*Original Article*

# Treatment of Spontaneously Hypertensive Rats With Rosiglitazone and/or Enalapril Restores Balance Between Vasodilator and Vasoconstrictor Actions of Insulin With Simultaneous Improvement in Hypertension and Insulin Resistance

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**Spontaneously hypertensive rats (SHRs) exhibit endothelial dysfunction and insulin resistance. Reciprocal relationships between endothelial dysfunction and insulin resistance may contribute to hypertension by causing imbalanced regulation of endothelial-derived vasodilators (e.g., nitric oxide) and vasoconstrictors (e.g., endothelin-1 [ET-1]). Treatment of SHRs with rosiglitazone (insulin sensitizer) and/or enalapril (ACE inhibitor) may simultaneously improve hypertension, insulin resistance, and endothelial dysfunction by rebalancing insulin-stimulated production of vasoactive mediators. When compared with WKY control rats, 12-week-old vehicle-treated SHRs were hypertensive, overweight, and insulin resistant, with elevated fasting levels of insulin and ET-1 and reduced serum adiponectin levels. In mesenteric vascular beds (MVBs) isolated from vehicle-treated SHRs and preconstricted with norepinephrine (NE) ex vivo, vasodilator responses to insulin were significantly impaired, whereas the ability of insulin to oppose vasoconstrictor actions of NE was absent (versus WKY controls). Three-week treatment of SHRs with rosiglitazone and/or enalapril significantly reduced blood pressure, insulin resistance, fasting insulin, and ET-1 levels and increased adiponectin levels to values comparable with those observed in vehicle-treated WKY controls. By restoring phosphatidylinositol 3-kinase–dependent effects, rosiglitazone and/or enalapril therapy of SHRs also significantly improved vasodilator responses to insulin in MVB preconstricted with NE ex vivo. Taken together, our data provide strong support for the existence of reciprocal relationships between endothelial dysfunction and insulin resistance that may be relevant for developing novel therapeutic strategies for the metabolic syndrome. *Diabetes* 55:3594–3603, 2006**

Vascular endothelium contributes importantly to regulation of cardiovascular and metabolic homeostasis (1,2). Reciprocal relationships between endothelial dysfunction and insulin resistance may help couple hemodynamic and metabolic abnormalities observed in important interrelated public health problems, including diabetes, obesity, hypertension, coronary heart disease, atherosclerosis, and the metabolic syndrome (3,4). In addition to its essential metabolic actions, insulin also has important endothelial-dependent vasodilator actions mediated by nitric oxide (NO) via phosphatidylinositol 3-kinase (PI 3-kinase)–dependent activation of endothelial NO synthase (5–9). These vasodilator actions of insulin contribute significantly to metabolic actions of insulin by increasing delivery of substrate and insulin to metabolic target tissues (10). Interestingly, insulin also has vasoconstrictor actions mediated by mitogen-activated protein kinase (MAPK)-dependent endothelial secretion of endothelin-1 (ET-1) (11–13). Insulin resistance is characterized by selective impairment in PI 3-kinase–dependent signaling pathways regulating metabolic actions of insulin in skeletal muscle (with intact MAPK signaling pathways) (14). In vascular endothelium, a similar selective impairment of PI 3-kinase pathways (with intact MAPK pathways) may contribute to endothelial dysfunction (13,15). Insulin resistance is accompanied by compensatory hyperinsulinemia that serves to overcome impairment in PI 3-kinase signaling to maintain euglycemia. However, this hyperinsulinemia is predicted to overdrive unaffected MAPK signaling that may promote pathological actions of insulin, including increased secretion of ET-1 (13,16), increased expression of vascular adhesion molecules (15,17–19), proliferation of vascular smooth muscle (20), increased expression of proinflammatory cytokines (21), and activation of cation pumps (22). These factors may shift the balance between vasodilator and vasoconstrictor actions of insulin and result in predisposition to hypertension in insulin-resistant states.

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ACh, acetylcholine; ARB, angiotensin II type 1 receptor blocker; ET-1, endothelin-1; MAPK, mitogen-activated protein kinase; MEK, MAP/extracellular signal–related kinase kinase; MVB, mesenteric vascular bed; NE, norepinephrine; PI 3-kinase, phosphatidylinositol 3-kinase; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat.

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Importantly, pathway-selective insulin resistance with impairment of insulin-stimulated PI 3-kinase signaling but not MAPK signaling is also present in vascular endothelium of insulin-resistant animal models (23–25). In spontaneously hypertensive rats (SHRs), a genetic model of hypertension with features of the metabolic syndrome, we recently demonstrated that metabolic insulin resistance is associated with impaired insulin-stimulated NO-dependent vasodilation (mediated by PI 3-kinase), as well as enhanced insulin-stimulated ET-1–dependent vasoconstriction (mediated by MAPK) (13). Pharmacological interventions in animals and humans support the existence of reciprocal relationships between insulin resistance and endothelial dysfunction. For example, ACE inhibitors and angiotensin II type 1 receptor blockers (ARBs) that improve endothelial function (resulting in lower peripheral vascular resistance and blood pressure in hypertensive subjects) also improve insulin sensitivity (26–31). Likewise, thiazolidinediones, ligands for peroxisome proliferator–activated receptor γ that improve metabolic actions of insulin in skeletal muscle, also improve endothelial function and reduce blood pressure in subjects with insulin resistance or diabetes (32–34). Taken together, these findings may help to explain pathophysiological mechanisms underlying hemodynamic and metabolic abnormalities in the metabolic syndrome. We hypothesize that reciprocal relationships between endothelial dysfunction and insulin resistance are in part due to imbalance between vasodilator and vasoconstrictor actions of insulin. To gain further insight into these mechanisms, we evaluated the ability of rosiglitazone, enalapril, or combination therapy to simultaneously improve blood pressure, insulin resistance, and endothelial dysfunction in SHRs by rebalancing insulin-stimulated production of vasoactive mediators. We tested effects of combination therapy in addition to monotherapies with an insulin sensitizer or ACE inhibitor because multiple interventions at different points in a coupled system may or may not have additive or synergistic effects. Results from our study have important implications for developing novel therapeutic strategies for diabetes, obesity, hypertension, and the metabolic syndrome.

## RESEARCH DESIGN AND METHODS

All procedures in animals were performed in accordance with Guidelines and Authorization for the Use of Laboratory Animals (Italian Government, Ministry of Health). Male SHRs (SHR/NHsd, haplotype RT1$^k$) and age-matched normotensive Wistar-Kyoto (WKY) control rats were obtained from Harlan Italy (Milan) and used in all studies. Eight-week-old animals were housed, handled, and trained for 1 week to minimize stress associated with blood pressure measurements. Nine-week-old SHRs were randomized into four groups and treated daily for 3 weeks by gavage with vehicle alone, enalapril (30 mg · kg$^{-1}$ · day$^{-1}$), rosiglitazone (5 mg · kg$^{-1}$ · day$^{-1}$), or enalapril plus rosiglitazone. Nine-week-old WKY controls were given vehicle alone. In all groups of animals, systolic blood pressure (SBP) was measured noninvasively using a tail cuff (Letica 5100; PanLab, Barcelona, Spain) according to standard procedures described previously (35). SBP values reported are the average of three sequential blood pressure measurements that were within 10 mmHg of each other. During the course of treatment, SBP was monitored twice weekly, the last time 24 h before death. Body weight was measured daily. Blood samples were obtained by cardiac puncture from rats fasted overnight, heparinized (200 IU i.p.; Pfizer), and then killed with ether. Serum concentrations of insulin, adiponectin, and ET-1 were measured by ELISA (Linco Research, St. Charles, MO; B-Bridge, Sunnyvale, CA; Cayman Chemical, Ann Arbor, MI, respectively). Plasma glucose concentrations were determined with a diagnostic glucometer (Accu-Chek Active; Roche Diagnostics, Mannheim, Germany). Insulin sensitivity was assessed using the quantitative insulin-sensitivity check index {QUICKI = 1/[log (insulin) + log (glucose)]} (36).

Drugs were obtained from the indicated sources: heparin (Pfizer); insulin (Novo Nordisk); and norepinephrine (NE), acetylcholine (ACh), enalapril, and rosiglitazone (Sigma-Aldrich). Stock solutions of NE (100 mmol/l) and ACh (10 mmol/l) were prepared with distilled water. Final dilutions of these drugs were prepared in modified Krebs-Henseleit solution immediately before use. Stock solutions of rosiglitazone in ethanol (1%) and enalapril in methanol (5%) were prepared. Final dilutions of these drugs were prepared in drinking water immediately before intragastric administration (~4 × dilution). Vehicle-treated WKY rats and SHRs received the same amount of ethanol or methanol as drug-treated animals.

**Evaluation of vascular function ex vivo.** Mesenteric vascular beds (MVBs) were isolated and removed from rats after 3-week vehicle or drug therapy as described previously (13). Briefly, MVBs 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% $O_2$ and 5% $CO_2$ (pH 7.4). A constant flow rate of 5 ml/min through the MVB 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 an equilibration period (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).

**Vasodilator and vasoconstrictor responses in MVB.** A steady-state perfusion pressure of ~120 mmHg was obtained 30–40 min after initial administration of NE and was maintained by continuous NE infusion (10 and 3 μmol/l in WKY rats and SHRs, respectively). Dose-response curves measuring vasodilation (decrease in perfusion pressure) in response to insulin were obtained by adding increasing concentrations of insulin (0.1 nmol/l to 3 μmol/l per 4-min perfusion) to the perfusate. For all vasodilation experiments, data from each curve were normalized to perfusion pressure obtained in WKY rats treated with a maximally stimulating dose of ACh (1 μmol/l, 100% representing initial steady-state perfusion pressure and 0% representing maximal reduction in response to ACh). In some experiments, insulin-induced relaxation was measured before and after 20-min treatment with wortmannin (100 nmol/l) or PD98059 (10 μmol/l). Dose-response curves measuring vasoconstriction (increase in perfusion pressure) in response to NE were obtained by adding increasing concentrations of NE (100 nmol/l to 50 μmol/l per 30-s perfusion) to the perfusate. These experiments were also repeated after 1 h of perfusion pretreatment with insulin (100 nmol/l). Relative changes in perfusion pressure at steady state reached with each dose of NE were measured and expressed in mmHg.

**Measurement of ET-1 in MVB perfusate.** MVBs isolated from WKY rats and SHRs were initially perfused with modified Krebs-Henseleit solution at a constant flow rate (5 ml/min) for 15–20 min. Flow rate was then decreased (30 μl/min) and ~1.8 ml perfusate was collected over the next hour for basal ET-1 measurements (dead space of MVB is ~400 μl). Flow rates were then increased (2 ml/min) for 1 h while insulin (100 nmol/l) was added to the perfusing solution. After this 1-h period, flow rate was decreased again (30 μl/min with insulin in the perfusate). Perfusate (~1.8 ml) was then collected over the next hour for additional ET-1 measurements. In a set of related experiments, additional samples were collected from vessels pretreated with wortmannin (100 nmol/l per 20 min) or PD98059 (10 μmol/l per 20 min) and then perfused with insulin. Samples were centrifuged to remove debris (1,000*g*, 10 min, 4°C), and ET-1 was measured in the supernatant with an ELISA kit (Cayman Chemical) according to the manufacturer's instructions.

**Statistical analysis.** Results were expressed as means ± SE of $n$ experiments ($n$ = number of rats). Two-way ANOVA for repeated measures and Student's $t$ tests (paired or unpaired) were used as appropriate. Values of $P < 0.05$ were considered to indicate statistical significance.

## RESULTS

**Biochemical and physiological parameters of WKY rats and SHRs after 3-week treatment with enalapril and/or rosiglitazone.** Similar to our previous report (13), when compared with age-matched WKY control rats, 12-week-old SHRs treated with vehicle alone were overweight and hyperinsulinemic but normoglycemic (Table 1). In addition, serum ET-1 concentrations in vehicle-treated SHRs were significantly higher than in WKY rats ($P < 0.004$). When compared with vehicle treatment, treatment of SHRs with enalapril for 3 weeks did not result in a significant difference in either body weight or fasting glucose levels. However, enalapril treatment of SHRs did cause a substantial and significant decrease in both serum concentrations of ET-1 and insulin when compared with

TABLE 1
Physiological and biochemical parameters in 12-week-old rats after a 3-week treatment with enalapril and/or rosiglitazone

| Strain | Treatment (3 weeks) | $n$ | Body weight (g) | Serum ET-1 (pg/ml) | Fasting glucose (mg/dl) | Fasting insulin (ng/ml) |
|---|---|---|---|---|---|---|
| WKY | Vehicle | 5 | 250 ± 8 | 4.2 ± 0.9 | 76 ± 12 | 0.9 ± 0.1 |
| SHR | Vehicle | 6 | 305 ± 8* | 7.1 ± 0.6* | 87 ± 4 | 5.5 ± 1.0* |
| SHR | Enal | 6 | 313 ± 10* | 5.4 ± 0.3† | 76 ± 6 | 2.3 ± 0.8*† |
| SHR | Rosi | 6 | 333 ± 6*† | 4.8 ± 0.8† | 71 ± 8 | 0.6 ± 0.1† |
| SHR | Enal + Rosi | 5 | 326 ± 6*† | 4.4 ± 0.3† | 77 ± 8 | 1.4 ± 0.4† |

Data are means ± SE of $n$ independent experiments. Nine-week-old rats were treated daily with vehicle alone, enalapril (30 $mg \cdot kg^{-1} \cdot day^{-1}$), rosiglitazone (5 $mg \cdot kg^{-1} \cdot day^{-1}$), or enalapril plus rosiglitazone for 3 weeks as described in RESEARCH DESIGN AND METHODS. *Significantly different from WKY treated with vehicle alone. †Significantly different from SHR treated with vehicle alone. Enal, enalapril; Rosi, rosiglitazone.

vehicle-treated SHRs ($P < 0.04$ and 0.01, respectively). Treatment of SHRs with rosiglitazone for 3 weeks, either alone or in combination with enalapril, resulted in a small increase in body weight (vs. vehicle-treated SHRs, $P < 0.01$) without significant changes in fasting glycemia. No significant differences between the weights of SHRs treated with rosiglitazone and combination therapy were observed. More importantly, treatment of SHRs with rosiglitazone, either alone or in combination with enalapril, resulted in a significant reduction in serum ET-1 levels comparable with that observed with enalapril treatment alone (vs. vehicle-treated SHRs, $P < 0.01$). In addition, treatment of SHRs with rosiglitazone, either alone or in combination with enalapril, caused substantial and significant decreases in serum concentrations of insulin (vs. vehicle-treated SHRs, $P < 0.005$). The magnitude of the decrease in fasting insulin levels caused by rosiglitazone treatment was larger than that caused by enalapril treatment ($P < 0.03$).

Fasting glucose and insulin data (Table 1) were consistent with our previous report (13) of insulin resistance in 12-week-old SHRs treated with vehicle (when compared with vehicle-treated WKY rats). Along with decreases we observed in fasting insulin levels (Table 1), treatment of SHRs with enalapril for 3 weeks significantly improved insulin sensitivity as assessed by QUICKI (Fig. 1*A*). Treatment of SHRs with rosiglitazone, either alone or in combination with enalapril, improved insulin sensitivity to levels comparable with those observed in WKY control rats treated with vehicle alone (Fig. 1*A*).

Adiponectin is a protein specifically secreted by adipose cells whose circulating levels are positively correlated with insulin sensitivity (37). Serum adiponectin levels in the treatment groups generally followed the pattern we observed for insulin sensitivity (Fig. 1*B*). That is, serum adiponectin levels of 12-week-old SHRs treated with vehicle alone were significantly lower than those of WKY control rats. Notably, treatment of SHRs with enalapril or rosiglitazone for 3 weeks significantly increased adiponectin levels when compared with levels from vehicle-treated SHRs.

Consistent with serum ET-1 levels (Table 1) and our previous report (13), SBP was substantially and significantly higher in untreated 9-week-old SHRs and 12-week-old vehicle-treated SHRs when compared with matched WKY control rats (Fig. 2). As expected, 3-week treatment of SHRs with the antihypertensive agent enalapril, either alone or in combination with rosiglitazone, effectively lowered SBP to normal levels comparable with those observed in WKY controls. SBP in these groups of animals progressively decreased during the first 2 weeks of enalapril or combination treatment (data not shown). Interestingly, significant reductions in SBP were also observed in SHRs treated with rosiglitazone alone. However, the magnitude of this change in SBP was not quite as large as

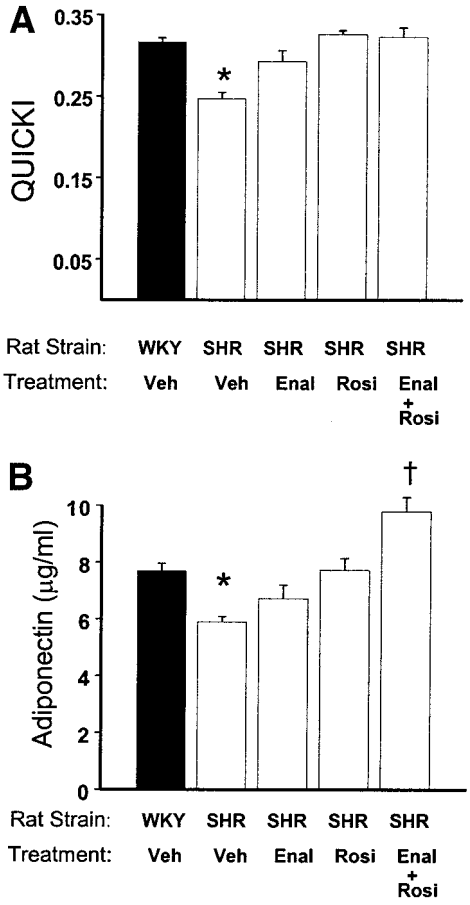


**FIG. 1. Insulin sensitivity and serum adiponectin levels are increased in SHRs after enalapril (Enal) and rosiglitazone (Rosi) treatment. Fasting blood samples were obtained after a 3-week therapy with the indicated drugs (means ± SE of at least five independent experiments for each group). *A*: Quantitative insulin sensitivity check index {QUICKI = 1/[log (insulin) + log (glucose)]} was significantly lower in vehicle (Veh)-treated SHRs when compared with WKY rats (*$P < 0.0002$). Enalapril, rosiglitazone, or combination therapy all significantly increased insulin sensitivity in SHRs (vs. vehicle-treated SHRs, $P < 0.007$). *B*: Immunoenzymatic measurement demonstrates significantly lower levels of serum adiponectin in vehicle-treated SHRs when compared with WKY (*$P < 0.0002$). Enalapril or rosiglitazone treatment increased adiponectin levels in SHRs (vs. vehicle-treated SHRs, $P < 0.03$ and 0.0003, respectively, by unpaired $t$ tests). Combination therapy with enalapril plus rosiglitazone significantly increased serum adiponectin to levels above those observed in WKY rats (†$P < 0.001$).**

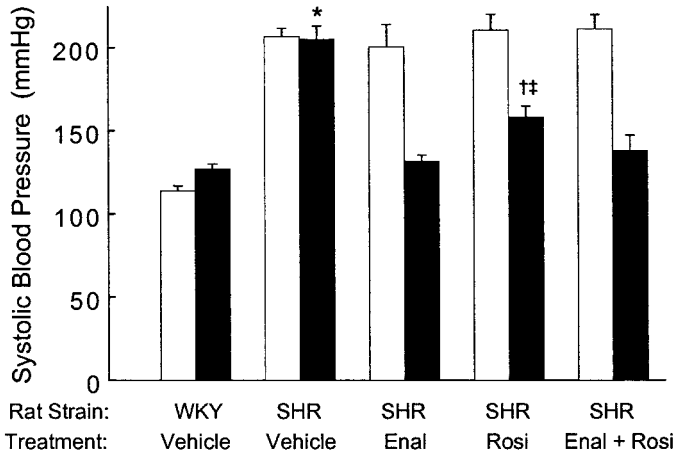


**FIG. 2. Elevated SBP in SHRs is significantly reduced by a 3-week treatment with enalapril (Enal) and/or rosiglitazone (Rosi). SBP was measured with a tail cuff before (□) and after (■) daily drug therapy for 3 weeks as described in RESEARCH DESIGN AND METHODS. SBP was higher in vehicle-treated SHRs than in WKY (*$P < 0.0005$). Treatment of SHRs with enalapril alone or enalapril plus rosiglitazone completely normalized SBP (vs. WKY treated with vehicle, $P > 0.22$). Treatment of SHRs with rosiglitazone alone also caused significant and substantial reduction in SBP (vs. SHRs treated with vehicle, †$P < 0.004$). However, reduction in SBP in response to rosiglitazone treatment of SHRs was not as substantial as that caused by enalapril (‡$P < 0.03$). Values shown are means ± SE of five independent experiments for each group of animals.**

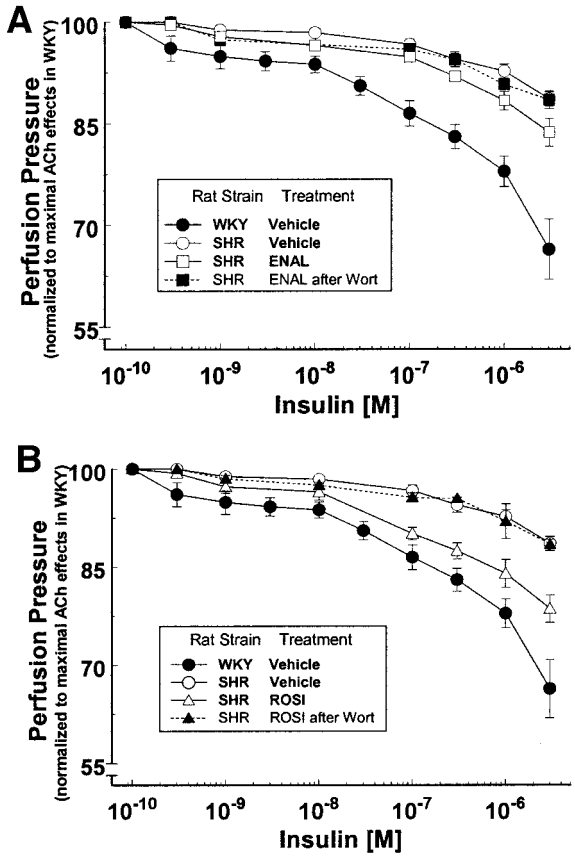


**FIG. 3. PI 3-kinase–dependent vasodilator actions of insulin are significantly improved in MVBs from SHRs treated with enalapril (ENAL) or rosiglitazone (ROSI). After daily drug therapy for 3 weeks, MVBs were studied ex vivo as described in RESEARCH DESIGN AND METHODS. Vasorelaxation was assessed in response to increasing concentrations of insulin in the perfusate (means ± SE of at least five independent experiments for each group). Vasodilator actions of insulin were significantly impaired in vehicle-treated SHRs (vs. WKY, $P < 0.001$). Treatment of SHRs with enalapril or rosiglitazone increased the ability of insulin to mediate vasorelaxation (vs. vehicle-treated SHRs, $P < 0.02$, $P < 0.0023$, respectively). Preincubation of vessels with wortmannin significantly inhibited vasodilator actions of insulin in MVBs from SHRs treated with either enalapril or rosiglitazone ($P < 0.001$). Statistical comparisons between dose-response curves were performed using two-way ANOVA for repeated measures.**

changes in treatment groups that included enalapril. Taken together, the biochemical and physiological changes we observed in SHRs treated with enalapril, rosiglitazone, or combination therapy suggest that these therapeutic interventions were sufficient to simultaneously ameliorate or normalize both metabolic and hemodynamic abnormalities characteristic of SHRs, a genetic model of hypertension with features of the metabolic syndrome.

**Effects of enalapril and/or rosiglitazone treatment on endothelial function in SHRs.** Insulin resistance in vascular endothelium may help to couple metabolic and hemodynamic abnormalities observed in SHRs (1,13). Therefore, we evaluated the ability of insulin to stimulate acute vasorelaxation in the MVBs of SHRs and WKY rats ex vivo. Consistent with our previous report (13), the ability of insulin to cause dose-dependent vasorelaxation in MVBs from 12-week-old vehicle-treated SHRs was significantly impaired when compared with MVBs from matched WKY control rats (Fig. 3, compare closed and open circles). In MVBs from SHRs treated with enalapril or rosiglitazone for 3 weeks (Fig. 3, open squares and open triangles), the ability of insulin to stimulate vasorelaxation was significantly improved when compared with MVBs from vehicle-treated SHRs. Similar results were observed with combination therapy (data not shown). However, insulin-mediated vasorelaxation in MVBs from SHRs treated with enalapril or rosiglitazone did not completely normalize when compared with MVBs from WKY control rats. Treatment of SHRs with rosiglitazone resulted in a slight improvement in the insulin responsiveness of MVBs with respect to vasorelaxation when compared with SHRs treated with enalapril. Importantly, pretreatment of MVBs from rosiglitazone- or enalapril-treated SHRs with the PI 3-kinase inhibitor wortmannin significantly inhibited the vasodilator effects of insulin in treated SHRs (Fig. 3, closed squares and closed triangles; $P < 0.001$). Taken together, these results suggest that 3-week therapy of SHRs with rosiglitazone or enalapril improves PI 3-kinase–dependent signaling, mediating greater insulin-stimulated vasodilation in MVBs.

We also evaluated the ability of insulin to oppose vasoconstrictor actions of NE (100–50 μmol/l) in MVBs from WKY rats and SHRs (Fig. 4). Consistent with our previous report (13), the ability of insulin pretreatment (100 nmol/l, 1 h) to oppose vasoconstrictor actions of NE was evident only in MVBs from WKY rats but not in MVBs from SHRs treated with vehicle alone (Fig. 4*A* and *B*). NE concentration response curves in the absence of insulin pretreatment in MVBs from SHRs treated with vehicle, enalapril, or rosiglitazone (Fig. 4*B–D*) were more responsive to vasoconstrictor actions of NE than MVBs from WKY rats (maximal perfusion pressure ~180 vs. ~125 mmHg, $P < 0.01$). The ability of insulin pretreatment to oppose NE-mediated vasoconstriction was evident only in MVBs from SHRs treated with rosiglitazone alone or in combination with enalapril but not in MVBs from SHRs treated with enalapril alone (Fig. 4*C–E*). Interestingly, in SHRs treated with enalapril plus rosiglitazone, maximal

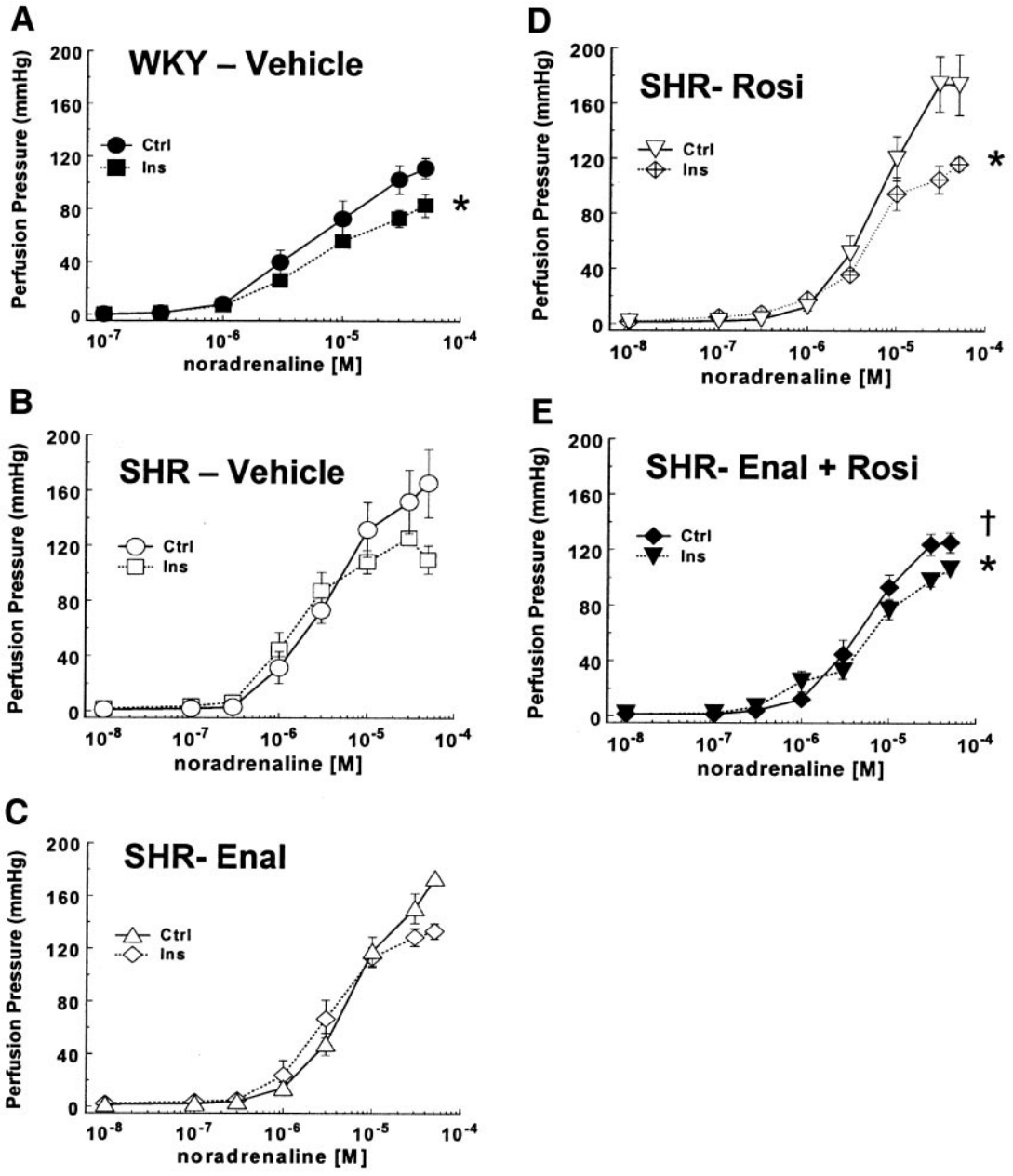


**FIG. 4. Effects of insulin to oppose norepinephrine-mediated vasoconstriction in SHRs become evident only after treatment with rosiglitazone (Rosi). MVBs isolated from rats after a 3-week treatment with the indicated drugs were stimulated with increasing concentrations of NE without or with preincubation with insulin (100 nmol/l, 1 h). *A*: In vehicle-treated WKY rats, vasoconstriction in response to NE was significantly reduced by preincubation with insulin (**P* < 0.01). By contrast, no effect of insulin preincubation was observed in vehicle-treated SHRs (*P* > 0.12) (*B*) or SHRs treated with enalapril (Enal) alone (*P* > 0.98) (*C*). However, vasoconstriction in MVBs in response to NE was significantly reduced by preincubation with insulin in SHRs treated with rosiglitazone (**P* < 0.004) (*D*) or enalapril plus rosiglitazone (**P* < 0.05) (*E*). Results shown are the means ± SE of five independent experiments for each group (i.e., five animals in each group). Statistical comparisons between dose-response curves were performed using two-way ANOVA for repeated measures. *Comparisons of entire dose-response curves within each panel. †Comparison of the entire NE dose-response curve without insulin pretreatment (control) in *E* and the corresponding control curves shown in *B*, *C*, and *D* (*P* < 0.04).**

responsiveness to vasoconstrictor actions of NE was significantly reduced when compared with vehicle-, enalapril-, or rosiglitazone-treated SHRs and comparable with that seen in MVBs from WKY control rats (Fig 4, compare *A*, *B*, and *E*). By contrast with maximal responsiveness, the sensitivity of MVBs to vasoconstrictor actions of NE was not substantially different among any of the treatment groups ($ED_{50}$ ~5.6 μmol/l). Taken together, results from our experiments in MVBs from SHRs and WKY rats suggest that 3-week treatment with enalapril, rosiglitazone, or combination therapy is sufficient to improve vasodilator actions of insulin.

**Effects of enalapril and/or rosiglitazone treatment on insulin-stimulated ET-1 secretion from MVBs in SHRs.** Elevated serum levels of ET-1 may contribute to hypertension and insulin resistance in SHRs (38,39). We previously demonstrated that ET-1 secretion from endothelial cells in response to insulin requires activation of MAPK (13). Therefore, in the present study, we investigated the ability of insulin (100 nmol/l, 1 h) to stimulate

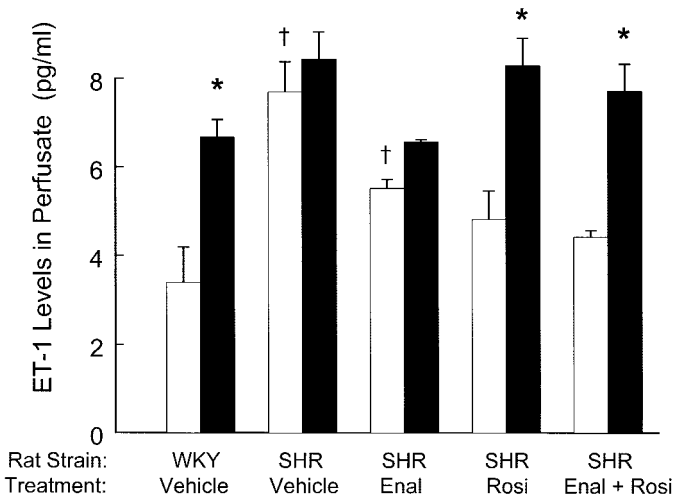


**FIG. 5. Insulin-stimulated secretion of ET-1 in SHRs normalizes after treatment with rosiglitazone. Perfusates from MVBs of WKY and SHRs treated for 3 weeks with the indicated drugs were collected before (□) and after (■) treatment with insulin (100 nmol/l, 1 h) as described in RESEARCH DESIGN AND METHODS. When compared with results obtained from WKY, basal ET-1 levels were elevated in perfusates from SHRs treated with vehicle (†$P < 0.002$) and SHRs treated with enalapril (Enal) (††$P < 0.03$). By contrast, basal levels of ET-1 in SHRs treated with rosiglitazone (Rosi) or combination therapy (Enal plus Rosi) were similar to basal levels of ET-1 in WKY treated with vehicle ($P > 0.12$). Insulin treatment resulted in a significant increase of ET-1 levels in perfusate from MVBs of WKY rats (vs. basal, *$P < 0.002$). By contrast, insulin did not stimulate a further significant increase in ET-1 levels in the perfusate of MVBs over the already elevated basal levels present in either SHRs treated with vehicle ($P > 0.28$) or SHRs treated with enalapril ($P > 0.08$). Importantly, the ability of insulin to stimulate significant increases in ET-1 levels in perfusate from MVBs of SHRs was restored after a 3-week treatment with either rosiglitazone alone (**$P < 0.01$) or rosiglitazone combined with enalapril (*$P < 0.002$). Results are the means ± SE for six independent experiments repeated in duplicate.**

ET-1 secretion from MVBs of WKY rats and SHRs (Fig. 5). As expected, acute insulin treatment of MVBs from WKY resulted in a significant twofold increase in the concentration of ET-1 in MVB perfusate. This insulin-stimulated increase in ET-1 levels was unaffected by pretreatment of MVBs with wortmannin (PI 3-kinase inhibitor) but completely abrogated by pretreatment of vessels with PD98059 (MAP/extracellular signal–related kinase kinase [MEK] inhibitor) (data not shown). Consistent with elevated plasma levels of ET-1 observed in vehicle-treated SHRs (Table 1), basal levels of ET-1 in MVB perfusate from vehicle-treated SHRs were substantially and significantly elevated when compared with MVBs from WKY rats (Fig. 5). Moreover, in MVBs from vehicle-treated SHRs, acute insulin treatment was unable to significantly increase the concentration of ET-1 in MVB perfusate. In MVB perfusate from SHRs treated with enalapril, basal levels of ET-1 were intermediate between those in MVB perfusate from WKY rats and SHRs treated with vehicle alone. Of note, in MVB perfusate from SHRs treated with rosiglitazone, either alone or in combination with enalapril, basal and insulin-stimulated levels of ET-1 were comparable with those observed in MVB perfusate from WKY rats. Interestingly, PD98059 pretreatment of MVBs from vehicle-treated SHRs significantly reduced the elevated basal levels of ET-1 (data not shown). Taken together, these data suggest that 3-week rosiglitazone therapy is sufficient to lower basal ET-1 levels and restore insulin action in the vasculature of SHRs with respect to ET-1 secretion.

**Comparisons between monotherapies and combination therapy.** Because ACE inhibitors and insulin sensitizers are targeting different points of coupling between metabolic and hemodynamic homeostasis, we compared monotherapies with each other and with combination therapy for each major outcome measure. Rosiglitazone treatment had a slightly larger effect on weight than enalapril, but this was comparable with combination therapy (Table 1; $P > 0.54$). With respect to ET-1 levels, all three treatments reduced serum ET-1 levels to a comparable extent. As might be expected, rosiglitazone therapy had the largest effect on reduce fasting insulin levels, and this was not statistically different from results with combination therapy (Table 1). Interestingly, combined therapy with enalapril and rosiglitazone caused serum adiponectin to significantly increase to levels even higher than those observed in WKY controls (Fig. 1). This effect of combination therapy was significantly greater than that observed with either enalapril or rosiglitazone treatment alone ($P < 0.003$ and 0.005, respectively). As might be expected, enalapril was more effective at reducing SBP than rosiglitazone, but combination therapy was not different than enalapril alone (Fig. 2, $P > 0.47$). In SHRs treated with combination therapy, maximal responsiveness to vasoconstrictor actions of NE was significantly reduced when compared with SHRs treated with vehicle, enalapril, or rosiglitazone and comparable with that seen in MVBs from WKY control rats (Fig. 4, compare *A*, *B*, and *E*). Finally, combination therapy was comparable with rosiglitazone monotherapy but more effective than enalapril monotherapy at normalizing basal and insulin-stimulated levels of ET-1 in MVB perfusate from SHRs (Fig. 5, $P < 0.01$). Thus, for the majority of the parameters evaluated, results from combined treatment with enalapril plus rosiglitazone did not significantly differ from the largest effect observed with either drug alone.

## DISCUSSION

Metabolic and hemodynamic homeostasis are coupled in part by vascular actions of insulin in endothelium. Impaired vascular actions of insulin contribute importantly to reciprocal relationships between endothelial dysfunction and insulin resistance (1). Thus, therapeutic interventions designed to improve metabolic insulin resistance are predicted to have beneficial effects on cardiovascular disorders characterized by endothelial dysfunction, whereas therapies aimed at ameliorating endothelial dysfunction are predicted to improve metabolic disorders associated with insulin resistance. In a previous study, we demonstrated that SHRs mimicking features of the human metabolic syndrome are characterized by impaired PI 3-kinase signaling in vascular endothelium regulating insulin-stimulated production of the vasodilator NO and enhanced MAPK signaling regulating insulin-stimulated secretion of the vasoconstrictor ET-1. In the present study, we used SHRs as a model of the metabolic syndrome with insulin resistance, overweight, and essential hypertension to test the hypothesis that insulin sensitizers and ACE inhibitors may simultaneously improve insulin sensitivity and lower blood pressure by restoring balance between opposing vascular actions of insulin in endothelium mediated by PI 3-kinase– and MAPK-dependent signaling pathways.

**Effects of enalapril and rosiglitazone therapy on the metabolic and hemodynamic phenotype of SHRs.**

Twelve-week-old SHRs treated with vehicle alone were overweight, hypertensive, hyperinsulinemic, normoglycemic, and insulin resistant, with elevated circulating ET-1 levels and decreased circulating adiponectin levels when compared with matched WKY control rats. Thus, 12-week-old SHRs mimic many of the essential features of the human metabolic syndrome (13,40). Of note, enalapril treatment of SHRs for 3 weeks not only lowered blood pressure and ET-1 levels as expected but also lowered fasting insulin levels while increasing insulin sensitivity and adiponectin levels. Similarly, rosiglitazone treatment of SHRs for 3 weeks not only lowered fasting insulin levels and increased insulin sensitivity and adiponectin levels as expected, but also resulted in substantial decreases in blood pressure and ET-1 levels. The surrogate index of insulin sensitivity that we use in this study (QUICKI) has been formally validated against glucose clamp studies in humans but not in rodents. Nevertheless, QUICKI seems to be generally useful in rodents (13,41). In addition, our results are also in line with results from studies of therapeutic interventions in human hypertension, diabetes, and metabolic syndrome using ACE inhibitors, ARBs, or thiazolidinediones (28,29,42–44). Thus, in SHRs, as in humans, therapy with either ACE inhibitors or insulin sensitizers simultaneously improves both metabolic and hemodynamic phenotypes. The fact that individual therapies targeting either endothelial dysfunction or insulin resistance simultaneously improves both metabolic and hemodynamic parameters (without additive effects of combination therapy) strongly supports a reciprocal relationship between endothelial dysfunction and insulin resistance that is important for linking metabolic and cardiovascular pathophysiology.

Hyperglycemia per se may contribute to both insulin resistance and endothelial dysfunction (1). However, our results cannot be explained by direct hypoglycemic effects of either enalapril or rosiglitazone because our SHRs were normoglycemic in the absence or presence of therapy with either drug. Rather, enhanced insulin sensitivity observed in SHRs treated with enalapril may be due to a reduction in cross-talk between angiotensin II signaling and insulin signaling pathways in both metabolic and vascular tissues (45). Reduction in serum levels of ET-1 in SHRs treated with enalapril may be due to inhibition of angiotensin II–dependent secretion of ET-1 from vascular endothelium (46). Because vasodilator actions of insulin contribute significantly to insulin-stimulated glucose uptake in skeletal muscle (47), decreased secretion of the vasoconstrictor ET-1 and enhanced insulin-stimulated production of the vasodilator NO from endothelium may contribute importantly to effects of enalapril to improve insulin sensitivity. Another potential mechanism for enalapril to improve insulin sensitivity may have to do with its ability to increase adiponectin gene expression and circulating adiponectin levels (48,49). This may be related to blocking inhibitory effects of the angiotensin II type 1 receptors on adiponectin expression mediated by increased oxidative stress (50). In addition, angiotensin II type 1 receptor blockade may activate peroxisome proliferator–activated receptor $\gamma$ to directly induce adiponectin expression (51). The reduction in blood pressure observed in SHRs treated with rosiglitazone may be due to its effects to improve endothelial dysfunction by enhancing insulin-stimulated production of NO (52,53), decreasing ET-1 levels (54), and decreasing expression of angiotensin receptors (55). Because reduction of blood pressure in SHRs treated with rosiglitazone is accompanied by substantial reduction in serum ET-1 levels, lowering of basal levels of ET-1 (secondary to reduced fasting insulin levels and improved insulin sensitivity) may be an important mechanism by which rosiglitazone lowers blood pressure in SHRs. Previous in vitro studies have demonstrated that thiazolidinediones inhibit transcription of ET-1 (54,56).

Combination therapy and monotherapy with either drug resulted in similar effects on hemodynamic and metabolic parameters, except for adiponectin levels where there was an additional effect of combination therapy to raise adiponectin levels even more than with monotherapy. Circulating levels of adiponectin are positively correlated with insulin sensitivity, and adiponectin levels typically increase after reduction of body weight (57). However, in SHRs treated with rosiglitazone, either alone or in combination with enalapril, body weight increased slightly when compared with vehicle-treated SHRs. Thus, enalapril and/or rosiglitazone treatment of SHRs improved insulin sensitivity and increased levels of adiponectin without a reduction in body weight. These results suggest that increases in adiponectin levels after enalapril and/or rosiglitazone therapy may be driving changes in insulin sensitivity rather than simply reflecting improvement in insulin sensitivity. Similar results have been observed in human studies with ACE inhibitors and ARBs with respect to increasing adiponectin levels (29,49,58,59). The ability of rosiglitazone to increase adiponectin levels may result from several mechanisms, including direct stimulatory effects on adiponectin expression (60) and/or indirect effects to antagonize the inhibitory actions of tumor necrosis factor-$\alpha$ on the adiponectin promoter (61). The mechanisms by which enalapril may increase circulating levels of adiponectin are unclear.

**Effects of enalapril and/or rosiglitazone treatment on endothelial function in SHRs.** To gain insight into mechanisms by which enalapril and/or rosiglitazone therapy may simultaneously improve the metabolic and hemodynamic phenotype of SHRs, we evaluated endothelial function in MVBs isolated from SHRs after 3-week therapy with drugs or vehicle. Although the doses of rosiglitazone and enalapril that we used in our study are higher than those usually used in humans, the doses that we used are typical for rodent studies (62–64). Given the large surface area and synthetic capacity of microvascular endothelium, small resistance vessels in the MVBs represent important determinants of plasma levels of endothelium-derived mediators and total peripheral vascular resistance. Thus, changes in endothelial function in MVBs in response to insulin may parallel, or even precede, changes in vascular reactivity in large conductance arteries. Because large conductance arteries do not contribute significantly to elevated peripheral vascular resistance related to hypertension in SHRs, we did not evaluate endothelial function in these vessels. Although it is possible that other important vascular beds may behave differently than MVBs with respect to their response to insulin, it was not technically feasible to evaluate those other vascular regions in our experimental paradigm.

As expected from the increased peripheral vascular resistance of SHRs, vascular reactivity (in terms of responsiveness to vasoconstrictors) is higher in MVBs from SHRs than in MVBs from WKY rats (13). Consistent with our previous study (13), in the present study, insulin-mediated vasorelaxation was impaired in MVBs from vehicle-treated SHRs when compared with MVBs from WKY control rats.

In our previous study, we found that the impaired vasodilator response to insulin in MVBs from untreated SHRs is not altered by pretreating MVBs with the PI 3-kinase inhibitor wortmannin but is significantly improved by pretreatment with the MEK inhibitor PD98059 or ET-1 receptor blockade with BQ-123/BQ-788 (13). This suggests an imbalance between vasodilator and vasoconstrictor actions of insulin mediated by pathway-specific insulin resistance with decreased PI 3-kinase signaling and increased MAPK signaling in endothelium (1). We have previously directly linked endothelial-dependent relaxation to insulin-stimulated activation of PI 3-kinase and endothelial NO synthase in MVBs by showing that these effects are inhibitable by L-NAME (NOS inhibitor), removal of endothelium, or wortmannin (PI 3-kinase inhibitor) (13). Importantly, after 3 weeks of enalapril and/or rosiglitazone therapy, the vasodilator response to insulin in MVBs from SHRs was significantly improved. Wortmannin pretreatment of MVBs from SHRs after a 3-week therapy with rosiglitazone or enalapril inhibited the vasodilator response to insulin. Interestingly, in MVBs from SHRs treated with the combination of enalapril and rosiglitazone, the ability of insulin to oppose NE-mediated vasoconstriction was restored along with a reduction in sensitivity to NE itself. That is, maximal vasoconstriction in response to NE in MVBs from SHRs treated with combination therapy was similar to that observed in MVBs from WKY control rats. Taken together, these results suggest that rosiglitazone and/or enalapril therapy enhances PI 3-kinase–dependent insulin signaling pathways in vascular endothelium of SHRs. This may in part contribute to the improved metabolic and hemodynamic phenotypes observed after rosiglitazone and/or enalapril therapy.

In addition to an improved vasodilator response to insulin, we also observed a significant decrease in basal ET-1 levels in perfusate from MVBs isolated from SHRs treated with rosiglitazone and/or enalapril. This was accompanied by a restoration of the ability of insulin to acutely stimulate an increase in ET-1 levels in perfusate from MVBs that was absent in MVBs from vehicle-treated SHRs. Because we only treated MVBs with one concentration of insulin in our studies, it was not possible to evaluate changes in the concentration dependence of insulin-stimulated secretion of ET-1 from MVBs isolated from SHRs after various therapies. We did not directly measure ET-1 secreted from large conduit vessels such as the aorta or femoral artery because circulating levels of ET-1 are likely to reflect the contribution of the larger mass of endothelium present in small resistance vessels. Pretreatment of MVBs from vehicle-treated SHRs with the MEK inhibitor PD98059 significantly decreased ET-1 levels in the MVB perfusate. Taken together, these results suggest that rosiglitazone and/or enalapril therapy decrease MAPK-dependent insulin signaling pathways in vascular endothelium of SHRs, leading to decreased circulating levels of ET-1. Thus, the impaired PI 3-kinase signaling and enhanced MAPK signaling in vascular endothelium that underlies the imbalance between vasodilator and vasoconstrictor actions of insulin in untreated SHRs (13) are ameliorated by 3-week therapy with rosiglitazone and/or enalapril. Our results suggest that ACE inhibitor and thiazolidinedione therapies result in rebalanced signaling through PI 3-kinase and MAPK pathways that are the upstream inputs to NO production and ET-1 secretion, respectively. Although we did not observe many synergistic or additive effects of combination therapy, there may still be some advantage to combination therapy because ACE inhibitors tend to be more effective at lowering blood pressure, whereas insulin sensitizers tend to be more effective at improving insulin sensitivity.

In summary, in SHRs, insulin resistance with compensatory hyperinsulinemia characterized by pathway-specific impairment in PI 3-kinase–dependent signaling and enhanced MAPK-dependent signaling in vascular endothelium may contribute to reciprocal relationships between endothelial dysfunction and insulin resistance that underlie both metabolic and hemodynamic abnormalities. Rosiglitazone and/or enalapril therapy in SHRs may simultaneously improve both blood pressure and insulin resistance, in part by restoring balance between vasodilator and vasoconstrictor actions of insulin (mediated by PI 3-kinase and MAPK signaling pathways, respectively) that serve to couple hemodynamic and metabolic homeostasis. These findings may be relevant to developing novel therapeutic strategies to treat multifaceted disorders, including the metabolic syndrome.

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