Increased Expression of Peroxisome Proliferator-Activated Receptor-α and -γ in Blood Vessels of Spontaneously Hypertensive Rats

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Abstract—Peroxisome proliferator-activated receptors (PPARs) are a family of ligand-activated transcription factors that include PPAR-α, PPAR-γ, and PPAR-δ. We hypothesized that PPAR expression in blood vessels could be reduced in hypertension to result in increased vascular growth and reduced apoptosis. We investigated the abundance of PPAR-α and PPAR-γ in aorta and mesenteric arteries from young (6-week-old) and adult (16-week-old) spontaneously hypertensive rats (SHR) compared with age-matched control Wistar-Kyoto rats (WKY). mRNA levels of PPAR-α and PPAR-γ were determined by reverse transcription–polymerase chain reaction. Protein expression was evaluated by Western blot and by immunohistochemistry. PPAR-γ was expressed in aortic and mesenteric vascular smooth muscle cells (VSMCs) from intact tissue and cultured cells. PPAR-α was expressed in intact vascular tissue but was almost undetectable in cultured VSMCs. In mesenteric arteries from adult SHR, PPAR-α and PPAR-γ mRNA levels were significantly greater than in WKY \((P<0.05)\). In aorta, PPAR-α mRNA was significantly \((P<0.05)\) more abundant in adult (but not in young) SHR than in WKY, whereas there was no difference in PPAR-γ mRNA between WKY and SHR. PPAR-α and PPAR-γ mRNA were greater in mesenteric arteries \((P<0.05)\) in young and adult SHR than in WKY. Expression of PPAR-α and PPAR-γ was similar in SHR and WKY in other tissues. In cultured mesenteric VSMCs, PPAR-γ mRNA was 3-fold higher in SHR than in WKY. Immunohistochemistry demonstrated that PPAR-γ resided constitutively in the cytoplasm in primary and low-passaged aortic and mesenteric VSMCs, whereas PPAR-α was almost undetectable. Thus, aorta and mesenteric resistance arteries from SHR in the prehypertensive and the established phase of hypertension exhibit increased expression of both PPAR isoforms, whereas other tissues do not. Changes (increases) in PPAR expression may play a compensatory role in the remodeling of blood vessels in SHR.

Key Words: PPAR ■ aorta ■ arteries ■ muscle, smooth ■ remodeling

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors\(^1\) that heterodimerize with retinoic acid–like receptor and become transcriptionally active by binding to specific DNA sequence elements termed PPAR response elements.\(^2\) To date, 3 different subtypes, PPAR-α, PPAR-β or -δ, and PPAR-γ, have been cloned. PPAR-α is predominantly expressed in tissues exhibiting high catabolic rates of fatty acids, such as liver, heart, kidney, and muscle, whereas PPAR-γ is adipose-tissue selective, where it triggers adipocyte differentiation and lipid storage by regulating the expression of genes critical for adipogenesis.\(^3\) Little is known about the functions of PPAR-δ, although it is the most ubiquitously expressed subtype. PPARs are activated by natural ligands such as fatty acids and eicosanoids.\(^4,5\) Furthermore, the lipid-lowering fibrates and the antidiabetic thiazolidinediones (TZDs) are synthetic ligands for PPAR-α and PPAR-γ, respectively.\(^4,6\)

The roles of PPARs in gene regulation have been studied primarily in liver and adipose tissue, and PPARs have been implicated in metabolic diseases such as obesity, diabetes, and atherosclerosis.\(^7\) However, the expression and function of PPARs in the vascular wall are still unclear. Even though PPARs have recently been shown to be expressed in human aortic vascular smooth muscle cells (VSMCs),\(^8\) in endothelial cells\(^9,10\) and in monocyte-derived macrophages\(^11\) of the vascular wall and have been shown to play a role in vascular pathophysiology, the level of expression of PPARs in VSMCs remains undefined. It is unknown whether PPAR expression may be altered in hypertension and play a pathophysiological role by regulating vascular growth. In spontaneously hypertensive rats (SHR), blood vessels exhibit abnormal structure due to vascular remodeling. Pathophysiological adaptation of the vascular wall in hypertension involves several processes, including VSMC growth,
apoptosis, and migration, as well as disposition of extracellular matrix proteins. We recently observed that docosahexaenoic acid and rosiglitazone (unpublished data, 2000), which are a PPAR-α activator and a PPAR-γ ligand, respectively, induce apoptosis in VSMCs. Furthermore, PPAR-α and PPAR-γ activators, polyunsaturated fatty acids, or fibrates and antidiabetic TZDs have been shown to have antihypertensive effects in different models. We hypothesized that there could be a function of PPARs at the level of the vascular wall, independent of their role in lipoprotein metabolism, that could modulate vascular remodeling in hypertension. We proposed the hypothesis that decreased PPAR expression in the vasculature of SHR could contribute to vascular growth in hypertension. We therefore investigated the abundance of PPAR-α and PPAR-γ in aorta and mesenteric arteries from 6-week-old and adult (16-week-old) SHR and age-matched Wistar Kyoto rats (WKY). Low-passage (1 to 3) cultured aortic and mesenteric VSMCs from SHR and WKY were also studied.

Our data show that both PPAR-α and PPAR-γ are expressed in aortic and mesenteric VSMCs from intact tissue and cultured cells in WKY and that they are expressed at a significantly greater level in SHR, indicating that PPARs may contribute to regulation of different genes in the vasculature in hypertension. Our hypothesis that low vascular PPAR expression in SHR may contribute to vascular remodeling was rejected, and a new hypothesis that PPAR expression is enhanced as a compensatory response to hypertensive vascular growth must be entertained.

Methods

Animal Experiments

The study was approved by the Animal Care Committee of the Clinical Research Institute of Montreal and was performed according to the guidelines of the Canadian Council for Animal Care. Male WKY and SHR at the age of 6 and 16 weeks were purchased from Taconic Farms (Germantown, NY) and group housed with free access to water and food. Systolic blood pressure (SBP) was measured by the tail-cuff method. Rats were killed by decapitation. Thoracic aorta and whole mesenteric bed were dissected from each animal. After fat, adventitia, and endothelium were rapidly removed from aorta and fat was removed from the mesenteric vasculature, tissues were immediately frozen in dry ice and kept at −70°C until studied.

Cell Culture

Aortic and mesenteric VSMCs from WKY and SHR age 16 weeks were isolated and cultured as previously described. Primary cell culture and cells at passages 1 to 3 were studied.

Reverse Transcription–Polymerase Chain Reaction Analysis of PPARs

RNA was extracted from frozen tissues or cultured VSMCs, and reverse transcription–polymerase chain reaction (RT-PCR) was performed as described previously. For amplification of PPAR-α cDNA, the sense primer 5′-ACTGTTTGCAAGATCTACAGACGTAAGAGATCTACAGAG-3′ and the antisense primer 5′-TGTTGCTGTA-3′. After amplification, PCR products were electrophoresed, and bands corresponding to RT-PCR products from PPAR-α, PPAR-γ, and GAPDH mRNA were visualized by ultraviolet light.

Western Blot Analysis of PPARs

Protein was extracted as previously described from frozen tissue and cells. Equal amounts of protein were separated by electrophoresis and transferred onto a PVDF membrane. Membranes were incubated with specific antibodies to PPAR-α and PPAR-γ (Santa Cruz Biotechnologies) at dilutions of 1:200 and 1:2000, respectively. Signals were visualized by autoradiography.

Immunohistochemical Assay of PPARs

Primary and low-passaged (1 to 3) cells were cultured on chamber slides. At subconfluence, cells were fixed, dried, and kept at −20°C until use. Cells were washed and blocked for 30 minutes with blocking solution containing 10% donkey serum (for PPAR-α) or goat serum (for PPAR-γ). After incubation with specific antibodies for PPAR-α and PPAR-γ (Santa Cruz Biotechnologies) at a dilution of 1:50 in specific blocking solution at 37°C for 1 hour, slides were washed and incubated with the secondary antibodies (FITC-conjugated anti-rabbit for PPAR-γ and anti-goat for PPAR-α) at a dilution of 1:2000 for 1 hour at room temperature. The slides were washed, mounted in 90% glycerol in PBS, and visualized with a Leitz DMR fluorescence microscope.

Data Analysis

Values are presented as mean±SEM of at least 3 rats or 3 independent experiments. Results were compared by Student’s t test or ANOVA, the latter followed by a Tukey-Kramer post hoc test. Values of P<0.05 were considered significant.

Results

Body Weight and SBP

Body weight of SHR was significantly lower than that of WKY, both in young (104±6 g, P<0.01) and in adult (320±1 versus 473±13 g, P<0.01) rats. SBP was measured by the tail-cuff method.

![Figure 1](http://hyper.ahajournals.org/doi/fig/1)

**Figure 1.** A, mRNA levels of PPAR-α in different tissues of adult WKY and SHR by RT-PCR. Upper panel, Representative photograph shows bands corresponding to RT-PCR amplification of mRNA of PPAR-α and GAPDH. Lower panel, Bar graph shows PPAR-α/GAPDH ratio. Error bars indicate SEM, n=3. *P<0.05 versus control (WKY), tP<0.05 versus liver (WKY), tP<0.05 versus liver (SHR). B, Representative Western blot of PPAR-α in different tissues of WKY and SHR. A indicates aorta; MA, mesenteric arteries; H, heart; Mu, muscle; K, kidney; L, liver; WF, white fat; and BF, brown fat.
slightly higher in young SHR than in WKY (131 ± 3 versus 113 ± 2 mm Hg, \( P < 0.05 \)). SBP was significantly greater in adult SHR than in WKY (187.5 ± 4.7 versus 119 ± 5 mm Hg, \( P < 0.01 \)).

Expression of PPAR-\( \alpha \) and PPAR-\( \gamma \) in Different Tissues of Adult WKY and SHR

Semiquantitative RT-PCR analysis demonstrated the presence of both PPAR-\( \alpha \) and PPAR-\( \gamma \) mRNA in many tissues of rat, including aorta, mesenteric arteries, heart, skeletal muscle, kidney, liver, white fat, and brown fat (Figures 1A and 2A). A similar pattern of PPAR-\( \alpha \) and PPAR-\( \gamma \) protein expression in different tissues was detected by Western blot analysis, as shown in Figures 1B and 2B, except in white fat, where protein could not be extracted. PPAR-\( \alpha \) was highly expressed in liver both in WKY and SHR, whereas PPAR-\( \gamma \) was expressed to a greater degree in adipose tissue (white and brown fat).

Compared with other tissues, mRNA and protein levels of PPAR-\( \alpha \) in aorta were almost undetectable in adult WKY. However, mRNA and protein levels of PPAR-\( \alpha \) in mesenteric arteries were expressed at similar levels as in the heart, skeletal muscle, kidney, and white adipose tissue in WKY. Aorta and mesenteric arteries showed a significantly higher mRNA level of PPAR-\( \alpha \) in adult SHR than in WKY (Figure 1A).

In WKY, mRNA and protein levels of PPAR-\( \gamma \) in aorta and mesenteric arteries were comparable to levels in the heart and skeletal muscle, whereas PPAR-\( \gamma \) mRNA and protein abundance in liver, white fat, and brown fat were significantly greater. mRNA and protein levels of PPAR-\( \gamma \) in mesenteric arteries and adipose tissue (white and brown fat) were significantly higher in SHR than in WKY (Figure 2A), whereas there were no significant differences between WKY and SHR in aorta, heart, muscle, kidney, and liver.

mRNA Levels of Vascular PPAR-\( \alpha \) and PPAR-\( \gamma \) in Young Versus Adult WKY and SHR

In aorta of young SHR, mRNA levels of PPAR-\( \alpha \) and PPAR-\( \gamma \) were similar to those in WKY (Figure 3A), in contrast to adult SHR, in which mRNA levels of PPAR-\( \alpha \) in aorta were increased 3-fold \( (P < 0.05) \) and PPAR-\( \gamma \) 1.2-fold compared with age-matched WKY levels (Figure 3A). In young SHR, as in adult SHR, mRNA levels of PPAR-\( \alpha \) and PPAR-\( \gamma \) in mesenteric arteries were significantly greater than in young WKY (Figure 3B).

Expression of PPAR-\( \alpha \) and PPAR-\( \gamma \) in Cultured Cells

Aortic and mesenteric low-passage–cultured VSMCs expressed abundant PPAR-\( \gamma \) mRNA and protein (Figures 4A

Figure 2. A, mRNA levels of PPAR-\( \gamma \) in different tissues of WKY and SHR by RT-PCR. Upper panel, Representative photograph shows bands corresponding to RT-PCR amplification of mRNA of PPAR-\( \gamma \) and GAPDH. Lower panel, Bar graph shows PPAR-\( \gamma \)/GAPDH ratio. Error bars indicate SEM. \( n=3 \). * \( P < 0.05 \) vs control (WKY), † \( P < 0.05 \) vs liver (WKY), ‡ \( P < 0.05 \) vs liver (SHR). B, Representative Western blot of PPAR-\( \gamma \) in different tissues of WKY and SHR. Abbreviations as in Figure 1.

Figure 3. A, mRNA levels of PPAR-\( \alpha \) and PPAR-\( \gamma \) in aorta of young (6-week-old) and adult (16-week-old) WKY and SHR, by RT-PCR. Upper panel, Representative photograph shows bands corresponding to RT-PCR amplification of mRNA of PPAR-\( \alpha \), PPAR-\( \gamma \), and GAPDH. Lower panel, Bar graph shows ratio \(( \times 10) \) of PPAR-\( \alpha \)/GAPDH and PPAR-\( \gamma \)/GAPDH. Error bars indicate SEM. \( n=3 \). * \( P < 0.05 \) vs controls. B, mRNA levels of PPAR-\( \alpha \) and PPAR-\( \gamma \) in mesenteric arteries of young (6-week-old) and adult (16-week-old) WKY and SHR, by RT-PCR. Upper panel, Representative photograph shows bands corresponding to RT-PCR amplification of mRNA of PPAR-\( \alpha \), PPAR-\( \gamma \), and GAPDH. Lower panel, Bar graph shows ratio \(( \times 10) \) of PPAR-\( \alpha \)/GAPDH and PPAR-\( \gamma \)/GAPDH. Error bars indicate SEM. \( n=3 \). * \( P < 0.05 \) vs controls.
and B). In mesenteric VSMCs, PPAR-α and PPAR-γ mRNA levels were 2-fold and 3-fold higher respectively in SHR than in WKY (P<0.05). In aortic VSMCs, the mRNA level of PPAR-α was significantly higher (P<0.05) in SHR than in WKY. However, the mRNA level of PPAR-γ in SHR was only slightly higher than that in WKY. Figure 5 demonstrates with immunohistochemistry that PPAR-γ was expressed in the cytoplasm in cells in primary culture, as well as after low passages of both aortic and mesenteric VSMCs. A faint band of PPAR-α mRNA could be detected with RT-PCR in both aortic and mesenteric VSMCs, but expression of PPAR-α was undetectable by Western blot analysis (Figure 4B).

**Discussion**

The present study demonstrates that both PPAR-α and PPAR-γ are expressed in various rat tissues, including blood vessels, heart, muscle, kidney, liver, and adipose tissue. There is a differential expression of PPAR-α and PPAR-γ during development in SHR, a genetic model of hypertension, compared with control WKY rats. We demonstrate for the first time that in aorta from young, prehypertensive SHR, PPAR-α and PPAR-γ levels are similar, whereas in mesenteric arteries from young SHR, PPAR-α and PPAR-γ levels were greater than in age-matched WKY. In established hypertension in adult SHR, however, PPAR-α and PPAR-γ levels in aorta and mesenteric arteries were greater than in age-matched WKY. Cell culture confirmed the expression of PPARs, particularly PPAR-γ, in VSMCs.

Expression of PPARs was primarily thought to be limited to tissues such as liver and fat, in which they participate in the regulation of lipid metabolism.18 Recently, it was suggested that PPAR activators not only regulate plasma cholesterol and triacylglycerol concentrations but also exert effects on the vascular wall, most likely to inhibit VSMC growth and VSMC migration.19–22 Treatment with PPAR-γ activators inhibited VSMC proliferation and migration19 and suppressed neointima formation in rat aorta after endothelial injury.20 The TZD PPAR-γ agonists troglitazone and rosiglitazone abrogated basic fibroblast growth factor–induced DNA synthesis and platelet-derived growth factor–directed migration of rat and human VSMCs.19 In addition, troglitazone downregulated c-fos expression.21 PPAR-mediated changes in VSMCs could contribute to vascular changes found in cardiovascular disease. Activation of PPAR-α and PPAR-γ induced apoptosis in different types of cells.11,23,24 In addition, we recently found (unpublished data, 2000) that both a
PPAR-α agonist (docosahexaenoic acid) and a PPAR-γ activator (rosiglitazone) induced VSMC apoptosis. The molecular mechanisms of apoptosis induction and growth inhibition by PPAR-α and PPAR-γ have yet to be clarified.

The identification of PPAR-γ in nonadipose tissues has suggested novel functions for this receptor, distinct from its well-characterized metabolic regulatory activity. PPAR-γ mRNA and protein were identified in rat aortic VSMCs, as well as in human endothelial cells and VSMCs. In agreement with those studies, we found that PPAR-γ expression was easily detectable in aorta and mesenteric arteries, both in intact tissue and in low-passage–cultured cells. Rat aorta and mesenteric artery–cultured VSMCs expressed PPAR-γ protein, consistent with their pattern of mRNA levels detected in intact tissue. PPAR-γ expression was substantially higher in VSMCs from SHR than in VSMCs from WKY. Levels of PPAR-γ protein in rat aorta appeared to be substantial, similar to those of adipose tissue.

PPAR-α expression has been demonstrated in human VSMCs. Our results demonstrate for the first time that PPAR-α can be detected in rat blood vessels. However, Western blot analysis or a highly specific immunofluorescence assay did not yield a detectable signal for PPAR-α, although mRNA was detectable by RT-PCR, which suggests that cells in culture have very low levels of expression of PPAR-α. Even though absence of expression of PPAR-γ was reported in human aortic smooth muscle cells, in the present study, PPAR-γ could be demonstrated clearly in aortic and mesenteric VSMCs from both WKY and SHR by RT-PCR, Western blot analysis, and immunohistochemistry. Thus, the level of expression of PPAR isoforms in the vascular wall appears to be species and cell-type dependent.

The present study also provides new insights concerning the in vivo expression of PPAR-α and PPAR-γ in genetic hypertension. Blood vessels express higher levels of both PPAR-α and PPAR-γ in SHR than in WKY. We demonstrate that PPAR-γ expression increases with age during development of hypertension. In young, prehypertensive SHR, PPAR-α and PPAR-γ levels were similar in aorta and greater in mesenteric arteries than in age-matched WKY. In adult SHR, however, in which hypertension is established, both PPAR-α and PPAR-γ levels in aorta and mesenteric arteries were greater in SHR than in age-matched WKY. The expression of vascular PPARs may have a function in vascular remodeling in hypertension, as well as in development of atherosclerosis. PPAR activation may modulate macrophage foam cell formation and apoptosis, as well as the inflammatory response of smooth muscle cells and macrophages. Our results suggest new directions for the investigation of potential roles of PPARs in blood vessels, where they may play an important role in vascular remodeling in hypertension and other cardiovascular diseases.

In summary, PPARs are modulated in SHR differentially with age and development of high blood pressure. Both PPAR-α and PPAR-γ are upregulated in mesenteric arteries of SHR before hypertension is established. Both isoforms are upregulated in both aorta and mesenteric arteries from adult SHR. In conclusion, the present study allows us to reject the hypothesis that PPAR-α or PPAR-γ expression is downregulated in VSMCs of SHR. PPAR-γ is expressed abundantly in blood vessels, particularly in resistance arteries, where it may play an important role in vascular changes during development of hypertension. More studies, both in vitro and in vivo, are needed to investigate the action of PPAR activators on vascular remodeling and the possible underlying mechanisms whereby PPARs may influence the development of hypertension.

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