro, insulin is able to amplify Ang II dependent ERK1/2 phosphorylation only in insulin sensitive cells. The lack of the synergistic effect of Insulin and Ang II on ERK1/2 phosphorylation in fibroblasts from insulin resistant individuals in the presence of a normal response to insulin alone suggests that short-term exposure to Ang II impairs insulin signaling also on ERK1/2, thereby extending insulin resistance to the ERK1/2 pathway. It has been demonstrated that long-term Ang II administration induces hypertension and insulin resistance by stimulating oxidative stress in Sprague-Dawley control rats, whereas endogenous Ang II suppresses insulin signaling in vascular smooth muscle.
cells from genetically hypertensive SHR rats by activating ERK1/2. Our data therefore strongly suggests that, in fibroblasts, hyperresponsiveness to Ang II blunts the effect of insulin on ERK1/2 signaling.

In conclusion, the present study shows that Ang II stimulates ERK1/2 to a greater extent in fibroblasts from insulin resistant than in insulin-sensitive hypertensive individuals and causes insulin resistance of ERK1/2 pathway in fibroblasts in vitro. Because ERK1/2 are involved in cellular proliferation and growth and extracellular matrix deposition, ERK1/2 hyperresponsiveness to Ang II may account for increased risk of cardiovascular damage in the insulin resistance syndrome. These results show how an exaggerated ERK1/2 phosphorylation by Ang II may play a pivotal role in cardiovascular remodeling in hypertensive individuals with insulin resistance.

References


