Rosiglitazone Attenuates Endothelin-1–Induced Vasoconstriction by Upregulating Endothelial Expression of Endothelin B Receptor

Jianwei Tian, Wing Tak Wong, Xiao Yu Tian, Peng Zhang, Yu Huang, Nanping Wang

Abstract—Thiazolidinediones improve insulin resistance and endothelial dysfunction. However, the mechanisms underlying the vasoprotective effects of thiazolidinediones remain to be fully elucidated. The present study aimed to examine the molecular mechanism for the anti-vasoconstrictive effects of rosiglitazone in response to endothelin (ET) 1. Mouse aortas were treated with rosiglitazone for 24 hours, and ET-1–induced vasoconstriction was assessed by wire myography. The results showed that rosiglitazone attenuated ET-1–induced contraction in mouse aortas; this effect was abolished by ET-B receptor (ET\textsubscript{B}R) antagonist, NO synthase inhibitor, and by the removal of endothelium. By using Northern blotting, real-time RT-PCR, Western blotting, and immunohistochemical techniques, we found that rosiglitazone upregulated expression of ET\textsubscript{B}R at both mRNA and protein levels in mouse aortas and human vascular endothelial cells. The induction of ET\textsubscript{B}R was prevented by peroxisome proliferator-activated receptor-\(\gamma\) antagonism. Luciferase reporter assay showed that rosiglitazone enhanced ET\textsubscript{B}R gene promoter activity. Furthermore, chromatin immunoprecipitation assays demonstrated that peroxisome proliferator-activated receptor-\(\gamma\) can directly bind to ET\textsubscript{B}R gene promoter. Moreover, in vivo treatment with rosiglitazone also attenuated the ET-1–induced vasoconstrictions and increased the ET\textsubscript{B}R expression in mouse aortas and mesenteric arteries. In conclusion, these results demonstrate that rosiglitazone attenuated ET-1–induced vasoconstriction through the upregulation of endothelial ET\textsubscript{B}R, which is a peroxisome proliferator-activated receptor-\(\gamma\) direct target. (Hypertension. 2010;56:129-135.)

Key Words: thiazolidinediones • vasoconstriction • ET\textsubscript{B}R • PPAR-\(\gamma\)

Endothelial dysfunction is associated with insulin resistance and type 2 diabetes mellitus. Thiazolidinediones (TZDs), like rosiglitazone and pioglitazone, are peroxisome proliferator-activated receptor (PPAR)-\(\gamma\) ligands and have been widely used for patients with type 2 diabetes mellitus. They exert cardiovascular benefit by improving glucose and lipid homeostasis and insulin resistance. In addition, TZDs also have direct protective effects on endothelial function independent of their insulin-sensitizing action.1–3

The endothelium is crucially involved in the maintenance of vascular homeostasis.4 Normal endothelial function mainly depends on the capacity of endothelial cells to produce NO, which is vasoprotective by inhibiting cytokine expression, smooth muscle cell migration, leukocyte adhesion, and platelet aggregation, as well as improving vasodilatory function.5 TZDs improve endothelial dysfunction. PPAR-\(\gamma\) activators inhibit thrombin-induced endothelin (ET) 1 production in human vascular endothelial cells.6 In vivo, treatment with rosiglitazone decreases blood pressure7 and partially restores the impaired relaxation of carotid arteries to acetylcholine without affecting the gene expression of major modulators of blood pressure, including endothelial NO synthase, angiotensin II type 1 receptors, and preproendothelin 1. PPAR-\(\gamma\) activators also modulate cardiac remodeling in mineralocorticoid-induced hypertension, which was associated with a decreased production of ET-1.8 In vitro experiments indicate that TZDs cause vasodilatation partially by decreasing the effect of ET-1.9,10 The Prospective Pioglitzone Clinical Trial in Macrovascular Events indicates that pioglitazone reduced the composite of all-cause mortality, nonfatal myocardial infarction, and stroke in type 2 diabetics who have a high risk of macrovascular events.11 However, the mechanisms underlying the vasoprotective effect of TZDs remain to be fully elucidated. In the present study, we observed the effect of rosiglitazone on ET-1–induced vasoconstriction and the underlying molecular mechanism.

Materials and Methods

Reagents and Chemicals
Polyclonal rabbit anti-ET-B receptor (ET\textsubscript{B}R) antibody was from Abcam. Polyclonal rabbit anti–ET\textsubscript{B}R and anti–PPAR-\(\gamma\) were from Santa Cruz Biotechnology. ET-1, acetylcholine, U46619, N\textsuperscript{6}-nitro-
l-arginine methyl ester (L-NAME), GW9662, and bisphenol A diglycidyl ether (BADGE) were from Sigma-Aldrich Co, rosiglitazone and troglitazone were from GlaxoSmithKline, and ABT627 and A192621 were from Abbott Laboratories.

Drug Treatment
Adult male C57BL/6J mice (10 weeks old) were housed under a 12-hour light/12-hour dark cycle and fed ad libitum. Mice received daily oral administration of 10 mg/kg 1 of rosiglitazone or vehicle via gastric gavage for 2 weeks. The animal protocols were approved by the institutional animal care and use committee and were consistent with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Blood Vessel Preparation
The mice were euthanized by cervical dislocation. Thoracic aortas and mesenteric resistance arteries were dissected out, cleaned of adhering connective tissue, and cut into several ring segments of ~2 mm in length each. Isolated mouse aortic rings were incubated in DMEM supplemented with 10% FBS, plus 100 IU/mL of penicillin and 100 μg/mL of streptomycin with rosiglitazone (1 or 10 μmol/L) or vehicle control for 24 hours, then transferred into Krebs solution and mounted in a myograph. Real-time changes in arterial tone were measured as described. Isometric Tension Measurement
Blood vessels were prepared as described previously. Briefly, each ring was suspended between 2 small tungsten wires in an organ chamber (Multi Myograph System) filled with Krebs-Henseleit solution and placed under a previously determined optimal resting tension of 3 mN for aortas and 1 mN for mesenteric resistance arteries and left for 90-minute equilibration. The concentration-tension of 3 mN for aortas and 1 mN for mesenteric resistance arteries were synthesized by RT-PCR, purified, and labeled with 32P-dCTP using the Prime-a-Gene Labeling System (Promega), as described. RNA Isolation and Northern Blotting
Total RNA was isolated from HUVECs using TRIzol reagent (Invitrogen), fractionated on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized to cDNA probes for human ETBR (1 or 10 μmol/L) or vehicle control for 24 hours, then transferred into Krebs solution and mounted in a myograph. Real-time changes in arterial tone were measured as described.12

Cell Culture
Human umbilical vein endothelial cells (HUVECs) and bovine aortic endothelial cells were isolated and cultured as described.13 RNA Isolation and Northern Blotting
Total RNA was isolated from HUVECs using TRIsol reagent (Invitrogen), fractionated on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized to cDNA probes for human ETBR and GAPDH cDNAs. The cDNA probes for ETBR and GAPDH were synthesized by RT-PCR, purified, and labeled with 32P-dCTP using the Prime-a-Gene Labeling System (Promega), as described.13

Real-Time Quantitative RT-PCR
Total RNA was reverse transcribed into cDNA using Moloney murine leukemia virus reverse transcriptase and oligo-dT primer. Real-time PCR involved SYBR-green dye and Taq polymerase; the results were analyzed with the Opticon Monitor analysis software. The sequences of the primers were listed in Table S1 (available in the online Data Supplement at http://hyper.ahajournals.org).

Protein Extraction and Western Blotting
Aortas were isolated and frozen in liquid nitrogen after rosiglitazone treatment and homogenized in radioimmunoprecipitation assay lysis buffer. Proteins were extracted from the vessels and HUVECs as described. Western analyses were performed with appropriate primary antibodies and a horseradish peroxidase–conjugated secondary antibody followed by ECL detection (Amersham Biosciences).

Adenoviral Infection
HUVECs were coinfected with AdPPAR-γ that encodes a mouse PPAR-γ1 fused to a minimal VP16 transactivation domain and AdTA that encodes a tetracycline-responsive transactivator and maintained in the presence or absence of tetracycline (0.1 μg/mL, a tet-off expression) for 48 hours as described.3 Immunohistochemistry
Cross-sections in 5-μm thickness were cut in paraffin-embedded aortic rings, treated with citrate buffer for antigen retrieval, incubated with 3% H2O2 to block endogenous peroxidase, and blocked in 5% normal goat serum. Anti-ETBR antibody was added and incubated overnight at 4°C, followed by Biotin-SP–conjugated secondary antibodies (Jackson Immunoresearch), then incubated with streptavidin–horseradish peroxidase conjugate (Zymed), and visualized by diaminobenzidine (Vector Labs).

Chromatin Immunoprecipitation Assay
Cells were cross-linked with 1% formaldehyde and quenched before harvest and sonication. The sheared chromatin was immunoprecipitated with 5 μg of anti–PPAR-γ antibody (or control IgG) and protein A Sepharose beads. The eluted immunoprecipitates were digested with proteinase K, and DNA was extracted and underwent PCR with the primers flanking the putative peroxisome proliferator response element binding motifs.

Plasmids, Transfection, and Reporter Assay
The pGL3/hETBR promoter-luciferase reporter was made by PCR cloning. Briefly, a 5′-flanking region (~1415 to +28) of the human ETBR gene was amplified from human genomic DNA using specific primers and subcloned into pGL3-basic. Plasmids were transfected into bovine aortic endothelial cells with the use of Lipofectamine 2000 (Invitrogen). The plasmid expressing β-galactosidase (pRSV-βgal) was cotransfected to normalize the transfection efficiency. At 48 hours after transfection, cell lysates were harvested to measure luciferase and β-galactosidase activities.

Statistical Analysis
Quantitative data are mean±SEM. Arterial contractions were expressed as active tension [tone developed/(2×ring length in millimeters)].13 Statistical analyses were performed with 1- or 2-way ANOVA or Student t test; Bonferroni post hoc tests were performed when >2 treatments were compared (GraphPad Prism, version 4.0, GraphPad), with statistical significance set at P<0.05. Nonquantitative results were representative of ≥3 independent experiments.

Results
Rosiglitazone Attenuates Vasoconstrictive Effect of ET-1
To investigate the effect of rosiglitazone on vasoconstriction induced by ET-1, male C57BL/6J mouse thoracic aortic rings were treated with rosiglitazone (10 μmol/L) for 24 hours, and the constrictive responses to ET-1 were examined. Original recordings in Figure 1A show that, in aortas with endothelium, ET-1 produced concentration-dependent contractions, which were significantly reduced by 24-hour treatment with rosiglitazone (Figure 1B). By contrast, the acute (30-minute) exposure to 10 μmol/L of rosiglitazone did not modulate ET-1–induced contractions (data not shown). Rosiglitazone (24-hour) treatment did not affect U46619 (a thromboxane A2 agonist)-induced contraction (Figure 1C; n=5). Likewise, the 60 mmol/L of K+–induced contraction was comparable in control (2.48±0.10 mN/mm) and rosiglitazone-treated (2.41±0.13 mN/mm) rings (P>0.05; n=4).
Inhibitory Effect of Rosiglitazone Is Abolished by ETBR Receptor Antagonist, NO Synthase Inhibitor, and Endothelium Denudation

To examine which ET receptor subtype contributed to the effects of rosiglitazone on ET-1–induced vasoconstriction, endothelium-intact and endothelium-denuded aortic rings treated with rosiglitazone were examined in the presence or absence of ETAR and ETBR antagonists. The specificities of ETAR and ETBR antagonists were also tested on ET-1–induced contractions. Finally, the role of endothelium-derived NO was examined in rings with endothelium pretreated with L-NAME, an endothelial NO synthase inhibitor.

The aortic contraction in response to ET-1 was mediated through ETAR, because the selective ETAR antagonist ABT627 (10 nmol/L) abolished the contraction in control and rosiglitazone-treated rings (Figure 2A). The attenuated ET-1–induced contraction of rosiglitazone-treated rings was restored by the presence of a selective ETBR antagonist, A192621 (10 nmol/L), whereas this antagonist did not modulate the evoked contractions in control rings (Figure 2B). The difference in the amplitude of contractions between control and rosiglitazone-treated rings was lost in rings that had been previously exposed to 100 µmol/L of L-NAME for 30 minutes (Figure 2C) or in rings without endothelium (Figure 2D).

TZDs Increase mRNA and Protein Levels of ETBR in Aortas and Endothelial Cells

To investigate how rosiglitazone affected the expression of ETBR, male C57BL/6J mice thoracic aortas were treated with 1 and 10 µmol/L of rosiglitazone for 24 hours and examined for the ETBR protein level. Western blotting showed an upregulation of ETBR with rosiglitazone treatment, which was much greater in endothelium-intact than in endothelium-denude aortas (Figure 3A), whereas the ETAR expression was unchanged (Figure 3B). Immunohistochemical staining also showed that ETBR was expressed in both endothelial cells and vascular smooth muscle cells at low levels in normal

Figure 1. A, Original records showing ET-1–induced constrictions in endothelium-intact mouse aortic rings pretreated with 10 µmol/L of rosiglitazone for 24 hours. B, Concentration-dependent contractions to ET-1 in rosiglitazone (1 to 10 µmol/L)-treated rings (n=4 to 7). C, Concentration-dependent contractions to U46619 in rosiglitazone (10 µmol/L)-treated rings (n=5). Results are mean±SEM of n experiments. ***P<0.001 vs control.

Figure 2. Concentration-dependent contractions to ET-1 in rosiglitazone-treated rings in the presence of 10 nmol/L of ABT627 (A), of 10 nmol/L of A192621 (B), of 100 µmol/L of L-NAME (C), and in rings without endothelium (D). Results are mean±SEM of 4 to 6 experiments.
mouse aortas (Figure 3C), and rosiglitazone treatment increased the ETαR expression, which was primarily confined to endothelial cells (Figure 3D).

To further investigate the effects of PPAR-γ activation on the expression of ETαR in endothelial cells, we first determined the ETαR mRNA level of HUVECs treated with troglitazone (10 μmol/L) or rosiglitazone (10 μmol/L) for 24 hours. The results showed that both PPAR-γ agonists upregulated the ETαR mRNA expression. ETαR mRNA was significantly increased in HUVECs with 24-hour treatment with troglitazone or rosiglitazone as detected by Northern blotting (Figure 4A). Moreover, rosiglitazone and troglitazone increased the ETαR mRNA expression in both a time- and concentration-dependent manner (Figure 4B to 4C and 4D to 4E). In addition, selective PPAR-γ antagonists GW9662 and BADGE abolished the stimulatory effects of TZDs on ETαR expression. In contrast, adenoviral overexpression of a constitutively active PPAR-γ increased ETαR mRNA and protein levels in HUVECs (Figure S1), indicating that the upregulation of ETαR by TZDs was PPAR-γ specific.

**PPAR-γ Binds and Activates the ETαR Promoter**

Sequence analysis using the transcription element search system (http://www.cbil.upenn.edu/tess) revealed 2 potential PPAR-responsive elements (PPRE) within the 2400-bp region upstream of the first exon of human ETαR gene.

![Figure 3. Western blotting for ETαR (A) and ETαR (B) in the vascular wall of control and rosiglitazone-treated mouse aortas. Immunohistochemical staining of ETαR in mouse aortas (C and D) and arrow indicates endothelial cells (EC). Results are mean±SEM of 3 experiments. *P<0.05.](http://hyper.ahajournals.org/)

![Figure 4. Northern blotting shows the effect of troglitazone and rosiglitazone on ETαR mRNA levels (A). Real-time quantitative RT-PCR shows the dose and time effects for rosiglitazone (B and C) or for troglitazone (D and E) in HUVECs. *P<0.05. Results are mean±SEM of the normalized mRNA levels from 3 independent experiments.](http://hyper.ahajournals.org/)
Rosiglitazone ETαR

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reporter and pRSV-1301/-1289 (PPRE1) in the flanking region of human infected with AdPPAR-0.05. Results are mean SEM of 5 experiments.

L-NAME. Western blotting also revealed that in vivo rosiglitazone treatment upregulated ETBR (E) but not ETAR (F) expression in mouse aortas. These inhibitory effects on aortas (B) and mesenteric resistance arteries (D) were abolished in the presence of 100 μmol/L L-NAME. In vivo rosiglitazone treatment also upregulated ETαR expression while leaving ETαR expression unaltered (Figure 6E and 6F) in mouse aortas. In addition, selective ETαR agonist sarafotoxin 6c induced vasodilatations in mesenteric resistance arteries only from rosiglitazone- but not vehicle-treated mice (Figure S2), suggesting a functional role of the TZD-induced ETαR in improving vascular response in vivo.

Chromatin immunoprecipitation assay indicated that PPAR-γ could directly bind to ETαR gene the PPRE located at −1301/−1289 (PPRE1) in the flanking region of human ETαR gene (Figure 5A), whereas the amplification with the primers flanking the −2146/−2134 site (PPRE2) or the −37/−23 site (RXRE) detected no binding for PPAR-γ. These results suggested that the PPRE1 could mediate the induction of ETαR gene by TZDs.

To further examine whether rosiglitazone activated the ETαR gene transcription activity, bovine aortic endothelial cells were transfected with pGL3/hETαR −1415-Luc plasmid containing human ETαR gene promoter region (ETαR gene 5'-flanking −1415 to +28 sequence that contains the PPRE1 motif) and then treated with rosiglitazone and/or pretreated with PPAR-γ-specific antagonists GW9662. Reporter gene luciferase assay shows that PPAR-γ agonist rosiglitazone enhanced ETαR gene promoter activity (P<0.05), and this effect was prevented by GW9662 (Figure 5B), indicating that ETαR is a direct target gene of PPAR-γ.

**In Vivo Rosiglitazone Treatment Attenuates ET-1–Induced Vasoconstrictions**

To further support the in vitro effects, mice were treated with rosiglitazone at 10 mg/kg for 2 weeks, and vascular reactivities were examined by myograph. ET-1–induced vasoconstriction was attenuated after rosiglitazone treatment in both the aortas and mesenteric resistance arteries (Figure 6A and 6C). In the presence of NO synthase inhibitor, the inhibitory effects of rosiglitazone treatment on ET-1–induced vasoconstrictions were abolished in aortas and mesenteric resistance arteries (Figure 6B and 6D). Furthermore, in vivo rosiglitazone treatment also upregulated ETαR expression while leaving ETαR expression unaltered (Figure 6E and 6F) in mouse aortas. In addition, selective ETαR agonist sarafotoxin 6c induced vasodilatations in mesenteric resistance arteries only from rosiglitazone- but not vehicle-treated mice (Figure S2), suggesting a functional role of the TZD-induced ETαR in improving vascular response in vivo.

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**Figure 5.** ETαR is a direct target gene of PPARγ. A. HUVECs were infected with AdPPAR-γ and AdtTA for 24 hours and then cross-linked and immunoprecipitated with anti–PPAR-γ antibody (AB) or the isotype-matched IgG. The immunoprecipitates or supernatants before immunoprecipitation (as positive control, INPUT) were PCR amplified using the primers flanking the motifs in the human ETαR gene as depicted in the top panel. B. Luciferase reporter assay was performed by transfection of pGL3/hETαR promoter-luciferase reporter and pRSV-β-galactosidase into bovine aortic endothelial cells. Transfected cells were treated with rosiglitazone or DMSO with or without GW9662 pretreatment. Luciferase activity was measured after 24 hours and normalized to β-galactosidase activity. *P<0.05. Results are mean±SEM of the normalized data from 3 independent experiments.
Discussion

In the present study, we demonstrate for the first time that rosiglitazone upregulates the expression of ET$_{B}$R in endothelial cells and attenuates ET-1–induced vasoconstriction induced through an endothelial ET$_{B}$R- and NO-related pathway. In addition, we show that PPAR-γ activation induced ET$_{B}$R expression in human endothelial cells.

ET-1 is a potent vasoconstrictive and growth-promoting factor and has been implicated in the pathogenesis of hypertension, atherosclerosis, restenosis after angioplasty, cardiac hypertrophy, and congestive heart failure. The ET family consists of 3 isoforms, including ET-1, ET-2, and ET-3. ET-1 is the predominant member generated by vascular endothelial cells and exerts physiological and pathophysiological function through 2 different type receptors: ET$_{A}$R and ET$_{B}$R. Although ET$_{A}$R is expressed mainly in vascular smooth muscle cells and mediates vasoconstriction and cell hypertrophy, ET$_{B}$R is predominantly located on endothelial cells and involved in the release of NO and prostacyclin, causing vasorelaxation, inhibiting platelet aggregation, and promoting the clearance of ET-1. On the other hand, ET$_{B}$R is slightly expressed in vascular smooth muscle cells and contributes to vasoconstriction and ET-1 clearance. Interestingly, it had been reported that PPAR-γ activation inhibited ET-1–induced cardiac hypertrophy and oxidized low-density lipoprotein-induced ET-1 secretion in endothelial cells. More recently, it was demonstrated that rosiglitazone treatment decreased blood pressure and was effective in preventing the progression of renal injury in deoxycorticosterone acetate-salt hypertension through reducing the overexpression of ET-1 in the kidney and that rosiglitazone decreased blood pressure and renal injury in a female mouse model of systemic lupus erythematosus accompanied by a reduction of urinary ET.

The mechanisms underlying the vasoprotective effects of TZDs remain largely unknown. The present study clearly shows that 24-hour treatment with rosiglitazone suppressed ET-1–induced and ET$_{A}$R-mediated contraction only in rings with endothelium. The attenuated contraction is most likely caused by the enhanced expression and function of ET$_{B}$R in the endothelial cells based on the following observations. First, the effect of rosiglitazone on the aortic contraction was acutely prevented by a selective ET$_{B}$R antagonist. Second, inhibition of NO production restored the reduced contraction in rosiglitazone-treated rings. Third, the reduced contraction disappeared in rings without a functional endothelium. The functional results are supported by an increased protein expression for ET$_{B}$R but not ET$_{A}$R in rosiglitazone-treated mouse aortas. By contrast, ET$_{A}$R is expressed in both endothelium and underlying vascular smooth muscle cells. It is reported that TZD treatments reduced blood pressure and ameliorated vascular dysfunction in genetically hypertensive rats. Our findings suggest additional benefit of the use of rosiglitazone in the preservation of endothelial function through the increased expression and activity of ET$_{B}$R and enhanced NO availability in endothelial cells.

The present study shows that TZDs upregulated ET$_{B}$R gene and protein expression through a PPAR-γ–dependent mechanism. Both agonists enhanced the ET$_{B}$R expression in HUVECs, as confirmed by Northern blotting, real-time RT-PCR, and Western blotting. The upregulation of ET$_{B}$R was prevented by cotreatment with PPAR-γ antagonists GW9662 and BADGE. Adenovirus-mediated overexpression of constitutively active PPAR-γ also increased the levels of ET$_{B}$R mRNA and protein in HUVECs. Likewise, rosiglitazone increased the ET$_{B}$R protein expression in mouse aortas, an effect prevented by the removal of endothelium. These findings indicate that the upregulation of ET$_{B}$R by TZDs in endothelial cells is PPAR-γ specific. Further results with reporter assay suggest that rosiglitazone increased ET$_{B}$R gene promoter activity in a PPAR-γ–dependent manner. The results with chromatin immunoprecipitation assays confirm that PPAR-γ directly bound to the PPRE site between −1301 and −1289 in the 5′-flanking region of human ET$_{B}$R gene. These observations suggest that the ET$_{B}$R gene is a direct target of transcriptional factor PPAR-γ.

Atherosclerosis and hypertension often coexist with and are common complications of diabetes mellitus. The prevalence of atherosclerosis and hypertension in diabetic patients is approximately twice compared with that of non-diabetics. Conversely, the chance of developing type 2 diabetes mellitus in atherosclerosis and hypertensive individuals is also 2- to 4-fold higher than that in normotensive individuals. It is noted that rosiglitazone treatment improved endothelial function and inflammation in patients with type 2 diabetes mellitus and that rosiglitazone lowered blood pressure and protected endothelial function in Zucker fatty rats. TZDs could exert beneficial effects against the occurrence of cardiovascular disease by enhancing ET$_{B}$R expression and NO production, which may be associated with a direct protection of endothelial function by inhibiting cytokine expression, smooth muscle cell migration, leukocyte adhesion, and platelet aggregation, as well as promoting endothelium-dependent relaxations. It was also suggested that TZDs improved blood flow by inhibiting neointimal formation after balloon injury in insulin-resistant rats. In light of the fact that the TZDs have both PPAR-γ–dependent and –independent effects, it is possible that the antihypertensive effect of TZDs may be mediated to the PPAR-γ–independent mechanisms. However, recent studies using tissue-specific PPAR-γ knockout or transgenic models have clearly demonstrated critical roles of vascular PPAR-γ in vascular relaxation and blood pressure regulation. In this study, we also observed that in vivo treatment with rosiglitazone attenuated ET-1–induced vasoconstrictions in both conduit and resistance arteries, of which the effects were mediated through NO-related pathway accompanied with an upregulation of ET$_{B}$R expression. Furthermore, selective ET$_{B}$R agonist sarafotoxin 6c caused vasodilatations in mesenteric resistance arteries of rosiglitazone-treated mice, which were sensitive to ET$_{B}$R and NO synthase inhibition. Collectively, these data further support the notion that TZDs increased expression of ET$_{B}$R and enhanced NO availability in endothelial cells, which offers an additional explanation of how TZDs improve endothelial function.

Perspectives

The present study demonstrates that ET$_{B}$R gene is a direct target gene of PPAR-γ and TZDs activate PPAR-γ and
inhibit ET-1–induced vasoconstriction through upregulation of endothelial ET\(_\alpha\)R and promotion of NO function in blood vessels. These results may provide novel insight into our understanding of the molecular mechanisms underlying the vasoprotective effects of TZDs in treatment of the vascular complications associated with a metabolic syndrome, such as hypertension.

**Sources of Funding**

This work was supported by the National Natural Science Foundation of China/Research Grants Council Joint Research Scheme (30931160434, N_CUHK428/09) and grants from the National Natural Science Foundation of China (30890041 and 30821001).

**Disclosures**

None.

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Hypertension. 2010;56:129-135; originally published online June 1, 2010;
doi: 10.1161/HYPERTENSIONAHA.110.150375
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Supplemental Data

Rosiglitazone Attenuates Endothelin-1-induced Vasoconstriction by Up-regulating Endothelial Expression of ETB Receptor

Short title: Tian, Wong. PPARγ reduces vasocontraction via ET₆R

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### Table S1. Oligonucleotides and Primers

**qRT-PCR Primers**

**ET<sub>B</sub>R**
Forward: 5'-GCCACCCACTAAGACCTCCT-3'  
Reverse: 5'-ATGCCTAGCACGAACACGAG-3'

**ET<sub>A</sub>R**
Forward: 5'-TTTG CCTCAAGATGGAACC-3'  
Reverse: 5'-TGTGGGCAATAGTTGTGCAT-3'

**GAPDH**
Forward: 5'-ACCACAGTCCATGCCATCAC-3'  
Reverse: 5'-TCCACCACCCTGTTGCTGTA-3'

**hETbR Promoter Cloning Primers**
Forward: 5'-GAGAGCTAGCAAGATGACGTGTTCCGTTGTT-3'  
Reverse: 5'-GAGAAGATCTCTGCGCTCCGCGAGTTTCA-3'

**ChIP Assay Primers**

**PPRE1**
Forward: 5' –AAAAGATCGAGCTGTTCC-3'  
Reverse: 5'-ACCCAAATCAAGGGCAAG-3'

**PPRE2**
Forward: 5'-GGACTCAGTGGAGGAAAT-3'  
Reverse: 5'-GAAATATGGTCTAGGGTTG-3'

**RXRE**
Forward: 5'-CCCCATCATCCTCCTCCCT-3'  
Reverse: 5'-CATGCTGCTACCTGCTCC-3'
Supplemental Figure S1. ET\textsubscript{B}R was up-regulated in a PPAR\textsubscript{\gamma}-specific manner. (a) HUVECs were treated with rosiglitazone or DMSO with or without pretreatment the antagonists. Western blotting (upper panel) and qRT-PCR (lower panel) results show that GW9662 abolished the effects of rosiglitazone on ET\textsubscript{B}R expression. (b) HUVECs were co-infected with Ad-PPAR\textsubscript{\gamma} and AdtTA with (mock infection) or without tetracycline. Total RNA and membrane proteins were isolated 48 h after the infection and subjective to qRT-PCR or western blotting analyses. *P<0.05. Results are means ± SEM of the normalized mRNA levels from 3 independent experiments.
Supplemental Figure S2. (a) Selective ET<sub>B</sub>R agonist sarafotoxin 6c (S6c)-induced vasodilatations in mesenteric resistance arteries only from rosiglitazone- but not vehicle-treated c57 mice. The vasodilatations in mesenteric resistance arteries induced by S6c were abolished by 100 μmol/L L-NAME (b) or 10nmol/L A192621 (c).