Effect of Peroxisome Proliferator–Activated Receptor-α and -γ Activators on Vascular Remodeling in Endothelin-Dependent Hypertension

Marc Iglarz, Rhian M. Touyz, Farhad Amiri, Marie-France Lavoie, Quy N. Diep, Ernesto L. Schiffrin

Objective—Peroxisome proliferator–activated receptors (PPARs) may modulate in vitro the vascular production of vasoactive peptides such as endothelin-1 (ET-1). Thus, we investigated in vivo the interaction between PPARs and ET-1 in deoxycorticosterone acetate (DOCA)–salt rats that overexpress vascular ET-1.

Methods and Results—Unilaterally nephrectomized 16-week-old Sprague-Dawley rats (Uni-Nx) were divided into 4 groups (n=6 each): control group, DOCA-salt group, DOCA-salt+PPAR-α activator (rosiglitazone, 5 mg · kg⁻¹ · d⁻¹), or DOCA-salt+PPAR-γ activator (fenofibrate, 100 mg · kg⁻¹ · d⁻¹). Systolic blood pressure was significantly increased in the DOCA-salt group (240±11 vs 121±2 mm Hg in Uni-Nx, P<0.01). Progression of hypertension was partially prevented by coadministration of rosiglitazone (172±3 mm Hg vs DOCA-salt, P<0.05) but not by fenofibrate. Both PPAR activators abrogated the increase in prepro-ET-1 mRNA content in the mesenteric vasculature of DOCA-salt rats. The media-to-lumen ratio was increased in DOCA-salt rats (10.3±0.9% vs 4.9±0.5% in Uni-Nx rats, P<0.01). Rosiglitazone and fenofibrate prevented the hypertrophic remodeling observed in DOCA-salt rats without affecting vascular stiffness. Rosiglitazone but not fenofibrate prevented endothelial dysfunction in pressurized mesenteric arteries. Finally, both rosiglitazone and fenofibrate prevented the vascular increase in superoxide anion production induced in DOCA-salt animals.

Conclusions—PPAR-α and -γ activators were able to modulate endogenous production of ET-1 and had beneficial vascular effects in endothelin-dependent hypertension. (Arterioscler Thromb Vasc Biol. 2003;23:●●●●●●●●)

Key Words: PPAR activators • rosiglitazone • fenofibrate • resistance arteries • DOCA-salt

Peroxisome proliferator–activated receptors (PPARs) are a family of ligand-activated transcription factors that have recently been found to be involved in the homeostasis of vascular biology. Indeed, PPAR-α and -γ isoforms have been characterized in multiple vascular cell types in rats and humans. Moreover, PPAR activators prevent in vitro vascular smooth muscle cell growth and inflammatory response and also induce apoptosis, suggesting at least a potential role in vascular remodeling.

A recent study from our laboratory demonstrated that both PPAR-α and -γ activators prevent vascular remodeling in angiotensin II–infused rats. As angiotensin II, endothelin-1 (ET-1) is a potent vasoconstrictor. It is produced within the vascular wall and plays a critical role in vascular hypertrophy in many models of hypertension, such as deoxycorticosterone acetate (DOCA)–salt rats or stroke-prone spontaneously hypertensive rats. Indeed, in those models of hypertension in which ET-1 plays a vasoconstrictor role, ET-1 was overexpressed in the vessel wall, and systolic blood pressure (SBP) was lowered by endothelin receptor antagonists. Moreover, in these experimental models, ET-1 receptor antagonists also regressed vascular growth and inflammation and improved endothelial dysfunction.

Interestingly, PPAR-γ activators are able to suppress ET-1 secretion from endothelial and vascular smooth muscle cells. In addition, ET-1 production by endothelial cells can be activated by many factors, such as insulin or thrombin, through c-jun fixation on the activator protein-1 site of the prepro-ET-1 promoter. Furthermore, Delerive et al recently demonstrated that PPAR-α and -γ bind c-jun, resulting in inhibition of ET-1 production in vitro.

The aim of our study was to assess in vivo the impact on vascular function and structure of PPAR-α (fenofibrate) and -γ (rosiglitazone) activators in a model of endothelin-dependent hypertension. Thus, we tested the hypothesis that PPAR activators would have potent blood pressure–reducing effects and would significantly improve vascular function and structure in DOCA-salt rats.
Methods

Animals

The present study was conducted according to recommendations from the Animal Care Committee of the Clinical Research Institute of Montreal and the Canadian Council of Animal Care. DOCA-salt hypertension was induced in Sprague-Dawley rats by the method of Ormsbee and Ryan.13 In brief, male Sprague-Dawley rats (Charles River, St Constant, Quebec, Canada) weighing 225 g were unilaterally nephrectomized under sodium pentobarbital anesthesia (40 mg/kg). Silicone rubber impregnated with DOCA (200 mg per rat, n = 6) was implanted subcutaneously, and rats were offered 1% saline to drink. Control rats (Uni-Nx, n = 6) were also unilaterally nephrectomized but received a silicone rubber implant without DOCA and tap water to drink. After surgery, rats were fed powdered diets containing rosiglitazone (5 mg/kg) in tap water to drink. SBP was measured weekly by tail-cuff method, and after 3 weeks of treatment, rats were killed by decapitation. The mesenteric bed was dissected, 1 segment was used for preparation of small arteries, and the rest was cleaned of fat, frozen in LN2, and kept at −80°C for reverse transcription–polymerase chain reaction (PCR). A portion of thoracic aorta was fixed in 4% paraformaldehyde and embedded in paraffin. Tissue sections (7 µm thick) were prepared for immunohistochemical analysis.

Preparation and Study of Small Arteries

A third-order superior mesenteric artery (2 mm) was placed on 2 glass microcannulas in a pressurized myograph under constant intraluminal pressure (45 mm Hg) with warm (37°C) physiological salt solution (PSS) and bubbled with 95% air and 5% CO2 to achieve a pH of 7.4. Vessels were used if they constricted >50% in response to potassium (125 mmol/L KCl) with norepinephrine (10−4 mol/L). Endothelium-dependent relaxation was assessed with cumulative doses of acetylcholine (10−10 to 10−4 mol/L) after preconstriction with 10−3 mol/L norepinephrine. Endothelium-independent relaxation was assessed with cumulative doses of sodium nitroprusside (10−7 to 10−4 mol/L). Contraction to norepinephrine was achieved with cumulative doses from 10−5 to 10−4 mol/L and expressed as a percentage of maximal response.

To study the vascular morphology, lumen and media dimensions were measured with the intraluminal pressure maintained at 45 mm Hg after deactivation of the vessels by perfusion with Ca2+-free PSS containing 10 mmol/L EGTA for 30 minutes. To study vascular mechanics, intraluminal pressure was increased stepwise from 10 to 140 mm Hg and media and lumen dimensions were measured at 3 different points.

Morphological and Mechanical Formulas

For definitions of parameters, see the article by Dobrin.14 Medial cross-sectional area (CSAn) is calculated as (π/4)(D2 − D2 i ), where D and D i are external and internal lumen diameters, respectively. Incremental distensibility is calculated as (I/ΔP)×(ΔD/ΔD i )×100, or the fractional change in lumen diameter (ΔD/ΔD i ) per change in intraluminal pressure (ΔP). Circumferential strain (ε) is (D − D i )/D, where D is the lumen diameter for a given intraluminal pressure, and D i is the original diameter at 3 mm Hg. Circumferential stress (σ) is (PD/2M), where P is the intraluminal pressure (dyne/cm²), and D and M are lumen diameter and media thickness, respectively. The elastic modulus was determined by fitting stress-strain data to σ = σεε, where σ is stress at D, and ε is a constant related to the rate of increase of the stress-strain curve. Tangential elastic modulus (ET) was calculated at several values of stress from the derivative of the aforementioned exponential curve: ET = Δσ/Δε. Remodeling index is calculated as 100× [(D1 − D2 i )/[(D3 − D4 i )]× [(D2 i − D3 i )]], where (D2 i ) and (D3 i ) are lumen diameters of control and treated vessels, respectively, and (D4 i ) is [(D2 i )−(4×CSAn/M)]11/3, where (D2 i ) is the external diameter of treated vessels and CSA, is the cross-sectional area of control vessels. Growth index is calculated as (CSAn − CSAn i )/CSAn, where CSAn and CSAn i are medial cross-sectional areas of control and treated vessels, respectively.

Determination of Resistance Artery Wall Composition

Second-order mesenteric arteries were pressurized at 70 mm Hg, fixed with 2% paraformaldehyde solution at room temperature for 60 minutes, removed from the cannula, and processed for histological analysis. Paraffin-embedded sections of mesenteric arteries were stained with Sirius red. Collagen in the media was quantified microscopically with the Northern Eclipse imaging program (EM-PIX Imaging Inc) and was determined by measuring the relative density per area in each Sirius red-stained section.

PreproET-1 mRNA Levels in Mesenteric Vasculature

Expression of the prepro-ET-1 gene in the mesenteric vasculature was studied by real-time PCR. Reverse transcription was performed in a 20-µL volume containing 2 µg RNA, 1.5 µL of 10 mmol/L dNTP, 6 µL BRL 5× buffer, 0.6 µL oligo(dT)12–18 primer (0.5 µg/µL), 1.5 µL of 200 U/µL M-MLV reverse transcriptase (GIBCO-BRL), 0.9 µL rRNasin (RNase inhibitor, 40 U/µL), and 3 µL dithiothreitol (0.1 mol/L) for 1 hour at 37°C. The reaction was stopped by heating at 95°C for 5 minutes. Standard real-time PCR was performed with a Stratagene Mx4000 system for relative quantification of vascular prepro-ET-1 mRNA. Primers were designed to generate short amplification products (147 bp for prepro-ET-1 and 107 bp for ribosomal protein S16, used as an internal standard), which spanned 1 intron region to avoid contamination by genomic DNA. Primers for prepro-ET-1 were as follows: sense 5′-GCTGTTGAGGGAAAGAAAAC-3′ and antisense 5′-CAACCGGGGCTCTGTAGTC-3′ and for S16, sense 5′-AGGIGGATTTGCTGTGGTSG-3′ and antisense 5′-GCTACAGGGGCTTTGAGATG-3′. To validate our real-time PCR protocol, gene-specific standard curves for prepro-ET-1 and S16 were generated from serial 10-fold dilutions of the cDNA. Linearity ranged from 1 to 1/1000 (for prepro-ET-1) and from 1/10 to 1/10000 (for S16). Slopes were similar (−3.51 for prepro-ET-1 vs −3.47 for S16), indicating a 3-fold increase in amplification product at every cycle (Figure 3A). Thus, 2 µL of 1/100 cDNA mixture was amplified by using specific primers. Real-time PCR was conducted with an initial denaturing interval (95°C, 15 minutes) and then 40 sequence cycles for prepro-ET-1 (94°C, 30 seconds), 60°C (45 seconds), and 72°C (30 seconds); for S16, 94°C (30 seconds), 55°C (45 seconds), and 72°C (30 seconds) with use of the Quantitect SYBR green PCR kit (Quaigen) and a final 0.5 µmol/L concentration of primers (run in duplicate). Amplification products were electrophoresed on 1% agarose gels containing ethidium bromide and visualized with UV light to check the fragment length and the absence of nonspecific products that could interfere with fluorescence signal produced by SYBR green (not shown). A melting-curve analysis was also performed to check the absence of primer dimers. Samples from Uni-Nx rat cDNAs were used as calibrators, and variations of prepro-ET-1 or S16 were calculated as a relative quantity compared with this group. Results are expressed as the ratio between prepro-ET-1 and S16 relative quantities.

In Situ Hybridization

Frozen sections (7 µm thick) of tissues were obtained on a Bright-Hacker cryostat and thaw-mounted on Superfrost glass slides for in situ hybridization. Specificity of labeling was established with the sense-strand prepro-ET-1 RNA probe of the same size and specific activity as the antisense-strand probe. Tissues from 3 different animals were sectioned, and at least 3 sections for each group were examined. The rat prepro-ET-1 sense or antisense RNA probes of 392 bp were prepared by the RNA transcription reaction using T7 or SP6 RNA polymerase, respectively, and a cDNA plasmid construct previously described in detail.15 Radiolabeled riboprobes were prepared using [35S]CTP (1250 Ci/mmole; Amersham) in the same labeling reaction.
Detection of Vascular O$_2^-$ by Lucigenin Chemiluminescence
The thoracic aorta was cleaned of adherent adipose tissue, and 5-mm-long rings were cut and incubated in HEPES buffer. As previously described,16 rings were maintained at 37°C for 30 minutes; rinsed; gently transferred to test tubes containing warmed Krebs’ buffer and lucigenin (5 μmol/L), an acridylium dinitrate; and allowed to equilibrate in the dark for 5 minutes. To measure NADPH oxidase activity, NADPH (10$^{-3}$ mol/L) was then added, and chemiluminescence was recorded every 1.8 seconds for 3 minutes with a luminometer (AutoLumat LB953, EG&G Berthold). Chemiluminescence was expressed as counts per second. Luminescence was also measured in tubes containing buffer and lucigenin without vascular rings, and these blank values were subtracted from the chemiluminescence signals obtained from the aortic rings. Tissue superoxide formation was expressed as counts per minute per dry tissue weight.

Data Analysis
Data are presented as mean ± SEM. One-way ANOVA followed by a Student-Newman-Keuls test and ANOVA for repeated measures were used as appropriate. A value of $P<0.05$ was considered significant.

**Results**

**Blood Pressure and Body Weight**
As shown in Figure 1, SBP was markedly increased in DOCA-salt rats after 3 weeks of treatment, compared with Uni-Nx rats (240±11 vs 121±2 mm Hg, $P<0.001$). Rosiglitazone partially prevented this increase (172±3 mm Hg, $P<0.05$, vs Uni-Nx and DOCA-salt), whereas the slight decrease in SBP induced by fenofibrate did not achieve statistical significance (215±16 mm Hg, NS vs DOCA-salt). Both rosiglitazone (315±6 g) and fenofibrate (299±8 g) prevented the weight loss observed in DOCA-salt rats (230±16 vs 334±7 g in Uni-Nx, $P<0.001$).

Vascular Morphology and the Mechanics of Mesenteric Arteries
DOCA-salt treatment resulted in a dramatic increase in wall thickness and media-to-lumen ratio in mesenteric resistance arteries (Table 1 and Figure 2). Cross-sectional area was also enhanced in DOCA-salt rats, indicating hypertrophic remodeling. Remodeling was partially prevented by coadministration of rosiglitazone and totally prevented by fenofibrate, as emphasized by the growth index (Table 1). Stiffness of the vessel wall, represented by the stress-versus-strain relation, and the stiffness of wall components (slope of the elastic modulus vs stress curve) were increased in DOCA-salt rats but were unaffected by rosiglitazone or fenofibrate administration (Figure 2).

Vascular Endothelin Content and Extracellular Composition
Real-time PCR analysis (Figure 3B) revealed an increase of prepro-ET-1 mRNA content in the mesenteric vasculature of DOCA-salt rats (5.4-fold vs Uni-Nx, $P<0.01$). Both rosiglitazone and fenofibrate abrogated this increase. In situ hybridization confirmed those results in another vascular territory, as shown in Figure 4, since prepro-ET-1 mRNA was increased within the endothelium and media of the thoracic aorta of DOCA-salt rats and was prevented by coadministration of rosiglitazone or fenofibrate. Sirius red staining of pressurized mesenteric arteries revealed an increased collagen content within the media of DOCA-salt rats (31.5±4.8% vs 20.2±3.6% in Uni-Nx, $P<0.05$). As shown in Figure 5, this increase was not modified by coadministration of either rosiglitazone (33.5±2.5%) or fenofibrate (35.9±1.6%).

<table>
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<tr>
<th>Morphological Characteristics of Mesenteric Resistance Arteries</th>
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<tr>
<td>Uni-Nx</td>
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<tr>
<td>Wall thickness, μm</td>
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<td>Media/lumen ratio, %</td>
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<td>CSA, μm$^2$</td>
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$n=6$ per group. ROSI indicates rosiglitazone; FEN, fenofibrate.

* $P<0.01$ vs Uni-Nx; † $P<0.05$ vs DOCA-salt.
Vascular Reactivity of Mesenteric Arteries

DOCA-salt rats had endothelial dysfunction, as demonstrated by a decreased response to acetylcholine (Figure 6). Rosiglitazone restored the vasodilatory response, whereas fenofibrate only improved the maximum response to acetylcholine. Response to sodium nitroprusside, a nitric oxide donor that triggers endothelium-independent relaxation, was similar in all groups (Figure 6). Response to the α-adrenergic agonist norepinephrine was slightly decreased in DOCA-salt rats. This alteration of the constrictor response was restored by both rosiglitazone and fenofibrate (Figure 6).

O$_2^-$ Production

NADPH-inducible generation of superoxide anion, assessed by the lucigenin assay, was increased 4-fold in the aorta of DOCA-salt rats compared with Uni-Nx rats (1613±463 vs 378±101 10$^3$ cpm/mg tissue, $P<0.01$). Treatment with rosiglitazone or fenofibrate prevented the increased activity of NADPH oxidase (488±116 and 592±101 10$^3$ cpm/mg tissue, respectively; NS vs Uni-Nx).

Discussion

The present study provides in vivo evidence of the beneficial vascular effects of PPAR activators in an ET-1–dependent model of hypertension. Although both PPAR activators prevented the vascular production of ET-1, rosiglitazone and fenofibrate induced different vascular effects, suggesting the involvement of several distinct mechanisms. Indeed, both PPAR activators prevented the vascular remodeling and the generation of superoxide anion within the vascular wall, but only rosiglitazone decreased SBP in DOCA-salt rats. Moreover, rosiglitazone had less effect on vascular growth compared with fenofibrate. Finally, the endothelial dysfunction observed in DOCA-salt rats was totally restored by rosiglitazone but not by fenofibrate.

Our previous studies demonstrated that ET-1 contributes to the development of high blood pressure and vascular growth in DOCA-salt rats.$^8,9$ As suggested by previous in vitro studies, both PPAR-α and -γ are potent inhibitors of ET-1 production by vascular cells.$^{10-12}$ Our study is the first to extend these results in vivo, because coadministration of rosiglitazone or fenofibrate prevented the increase in vascular prepro-ET-1 mRNA in DOCA-salt rats. Despite inhibition of prepro-ET-1 mRNA production, both PPAR activators failed to normalize SBP, although rosiglitazone induced a decrease of 68 mm Hg and fenofibrate, 25 mm Hg (nonsignificant), in DOCA-salt rats. This agrees with the blood pressure–lowering effect of selective endothelin receptor blockers, suggesting that PPAR activators may be antagonizing ET-1 action. Likewise, this is in agreement with the notion that ET-1 participates in the pathophysiology of this hypertensive model, in concert with other mechanisms that are not antagonized by either endothelin receptor blockers or PPAR activators.$^8$
Interestingly, PPAR activators presented distinct vascular effects that could, in part, explain their different impacts on blood pressure. We previously showed that resistance arteries of DOCA-salts rats undergo hypertrophic remodeling (ie, increased media-to-lumen ratio and cross-sectional area), fibrosis, and endothelial dysfunction. Fenofibrate and rosiglitazone prevented this vascular remodeling, although rosiglitazone had less effect, as suggested by the growth index.

However, as mentioned earlier, there is growing evidence that PPARs are involved in vascular remodeling because they participate in various pathophysiological processes such as growth, apoptosis, and inflammation. In addition, in vitro studies suggest that thiazolidinediones could present calcium-blocking properties and thus, may prevent vascular growth through a decrease of calcium-dependent pathways. We recently reported that PPAR activators prevented vascular

![Figure 3.](image-url)
hypertrophy in angiotensin II–infused rats. Thus, the present findings support the hypothesis that PPARs modulate vascular remodeling through interaction with paracrine hormones such as ET-1.

Despite their preventive action on hypertrophic remodeling in DOCA-salt rats, both fenofibrate and rosiglitazone failed to decrease the stiffness of resistance arteries. Interestingly, analysis of the collagen content in the media revealed that PPAR activators did not prevent the collagen deposition induced by DOCA-salt treatment. Taken together, the composition of the extracellular matrix in fenofibrate- and rosiglitazone-treated animals could explain the lack of effect of these agents on arterial stiffness in DOCA-salt rats and hence, their partial effect on blood pressure. It should be noted, however, that the impact of both treatments on vascular remodeling appears to be pressure independent and underscores the trophic importance of local factors such as ET-1.

As mentioned previously, inflammation and oxidative stress in particular have been involved in the remodeling process. In view of this, we measured the NADPH oxidase–dependent production of superoxide anion in the aorta. As in other murine models of hypertension, we found an increased production of superoxide anion in the aorta of DOCA-salt rats. Although both PPAR activators abrogated this increase, we cannot conclude whether this effect was due to normalization of vascular ET-1 mRNA content or a direct effect of PPAR activators on NADPH oxidase activity. Indeed, ET-1 has been shown to activate NADPH oxidase in endothelial cells. On the other hand, PPARs suppress nuclear factor-κB activity, a major mediator of the oxidative stress–signaling pathway.

Another differential effect between fenofibrate and rosiglitazone was their impact on endothelial function. As in previous studies, DOCA-salt rats presented endothelial dysfunction, as revealed by a blunted response to acetylcholine associated with unaltered sodium nitroprusside responses. Only rosiglitazone fully restored the vasodilatory response to acetylcholine. With our data, we cannot conclude whether the lack of effect of fenofibrate on blood pressure might explain the endothelial dysfunction or vice versa. However, the blunting of vascular superoxide anion production by both PPAR activators could have resulted in increased nitric oxide bioavailability and improved relaxation to acetylcholine. This notion is supported by previous work that showed that troglitazone, another PPAR-γ activator, improved flow-induced dilation through inhibition of oxidative stress. It is important to note that vasodilatory response in resistance arteries involves agents other than nitric oxide, such as cyclooxygenase products and endothelium-derived hyperpolarizing factors. The heterogeneity of this response might explain the discrepancy between the effects of fenofibrate and rosiglitazone on endothelial function. Because fenofibrate failed to fully restore endothelial function, this could in part contribute to the lack of effect of the PPAR-α activator on SBP, compared with rosiglitazone.

In conclusion, this study provides evidence that PPAR activators are able to modulate endogenous production of a vasoactive peptide, ET-1, and to prevent vascular remodeling in association with decreased superoxide production in vivo in DOCA-salt rats, a model of endothelin-dependent hypertension.

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References


**Figure 5.** A, Representative photomicrographs show collagen contents in mesenteric sections stained with Sirius red (original magnification, ×200). B, Quantification of collagen content in mesenteric arteries. Results are presented as mean±SEM. n=6 per group. ∗P<0.05 vs Uni-Nx, 1-way ANOVA.

**Figure 6.** Pharmacological study of mesenteric resistance arteries. Upper left: Endothelium-dependent relaxations to acetylcholine. Upper right: Endothelium-independent relaxation to sodium nitroprusside. Bottom: α-Adrenergic-dependent response to norepinephrine. Results are expressed as mean±SEM of percentage of maximal relaxation (top) or maximal contraction to KCl+norepinephrine (bottom). n=6 per group. ∗P<0.05 vs Uni-Nx, ANOVA repeated measures; †P<0.05 vs Uni-Nx, 1-way ANOVA.
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