Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production.

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METHODS

Animal experiments. All procedures were performed in conformity with “Guidelines and Authorization for the Use of Laboratory Animals” (Italian Government, Ministry of Health). Male 12-wk-old spontaneously hypertensive rats (SHR/NHsd, haplotype RT1b; n = 90) and their age-matched normotensive Wistar-Kyoto controls (WKY, n = 90) obtained from Harlan Italy (Milan, Italy) were used in all studies. Systolic blood pressure (SBP) was measured using a tail cuff (Letica 5100; PanLab, Barcelona, Spain) and was monitored for the last time 24 h before death. Blood samples were obtained by cardiac puncture from rats fasted overnight, heparinized (200 IU ip; Pfizer), and then euthanized with ether. Plasma concentrations of insulin were measured using an ELISA kit (Linco Research, St. Charles, MO). Plasma glucose concentrations were determined with a diagnostic autoanalyzer (Accu-Chek Active, Roche Diagnostics, Germany). Insulin sensitivity was assessed using the quantitative insulin-sensitivity check index (QUICKI) (27).

Ex vivo perfusion system. The mesenteric vascular bed (MVB) was isolated and removed from rats as described previously (47). MVB mounted in a temperature-controlled moist chamber (type 834/1; Hugo Sachs Elektronik, March-Hungstetten, Germany) were perfused with modified Krebs-Henseleit solution continuously gassed with a mixture of 95% O2 and 5% CO2 (pH 7.4). A constant flow rate of 5 ml/min was maintained using a peristaltic pump (ISM 833; Hugo Sachs Elektronik). Drug solutions were infused into the perfusate proximal to the arterial cannula using another peristaltic pump. After equilibration (30–40 min), changes in perfusion pressure were measured with a pressure transducer system (SP 844 Capto, Horten, Norway) and recorded continuously using data acquisition and analysis equipment (PowerLab system; ADInstruments, Castle Hill, Australia). Endothelium removal was performed by flushing vessels intermittently with air for several minutes as previously described (37) and was verified by complete lack of vasodilation induced by infusion of 1 μM acetylcholine (ACh; Sigma Aldrich).

Measurement of vascular responses in MVB. Perfusion pressure (PP) at ~120 mmHg was obtained at the steady state 30–40 min after initial administration of norepinephrine (NE) and was maintained by continuous infusion with NE (10 μM and 3 μM in WKY and SHR rats, respectively). Dose-response curves measuring vasodilation (decrease in PP) in response to ACh or insulin (Novo Nordisk) were obtained by adding increasing concentrations of ACh (0.1 nM–3 μM/30 s perfusion) or insulin (0.1 nM–3 μM/4 min perfusion) to the perfusate. For all vasodilation experiments, data from each curve were normalized by defining 100% as the initial steady-state PP and 0% as the maximal reduction in PP obtained in WKY treated with a maximally stimulating dose of ACh. In some experiments, insulin dose–response curves were repeated after pretreatment with genistein (50 μM, 30 min), Nω-nitro-l-arginine methyl ester (l-NAME) (100 μM, 30 min), charybdotoxin (30 μM, 30 min), or indomethacin (10 μM, 30 min) or after endothelium removal. In other experiments, insulin-induced relaxation was measured before and after 20-min treatment with wortmannin (100 nM), PD-98059 (10 μM; Alexis Biochemicals), or BQ-123 and BQ-788 (1 μM; Neosystem, Strasbourg, France). Dose-response curves measuring vasoconstriction (increase in PP) in response to NE were obtained by adding increasing concentrations of NE (100 nM–50 μM/30 s perfusion) to the perfusate. These experiments were also repeated after a 1-h perfusion pretreatment with insulin (1 μM), insulin plus wortmannin (100 nM), insulin plus PD-98059 (10 μM), or insulin plus ET-1 receptor antagonists BQ-123 and BQ-788 (1 μM). Analogous experiments were repeated using 10 nM insulin. Relative changes in PP at the steady state reached with each individual dose were measured and expressed in millimeters of Hg.

Cell culture, immunoblotting, and ELISA assays. Bovine aortic endothelial cells (BAEC) in primary culture (Cell Applications, San Diego, CA) were grown in EGM-2 (endothelial cell growth medium) as described previously (35) and used between passages 3 and 5. For immunoblotting experiments and ELISA assays, BAEC were serum-starved overnight with EBM-A [red phenol-free endothelial basal medium from Clonetics, supplemented with 1% platelet-deprived horse serum (Sigma)] before initiation of experiments. BAEC were stimulated with insulin (100 nM, 5 min) with or without pretreatment with either wortmannin (100 nM, 30 min) or PD-98059 (10 μM, 30 min). Levels of ET-1 in the media were measured with a sensitive ELISA kit (Assay Designs, Ann Arbor, MI) according to the manufacturer’s instructions. Cell lysates were prepared using 300 μl of lysis buffer [100 mM NaCl, 20 mM HEPES, pH 7.9, 1% Triton X-100, 1 mM Na2VO3, 4 mM sodium pyrophosphate, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, and the Complete protease inhibitor mixture (Roche Applied Science)]. Samples (50 μg of total protein) were separated by 8% SDS-PAGE and immunoblotted with antibodies against Akt (Upstate Biotechnology, Lake Placid, NY), phospho-Akt(473), MAPK, or phospho-MAPKThr202/Tyr204 (Cell Signaling Technology, Beverly, MA) according to standard methods as previously described (35, 39). Blots were quantified by scanning densitometry.

Drugs. Stock solutions of each drug were prepared in distilled water except for indomethacin (dissolved in distilled water containing 4% NaHCO3 and sonicated before use) and genistein, BQ-788, PD-98059, and wortmannin (dissolved in DMSO). Final dilutions of all drugs were prepared in modified Krebs-Henseleit solution immediately before use. None of the vehicles used (including DMSO) at the final dilutions induced any significant vascular effects (assessed by appropriate controls in preliminary studies).

Statistical analysis. Results are expressed as means ± SE of n experiments, with n representing the number of rats. Two-way ANOVA for repeated measures and Student’s t-test (paired or unpaired) were used as appropriate. Values of P < 0.05 were considered to indicate statistical significance.

RESULTS

Physiological parameters. We used 12-wk-old male SHR as a genetic model of hypertension and compared them with male WKY control rats. Consistent with previous studies (37, 40), our SHR had significantly higher SBP and weight than age-matched WKY rats (Table 1). Although SHR were hypertensive, they were not diabetic, because fasting plasma glucose concentrations were normal and comparable to those of WKY rats. However, SHR had a significant fivefold increase in fasting serum insulin concentrations (compared with WKY rats), indicative of metabolic insulin resistance. Indeed, QUICKI [a surrogate index of insulin sensitivity (27)] was significantly lower in SHR compared with WKY rats (Table 1).

Endothelial response to vasodilators. For evaluation of endothelium-dependent vasodilation ex vivo, MVB preparations were first preconstricted with NE to achieve a baseline PP of ~120 mmHg. To obtain comparable baseline PP in SHR and WKY rats, we perfused with 3 and 10 μM NE, respectively.

Table 1. Physiological parameters of 12-wk-old SHR and WKY rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight</th>
<th>Systolic Blood Pressure</th>
<th>Fasting Glucose</th>
<th>Fasting Insulin</th>
<th>QUICKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>250±8.10</td>
<td>131.8±10.0</td>
<td>73.2±4.56</td>
<td>0.93±0.14</td>
<td>0.30±0.19</td>
</tr>
<tr>
<td>SHR</td>
<td>300±10.2*</td>
<td>202.2±10.7*</td>
<td>62.0±3.76</td>
<td>4.99±1.01†</td>
<td>0.25±0.23*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 90 for each group. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; QUICKI, quantitative insulin-sensitivity check index. *P < 0.05; †P < 0.001 vs. WKY (all statistical comparisons were made using 2-tailed unpaired Student’s t-test).
After preconstriction, we introduced increasing concentrations of ACh into the perfusate at short intervals to elicit vasodilation (measured as a decrease in PP). In MVB from SHR rats, ACh (0.1 nM to 3 μM) caused a dose-dependent decrease in PP that was similar to that observed in WKY rats (Fig. 1).

We next evaluated the vasodilator responses to insulin. In WKY rats, the maximal vasodilation achievable with insulin was only ~50% of the maximal ACh response (cf. Figs. 1 and 2). Interestingly, in SHR, the vasodilator response to insulin (0.1 nM to 3 μM) was significantly blunted compared with that in WKY rats (Fig. 2). In both SHR and WKY rats, the vasodilator effect of insulin was blocked by pretreatment with genistein (tyrosine kinase inhibitor) or endothelium removal (data not shown). In addition, pretreatment with L-NAME (an inhibitor of all NOS isoforms) significantly inhibited vasorelaxation in response to insulin in WKY, but not SHR, rats (Fig. 3, A and B). Pretreatment with L-NAME plus charybdotoxin plus indomethacin (Fig. 3, C and D) significantly inhibited the vasodilator response to insulin in both SHR and WKY rats, suggesting that insulin stimulates several endothelium-derived relaxing factors. Because this inhibition was not complete, our data suggest that other vasodilator substances in addition to NO may be released by the endothelium in response to insulin. The residual amount of insulin-induced vasorelaxation in the presence of these inhibitors may reflect some unknown endothelium-derived relaxing factors contributing to vasorelaxant effects of insulin.

**Effect of insulin to oppose NE-mediated vasoconstriction.**

Perfusing MVB from WKY rats with increasing concentrations of NE (100 nM to 50 μM) resulted in a dose-dependent increase in vasoconstriction [maximal PP = 107 ± 9 mmHg, half-maximal effective dose (ED$_{50}$) = 5.8 μM; Fig. 4A].
Pretreatment with insulin (1 μM, 1 h) significantly reduced NE-mediated vasoconstriction, in WKY rats (Fig. 4A), consistent with vasodilator actions of insulin (cf. Fig. 2). Similar results were obtained with the use of a lower dose of insulin (10 nM; data not shown). MVB from SHR were significantly more sensitive and responsive to the vasoconstrictor actions of insulin without (circles) or with (squares) pretreatment with N'\(^{-}\)nitro-L-arginine methyl ester (L-NAME; 100 μM, 30 min). Ins, insulin. Results are means ± SE of 5 (WKY) and 6 (SHR) independent experiments. Data from each curve were normalized as in Figs. 1 and 2 and then stimulated with increasing concentrations of insulin without (circles) or with (squares) pretreatment with N'\(^{-}\)nitro-L-arginine methyl ester (L-NAME; 100 μM, 30 min). Ins, insulin. Results are means ± SE of 5 (WKY) and 6 (SHR) independent experiments. Additional experiments shown in Fig. 4 were repeated with an additional treatment arm in which MVB were pretreated with wortmannin (100 nM) plus insulin (1 μM) for 1 h before NE treatment. The addition of wortmannin completely blocked the ability of insulin to oppose the vasoconstrictor effects of NE in WKY rats (Fig. 4B). Pretreatment with wortmannin alone did not significantly affect the NE dose-response curve in either WKY or SHR rats (data not shown). These results suggest that PI3-kinase-dependent pathways are essential for the actions of insulin to oppose NE-mediated vasoconstriction. In SHR, pretreatment with insulin alone or wortmannin plus insulin did not alter the NE dose-response curve for vasoconstriction (Fig. 4B). Moreover, wortmannin pretreatment resulted in a significant reduction in direct insulin-mediated vasodilation only in WKY rats, not in SHR (Fig. 4C).

Roles of MAPK and ET-1. In addition to vasodilator actions mediated by PI3-kinase dependent activation of eNOS (61), insulin also has vasoconstrictor actions mediated by increased secretion of ET-1 (5). To investigate the role of MAPK-dependent signaling in the vascular response of SHR to insulin, we evaluated the insulin dose-response curve for vasodilation in the absence and presence of PD-98059 [a specific inhibitor of MAPK/ERK kinase (MEK)]. Pretreatment of WKY MVB with PD-98059 did not significantly alter the insulin dose-response curve (Fig. 5A). By contrast, in SHR MVB, pretreatment with PD-98059 significantly enhanced the vasodilator actions of insulin, resulting in a dose-response curve that was comparable to that observed in the WKY MVB. These results provide further support for the notion that the impaired vasodilator response to insulin in SHR may be due to increased signaling through MAPK-dependent pathways. To further evaluate the contributions of MAPK signaling to the vascular phenotype of SHR, we repeated NE dose-response curves in MVB from SHR and WKY rats without or with pretreatment with insulin or insulin plus PD-98059 (Fig. 5B). In WKY rats, PD-98059 did not significantly alter the ability of insulin to oppose the vasoconstrictor actions of NE (Fig. 5B). By contrast, inhibition of MAPK-dependent pathways in MVB from SHR unmasked a significant effect of insulin to oppose NE-mediated vasoconstriction (Fig. 5B). Indeed, the NE dose-response curve from SHR MVB pretreated with insulin and PD-98059 was comparable to that from WKY MVB without
Insulin pretreatment. Pretreatment with PD-98059 alone did not significantly affect NE-mediated vasoconstriction in MVB from either SHR or WKY rats (data not shown).

When we repeated these experiments using BQ-123 and BQ-788 (ET-1 receptors blockers) instead of PD-98059, we observed similar results (Fig. 6). That is, pretreatment of SHR MVB with ET-1 receptors blockers unmasked a significant effect of insulin to oppose NE-mediated vasoconstriction such that the dose-response curve was comparable to that of WKY MVB treated with NE alone. Moreover, pretreatment of SHR MVB with ET-1 receptor blockers also increased the sensitivity and responsiveness of MVB to the vasodilator actions of insulin (Fig. 7). Pretreatment with ET-1 receptor blockers alone did not significantly affect NE-mediated vasoconstriction in MVB from either WKY or SHR (data not shown).

**Insulin-stimulated ET-1 secretion is mediated by MAPK signaling.** To provide more definitive evidence that MAPK-dependent insulin signaling pathways are mediating ET-1 secretion in vascular endothelium, we studied insulin-stimulated ET-1 secretion from BAEC in primary culture. We used an ELISA assay to measure levels of ET-1 in the conditioned media from BAEC under basal conditions and after insulin stimulation (100 nM, 5 min) without or with wortmannin (100 nM, 30 min) or PD-98059 (10 μM, 30 min) pretreatment (Fig. 8A). Insulin treatment induced a twofold increase in the levels of ET-1 in conditioned media from BAEC. Pretreatment with wortmannin had no significant effect on insulin-stimulated ET-1 secretion (*P > 0.2). By contrast, pretreatment with PD-98059 completely blocked this effect of insulin. That is, levels of ET-1 after insulin stimulation in the presence of PD-98059 were not significantly different from basal ET-1 levels (*P > 0.5). Thus blockade of MAPK-dependent pathways (but not PI3-kinase-dependent pathways) abrogates insulin-stimulated secretion of ET-1 in vascular endothelial cells. We also immunoblotted cell lysates from these experiments with antibodies against phospho-Akt, Akt, phospho-MAPK, and MAPK to monitor the PI3-kinase- and MAPK-dependent insulin signaling under these conditions (Fig. 8B). As expected, insulin-stimulated phosphorylation of Akt was completely blocked by wortmannin pretreatment, whereas MAPK phosphorylation was unaffected (Fig. 8B, cf. lanes 2 and 3). Moreover, insulin-stimulated phosphorylation of MAPK was completely blocked by PD-98059 pretreatment, whereas Akt phosphorylation was unaffected (Fig. 8B, cf. lanes 2 and 4). Taken together, these results directly demonstrate, for the first time, that insulin-stimulated secretion of ET-1 in endothelial cells is mediated by MAPK signaling.

**Fig. 4.** Insulin opposes vasoconstrictor actions of NE in WKY but not SHR by a phosphatidylinositol (PI) 3-kinase-dependent mechanism. A: MVB isolated from 12-wk-old WKY (closed symbols) and SHR (open symbols) were stimulated with increasing concentrations of NE without (circles) or with (squares) pretreatment with insulin (1 μM, 1 h). Results are plotted as means ± SE of 16 (WKY) and 16 (SHR) independent experiments. Vasoconstriction in response to NE was significantly reduced by preincubation with insulin in WKY (*P < 0.005) but not SHR (*P > 0.26). B: stimulation with NE was sequentially repeated after pretreatment with insulin alone (1 μM, 1 h; squares) and insulin plus wortmannin (100 nM, 1 h; triangles). Results are plotted as means ± SE of 4 (WKY) and 5 (SHR) independent experiments. Wortmannin completely blocked the effect of insulin to oppose NE-mediated vasoconstriction in WKY (*P < 0.005) but not SHR (*P > 0.18). C: MVB isolated from 12-wk-old WKY (closed symbols) and SHR (open symbols) were precontracted with NE as in Fig. 2 and then stimulated with increasing concentrations of insulin without (circles) or with (squares) pretreatment with wortmannin (100 nM, 20 min). Results are means ± SE of 4 (WKY) and 5 (SHR) independent experiments. Data from each curve were normalized as in Figs. 1 and 2. Wortmannin significantly inhibited insulin-mediated vasorelaxation in WKY (*P < 0.041) but not SHR (*P > 0.32).
cells is mediated by MAPK-dependent signaling pathways independent of PI3-kinase-dependent signaling.

**DISCUSSION**

Hypertension and insulin resistance are disorders of hemodynamic and metabolic homeostasis, respectively, that are interrelated and that both have strong genetic components (25, 30). In this study, we used the SHR as a genetic model of essential hypertension with insulin resistance to characterize defects in endothelial insulin action that may contribute to hypertension. The MVB is a resistance region whose ability to modulate vascular tone may reflect and predict pathophysiological changes in hemodynamic homeostasis (9, 41). Although other vascular beds may be subject to distinct regulation, results obtained from MVB may have physiological relevance for the entire cardiovascular system (10, 54). In addition, abnormalities in the gastrointestinal circulation that we describe may be relevant to intestinal malabsorption of nutrients in diabetes and other insulin resistant states (14, 19). In future studies, it will be important to investigate the effects of insulin on the microcirculation of skeletal muscle and other vascular beds that contribute more directly to metabolic and...
vascular homeostasis. Our results suggest several mechanisms for insulin resistance in the vascular endothelium to link the pathophysiology of the metabolic syndrome with hypertension. We studied SHR and their age-matched WKY controls at 12 wk of age when the SHR were significantly hypertensive, insulin resistant, and overweight but not frankly diabetic. This is a period in which the pathophysiology of SHR reflects the metabolic syndrome in humans. Although SHR are frequently smaller than WKY rats, the differences in body weight between SHR and WKY rats observed in our study are in agreement with growth charts obtained from our supplier (Harlan Italy). Thus the relative overweight observed for SHR in our study may reflect the age and genetics of these animals. Elevated peripheral vascular resistance is an important component of the development of hypertension in SHR (18). Accordingly, we found that SHR were more sensitive than WKY rats to vasoconstrictive effects of NE and required a lower dose of NE to achieve the same perfusion pressure in the MVB. Nevertheless, when MVB from SHR and WKY rats were evaluated at a comparable perfusion pressure, the dose-dependent vasodilator effects of ACh were similar.

An alternative approach for assessing vasorelaxation in response to ACh and insulin would have been to precontract mesenteric arteries from SHR and WKY rats to their respective NE EC₇₀. However, the advantage of our approach (i.e., normalizing vasorelaxation to results obtained in control animals) is that we can directly compare differences between pathological and physiological conditions. This minimizes confounding factors that may be present. Moreover, our approach using equieffective concentrations of NE is a generally accepted method (28). Indeed, this approach is particularly relevant for our studies, because under normal pressure conditions, vasorelaxation in SHR in response to ACh is normal while the response to insulin is impaired. Because the vasodilator response of vascular smooth muscle to NE is pressure dependent, it is possible that a defect in the vasodilator response to ACh may be detectable if vessels are studied under hypertensive conditions. However, it is difficult to make a fair comparison under these conditions because the maximal perfusion pressure in response to NE in WKY rats cannot reach hypertensive levels (see Figs. 4, 5B, and 6). Our results are in agreement with those of Wick et al. (58), who found that the vasodilator response to ACh in mesenteric vessels from 12-wk-old SHR is normal. By contrast, other studies have found impaired vasodilator responses to ACh in mesenteric vessels of SHR that were ~30 wk old (4, 52). It is likely that increased age and chronic hypertension present in 30-wk-old SHR may account for differences between our results in 12-wk-old SHR and results from these older animals.

ACh is a classic cholinergic agonist that activates eNOS by a calcium-dependent mechanism (55). Insulin has calcium-independent vasodilator actions that are mediated by a PI3-kinase-dependent mechanism involving phosphorylation of eNOS by Akt (35). Interestingly, in our study, the vasodilator actions of insulin mediated by endothelium-derived NO (albeit weaker than ACh) were significantly impaired in MVB from SHR compared with WKY rats. That is, the vasodilator effects of insulin in MVB preconstricted with NE were reduced in SHR compared with WKY rats. As mentioned previously, the vasoconstrictor response of vascular smooth muscle to NE is pressure dependent, so it is possible that the vasodilator response to insulin may be different if vessels are studied under hypertensive conditions. However, maximal perfusion pressure in response to NE in WKY rats cannot reach hypertensive levels, so it is difficult to make this comparison (see Figs. 4, 5B, and 6). Interestingly, the ability of insulin to oppose NE-mediated vasoconstriction was present only in WKY (but not SHR) rats. Using wortmannin, an inhibitor of PI3-kinase known to block insulin-stimulated production of NO in endothelium (61), we demonstrated that both direct insulin-induced vasodilation and effects of insulin to oppose NE could be inhibited only in WKY (but not SHR) rats. In states of insulin resistance, PI3-kinase-dependent pathways are impaired in metabolic targets of insulin such as skeletal muscle (29, 59). Our results suggest that insulin resistance in SHR is also
accompanying defects in PI3-kinase-dependent pathways in endothelium that impair vasodilator actions of insulin. This may be one mechanism whereby insulin resistance contributes to elevated peripheral vascular resistance and hypertension in SHR. Our findings are consistent with previous studies showing that PI3-kinase-dependent pathways are impaired in vascular endothelium from insulin-resistant Zucker fatty rats (26) and that adenoviral expression of Akt in the carotid artery of SHR ameliorates endothelial dysfunction (23). The isolated endothelial dysfunction with respect to insulin that we observed may be an early feature of vasomotor abnormalities characteristic of the hypertensive process that precedes a generalized endothelial dysfunction (with abnormal response to ACh) found in well-advanced essential hypertension (20, 44, 45).

Prolonged treatment with insulin in vivo increases VEGF release from human adipocytes (13) as well as from rat aortas (28). In vitro, stimulation with insulin for 8, 24 or 48 h increases VEGF protein expression and secretion from smooth muscle cells in human and rat arteries (11). In our study, we did not directly assess effects of insulin on VEGF secretion. However, in our ex vivo experiments, acute stimulation with insulin did not exceed 2 h. Thus it seems unlikely that the insulin effects we observed were due to increased VEGF expression. Nevertheless, because pretreatment of vessels with l-NAME did not completely inhibit the vasodilator actions of insulin in either WKY or SHR (whereas endothelium removal completely inhibited these actions), it is likely that other vasoactive substances derived from endothelium (e.g., endothelium-derived hyperpolarizing factor, cyclooxygenase, or cytochrome P-450 metabolites) may contribute to vasodilator actions of insulin (24). The residual insulin-induced vasorelaxation that we detected in the presence of l-NAME, charybdo-toxin, and indomethacin suggests that some unknown endothelium-derived factors may also participate in insulin-mediated vasorelaxation.

In addition to impairment in PI3-kinase-dependent insulin signaling, previous studies have implicated increased signaling through MAPK-dependent pathways in the pathological vascular effects of compensatory hyperinsulinemia that accompanies metabolic insulin resistance (3). In the vasculature, activation of MAPK mediates cellular growth as well as the ability of endothelial cells, vascular smooth muscle cells (VSMC), and monocytes to migrate. In addition, secretion of plasminogen activator inhibitor-1 (PAI-1, a prothrombotic factor) is increased ET-1 secretion independently of PI3-kinase-dependent pathways. In experiments with primary human endothelial cells, we have shown, for the first time, that MAPK-dependent insulin signaling pathways mediate increased ET-1 secretion independently of PI3-kinase-dependent pathways. In addition, genistein (tyrosine kinase inhibitor that blocks signaling by the insulin receptor tyrosine kinase) presumably blocked both PI3-kinase- and MAPK-dependent actions, resulting in inhibition of vasodilator actions of insulin. By contrast, pretreatment with PD-98059 (MEK inhibitor) demonstrated the specific role of the MAPK pathway in mediating insulin-dependent contractile effects through ET-1. Taken together, our ex vivo and in vitro results suggest that insulin-dependent MAPK signaling leading to increased ET-1 secretion is pathologically elevated in the endothelium of SHR. In WKY rats that are not insulin resistant, the blockade of MAPK does not affect the vasodilator actions of insulin and the blockade of ET-1 receptors has minimal effects. Thus there is a pathway-selective insulin resistance in vascular endothelium of SHR that simultaneously impairs PI3-kinase-dependent pathways and augments MAPK-dependent pathways. This is similar to what we previously observed in primary human endothelial cells that were made insulin resistant (36). Thus an additional mechanism that may contribute to hypertension in SHR is the elevation of MAPK-dependent signaling that drives increased ET-1 secretion. This is consistent with human studies in which blockade of ET-1 receptors resulted in significant vasodilator response to insulin in hypertensive patients but not in normotensive controls (5–7, 33, 34).

In human essential hypertension, an imbalance between vasodilator and vasoconstrictor substances has been described that may be due to decreased bioavailability of NO (secondary to reduced eNOS expression, reduced NO production, and/or increased NO catabolism) as well as increased secretion of ET-1 (31). Insulin signaling pathways in endothelium related to NO production share important features in common with metabolic insulin signaling pathways (39, 60). In addition, many secondary forms of hypertension lack insulin resistance, suggesting that abnormal insulin signaling may play an important pathophysiological role in essential hypertension (50). Of note, levels of ET-1 stimulated by insulin or triglycerides are higher than normal in subjects with the metabolic syndrome (16). The defects that we demonstrate in endothelial insulin signaling in SHR with impaired PI3-kinase-dependent signaling and augmented MAPK-dependent signaling provide a mechanistic explanation for the imbalance between the endothelium-derived vasodilator NO and the vasoconstrictor ET-1 that may conspire to elevate peripheral vascular resistance and contribute to hypertension. Thus our findings suggest several related mechanisms for insulin resistance to contribute to hypertension in a genetic model and may be relevant to understanding why insulin resistance is linked to human essential hypertension.
REFERENCES


