Peroxisome proliferator-activated receptor gamma (PPARγ, NR1C3) is a ligand-activated transcription factor belonging to the retinoic acid receptor and thyroid hormone receptor family of nuclear receptors. Classically, these transcription factors regulate expression of target genes by binding to PPAR response elements (PPRE) in the 5’ flanking region of target genes as a heterodimer with retinoid X receptors (RXR). PPARγ first gained prominence when it was discovered that it was expressed specifically in adipocytes and its expression induced their differentiation. It has now become clear that although expression of PPARγ may be the highest in adipose tissue, it is expressed in many, if not all, cell types and tissues. Among the many roles ascribed to PPARγ in adipose tissue, it is expressed in many, if not all, cell types and tissues. The significance of PPARγ expression in non-adipose tissues is now being increasingly recognized. Other tissue-specific functions for PPARγ have been proposed, and recent experiments suggest that PPARγ may have a role in the development of atherosclerosis and may be involved in the regulation of arterial pressure.

Studies of PPARγ Activation: Blood Pressure-Lowering Effects of TZD in Humans

This section briefly describes a subset of clinical studies in which the blood pressure effects of TZD were assessed. Early studies focused on the TZD troglitazone, which was ultimately discontinued from clinical use because of hepatotoxicity. In a 12-week study of 18 nondiabetic obese patients using twice-daily troglitazone, Nolan et al reported a 4- to 5-mm Hg decrease in systolic blood pressure and DBP along with an increase in insulin sensitivity. A decrease in DBP was observed in 154 type 2 diabetes mellitus patients, and a 6- to 8-mm Hg decrease in DBP and systolic blood pressure was observed in 22 type 2 diabetes mellitus patients receiving troglitazone. Troglitazone was also reported to blunt the increase in arterial pressure caused by mental stress. More recent experiments used 2 other clinically approved TZD, pioglitazone and rosiglitazone. Fullert et al reported a 6-mm Hg greater reduction in DBP over placebo in the first placebo-controlled, double-blinded study designed to test the efficacy of pioglitazone in hypertensive patients without diabetes. In total, 48 patients were examined (23 treated with pioglitazone). The decrease in arterial pressure occurred in conjunction with an improvement in insulin sensitivity, and decreases in insulin resistance (measured by HOMA), fasting insulin, and glucose. A similar decrease in DBP and systolic blood pressure was found in other studies of diabetic patients treated with pioglitazone or rosiglitazone. Rosiglitazone-induced improvements in arterial elasticity were reported in one study and increased endothelial-dependent...
vasodilation was reported in another. In perhaps the first adequately powered study, a large clinical trial of 5238 type 2 diabetes mellitus patients called PROActive (PROspective pioglitAzone Clinical Trial In macroVascular Events), in which blood pressure was not the primary end point, pioglitazone lowered systolic blood pressure by 3 mm Hg. Although the blood pressure-lowering effect of TZD is generally modest, even small decreases in arterial pressure can lower the risk of stroke and ischemic heart disease.

**Blood Pressure and Vascular Effects of TZD in Animal Models**

Complementing the studies summarized is a growing body of data reporting vascular protective effects of TZD administration in animal models. Early studies using the Zucker fatty rat as a model of the metabolic syndrome reported that administration of rosiglitazone prevented the increase in arterial pressure over time compared to lean controls. Rosiglitazone also partially corrected endothelial dysfunction in the mesenteric arteries of these rats. Similarly, pioglitazone lowered arterial pressure in fructose-fed rats and blunted the contractile responses to a number of agonists. In the DOCA-salt model of hypertension in Sprague-Dawley rats, rosiglitazone blunted the increase in arterial pressure, prevented endothelial dysfunction, and prevented the increase in endothelin expression that accompanies hypertension in the model. Both rosiglitazone and pioglitazone significantly attenuated the pressor response and the structural changes in resistance arteries associated with angiotensin (Ang) II treatment. Rosiglitazone also lowered blood pressure and improved endothelial function in an established model of hypertension in transgenic mice overexpressing the human renin and angiotensinogen genes. Notably, the latter 2 reports are consistent with the observation that PPARγ activation lowers expression of the Ang AT1 receptor, although it is also possible that these effects are manifest by antagonism of Ang-induced growth and inflammation. In vascular smooth muscle cells (VSMC), PPARγ activation decreased Ang-induced DNA synthesis, blunted the activity of ERK1/2 and Akt by Ang, and blunted the activation of other growth promoting intracellular signaling molecules. There is also evidence supporting a mutual inhibitory circuit by which PPARγ–Ang may control cell growth and inflammation. In this regulatory circuit, not only does PPARγ antagonize the actions of Ang but also Ang can exhibit reciprocal antagonism of PPARγ through Ang-stimulated Bcr kinase-mediated phosphorylation of PPARγ.

An unexpected physiological interaction between PPARγ and the renin-angiotensin system comes from the novel finding that telmisartan, an Ang receptor blocker, and other Ang receptor blockers may act as partial PPARγ agonists. Interestingly, meta-analysis suggests that telmisartan may reduce the risk for new-onset diabetes when compared with placebo, although it is unclear if this occurs through direct effects on PPARγ. Consequently, the interaction between PPARγ and the renin-angiotensin system occurs at multiple levels, some of which may be relevant to the treatment of metabolic disturbances associated with hypertension and the metabolic syndrome.

**PPARγ as a Critical Regulator of Blood Pressure and Vascular Function: Genetic Evidence**

Perhaps the most compelling single piece of evidence implicating a functional role for PPARγ as a regulator of arterial pressure and as a candidate in hypertension comes from a genetic study by O’Rahilly’s laboratory. Barroso et al reported an analysis of 85 unrelated subjects with insulin resistance in which mutations (P467L, V290M) in the ligand binding domain of PPARγ were identified in 2 subjects. Positions 290 and 467, while physically separated in the primary sequence, lie near helix 12 in the 3-dimensional structure. Helix 12 is important in the association of PPARγ with both ligand and transcriptional coactivators. Both probands in the study were female and exhibited severe hypertension in addition to a history, in 1 patient, of gestational diabetes and preeclampsia. Both patients and one affected offspring required antihypertensive therapy. Characterization of the mutations revealed them to act dominant-negatively, that is, they not only exhibited impaired transactivation themselves but also had the capacity to inhibit the activity of wild-type PPARγ. Other dominant-negative mutations in the DNA-binding domain of PPARγ that caused insulin resistance, partial lipodystrophy, hepatic steatosis, marked dyslipidemia, and, in some patients, early-onset hypertension were identified in a separate study. Like the mutations that cause some Mendelian forms of inherited hypertension, these PPARγ mutations are rare. Therefore, these results suggest that PPARγ either participates directly in or is part of a biological pathway regulating arterial pressure. Interestingly, the most common mutation in PPARγ (P12A) has been reported to be associated with blood pressure in type 2 diabetes mellitus subjects, obese type 2 diabetes mellitus subjects, and in subjects with hypertension, although this conclusion remains controversial and is disputed by other studies.

In an effort to study in detail the effect these dominant-negative mutations in PPARγ have on metabolism and cardiovascular function, Tsai et al and Gray et al modeled the effect of the P467L PPARγ mutation by generating knockin mice replacing 1 normal PPARγ allele with a P465L (the mouse equivalent of P467L) allele. Gray et al reported decreased thermogenic capacity without inducing insulin resistance in mice on an otherwise normal genetic background. However, in a leptin-deficient (ob/ob) genetic background, the P465L PPARγ mutation by generating knockin mice replacing 1 normal PPARγ allele with a P465L (the mouse equivalent of P467L) allele. Gray et al reported decreased thermogenic capacity without inducing insulin resistance in mice on an otherwise normal genetic background. However, in a leptin-deficient (ob/ob) genetic background, the P465L mutation exaggerated the insulin resistance and metabolic disturbances caused by deficiency of leptin. In their study, P465L knockin mice exhibited normal blood pressure and a similar pressor response to Ang but exhibited accelerated cardiac fibrosis and increased expression of profibrotic genes compared with wild-type mice also receiving Ang. These data suggest that Ang-induced hypertension and simultaneous PPARγ interference (or deficiency) may have synergistic detrimental effects causing end-organ damage. Tsai et al reported that interference with PPARγ signaling throughout the whole body resulted in hypertension at baseline. We confirmed a 10-mm Hg increase in arterial pressure in this line of P465L knockin males but not female mice. Hypertension was also reported in heterozygous knockin mice carrying the L466A dominant-negative muta-
tion in PPARγ. In aggregate, these data strongly support a role for PPARγ in the regulation of arterial pressure. Presumably, the beneficial effects are mediated by changes in gene expression induced by PPARγ ligands and, alternatively, opposite changes when transcriptional regulation of PPARγ targets is interfered with.

In addition to increased blood pressure, we also demonstrated that global (nontissue-specific) interference with PPARγ signaling resulted in impaired endothelial-dependent vasodilation in the cerebral circulation in vitro and in vivo. Cerebral vessels exhibited increased hydroethidine staining, and the impairment in vasodilation was restored to normal in response to a scavenger of superoxide. These results implicate increased superoxide production and oxidative stress in response to PPARγ interference. This is consistent with the antioxidant actions of PPARγ. Interestingly, Meredith et al. using the same P465L mice, reported increased proliferation and migration of smooth muscle cells in culture and a marked increase in the vessel wall. In a follow-up study by the same group, loss of Tel2-cre knockout of PPARγ null mice. These "EC-PPARγ-null" mice had normal blood pressure at baseline but exhibited high-fat diet-induced hypertension. This provided important evidence suggesting a protective effect of endothelium. Our data using genetic interference are largely consistent with studies of genetic deficiency in which vascular function was normal in the aorta and basilar (a cerebral) artery under baseline conditions. However, after feeding a high-fat diet for 12 weeks, endothelial responses to acetylcholine and A23187 became impaired in mice expressing the V290M or P467L dominant-negative mutants of PPARγ. There was no change in endothelial function in nontransgenic littermate controls in response to high-fat, and there was no change in mice expressing wild-type PPARγ. The impairment in basilar artery function was reversed in high-fat diet fed mice after administration of tempol. This along with increased expression of Nox genes and decreased expression of catalase and SOD1 implicate oxidative stress. We also demonstrated that mice expressing endothelial-specific dominant-negative PPARγ exhibited an exaggerated pressor response to Ang. These data imply that endothelial
PPARγ plays a protective role under conditions of stress. What remains unclear is whether the dysfunction is caused by local factors or systemic factors in response to PPARγ interference during high-fat diet.

To be fair, there may be weaknesses associated with the use of dominant-negative PPARγ attributable to cross-interference with other PPAR family members. Diminishing this concern is microarray data from our laboratory showing that 71 probes sets representing 37 experimentally validated PPARγ target genes exhibit the expected pattern of expression in the aorta in response to the P465L dominant-negative mutant (ie, an increase in expression in response to rosiglitazone but decreased expression in response to P465L PPARγ). These data are available at the Gene Expression Omnibus at the NCBI (GEO array platform, GPL1261; series accession, GSE8949).

In aggregate, therefore, most studies of endothelial-specific PPARγ deficiency and interference strongly suggest that PPARγ plays a protective role in the endothelium. This may be necessary under conditions of stress such as in response to high-fat diet when endogenous or exogenous PPARγ ligands activate PPARγ to induce an antioxidant, anti-inflammatory, and prometabolic state. This protection may be blunted or ablated when PPARγ function is impaired, resulting in vascular impairment under conditions when PPARγ would be normally activated.

Role of PPARγ in Vascular Muscle
Like the endothelium, PPARγ is expressed in VSMC. Treatment of VSMC in culture with TZD inhibits proliferation and migration through a mechanism involving inhibition of cyclin-dependent kinases and halting progression through the cell cycle. PPARγ expression is increased in neointimal VSMC after injury, and the antiproliferative effect of PPAR may account for suppression of neointimal growth of VSMC after injury. That PPARγ is required to mediate insulin signaling in VSMC suggests it may also play a role in cellular metabolism in these cells. PPARγ activators also inhibit expression of the Ang AT1 receptor in VSMC.

Using a similar methodology as for the endothelium, we examined the importance of PPARγ signaling in VSMC in vivo by targeting expression of wild-type and dominant-negative PPARγ using the smooth muscle-specific promoter smooth muscle myosin heavy chain. We demonstrated that mice expressing the P467L or V290M dominant-negative mutations in vascular muscle (S-P467L or S-V290M) exhibited severely impaired vasodilation to acetylcholine and cGMP. The mice also exhibited increased arterial pressure. Unlike other forms of hypertension in which vasodilation is normal in response to NO donors, the response of the aorta of S-P467L mice to nitroprusside was also markedly impaired. This initially suggested the possibility that the loss of PPARγ might render the smooth muscle resistant to the effects of NO. However, recent unpublished results from our laboratory suggest that endogenous NO acts to buffer vasoconstrictor effects. S-P467L mice also exhibit a markedly augmented vasoconstrictor response to endothelin-1, mediated by ET-A receptors, which is dependent on Rho kinase. Current studies in the laboratory are testing the hypothesis that Rho kinase activity is elevated, and this elevation interferes with the dilatory actions of NO. Other studies are investigating the importance of PPARγ in resistance vessels. There were no abnormalities in mice expressing wild-type PPARγ selectively in VSMC, once again proving the effects are mediated by the dominant-negative actions of PPARγ.

As for PPARγ in the endothelium, a number of investigators have generated smooth muscle cell-specific knockouts of PPARγ using the Cre-loxP system. Wang et al reported moderately increased arterial pressure during the light cycle but normal pressure during the dark cycle. As noted, the circadian variation in blood pressure was blunted in the smooth muscle cell-PPARγ-null mice, but not to the extent as reported in EC-PPARγ-null mice. Loss of VSMC PPARγ had no effect on the blood pressure-lowering effects of TZD in DOCA-salt hypertension. Despite an increase in arterial pressure during the day, S-null mice exhibited improved responses to endothelial-dependent vasodilation. It is noteworthy, however, that the control strains (PPARγ-lox/lox Tie-2-Cre/minus vs PPARγ-lox/lox SM22-cra/minus) for the EC-PPARγ-null and smooth muscle cell-PPARγ-null experiments, respectively, exhibited substantially different responses to acetylcholine, a finding that went unexplained.

When Does a Null Not Act Like One?
The opposite results of blood pressure caused by smooth muscle cell-specific dominant-negative PPARγ and PPARγ-deficiency are at first surprising. But are these results really contradictory? Is there a molecular explanation that reconciles these results? The answer to this question requires consideration of the mechanism of PPARγ action. Unlike steroid hormone receptors, such as glucocorticoid receptor, that reside in the cytoplasm waiting for ligand to induce nuclear translocation, PPARγ resides in the nucleus even in the absence of ligand. As illustrated in Figure 1A, PPARγ forms a heterodimer with RXR and binds to a PPAR response element (PPRE) in chromatin in the regulatory region of a PPARγ target gene even in the unliganded state. The unliganded PPARγ-RXR-PPRE complex recruits corepressors and histone deacetylases, which prevent transcription. This is not a static process, because the complex cycles through DNA bound and unbound states. Consequently, the rate of transcription under these circumstances reflects the occu-
pancy of the site by the PPARγ-RXR-corepressor complex and the binding of other stimulatory transcription factors and RNA polymerase to other sequences in the regulatory region of the gene. The end result of this is the basal or steady-state level of transcription we would measure by quantitative reverse-transcription polymerase chain reaction or Northern blot. Figure 1B illustrates that ligand binding, either endogenous or exogenous (ie, TZD), causes dismissal of the corepressors and a recruitment of coactivators. The coactivator complex facilitates a conformation of chromatin favorable for transcription. This leads to a marked increase in the rate of transcription and an increase in steady-state levels of the mRNA of the target genes. Microarray analysis of aortic RNA comparing C57BL/6 mice fed a diet lacking or containing rosiglitazone resulted in the identification of many genes induced 2- to 8-fold.67 These include classic PPARγ targets such as fatty acid-binding protein 4 (FABP4, also known as aP2) and novel PPARγ targets. However, the presence of a dominant-negative mutant of PPARγ results in a competition with wild-type PPARγ for access to RXR and the PPRE. The balance of this competition will be determined by the relative expression of wild-type and mutant PPARγ. As illustrated in Figure 1C, replacement of wild-type PPARγ with P467L PPARγ causes increased occupancy of the PPRE by the PPARγ-RXR-corepressor complex. Li and Leff68 demonstrated that the recycling rate of dominant-negative PPARγ on and off chromatin is reduced thus increasing its occupancy. This further reduces baseline transcription of actively repressed PPARγ target genes. Our recent data suggest that genes in aorta whose transcription is induced by TZD are repressed by dominant-negative PPARγ. Moreover, the complexes containing dominant-negative PPARγ are unresponsive to ligand unless it is present in saturating concentrations.30,69 This scenario likely governs the largest class of PPARγ targets induced in response to PPARγ ligand. If this hypothesis is true and accurate, then one has to consider the possibility that a null mutation in PPARγ might provide an intermediate phenotype between PPARγ-mediated repression and ligand-mediated activation. This intermediate effect is illustrated in Figure 1D and was first suggested by Duan et al70 to explain hypotension in PPARγ-deficient mice rescued from lethality. In this model, PPARγ deficiency results in a loss of active repression, that is, an inability to recruit corepressors. Even though PPARγ deficiency also ablates ligand-mediated activation, the resultant transcriptional activity is expected to be intermediate
between the fully repressed and fully activated state. This hypothesis could potentially explain the increase in β2-

adrenergic receptor expression noted in smooth muscle cell-

PPAR γ-null mice.65 New studies comparing the gene expression

profile of aorta from smooth muscle cell-PPAR γ-null with aorta from TZD-treated mice and with mice carrying the

P465L mutation will test the hypothesis directly.

What is the state of transcription complexes bound to the

PPRE in a state of PPAR γ deficiency? To answer this, one

can take a clue from whole-genome transcription factor-

binding studies.71,72 In 3T3-L1 preadipocytes, an association

of RXR with the PPRE, occurs in chromatin before PPAR γ is

expressed. It is unclear, however, if these are RXR ho-

modimers (as diagrammed in Figure 1D), RXR monomers, or

RXR heterodimerized with other members of the ligand-acti-

modimers, or even RXR in a state of repression.71 This suggests that RXR, other

PPAR (PPARα or PPAR δ), or other members of the NR1 family

may bind to the PPRE in the absence of PPAR γ.

The predicted increase in target gene expression in PPAR γ deficiency therefore may provide a level of protection similar to that induced by TZD. This explanation, if true, would reconcile published results and continue to support a hypothesis of vascular protection by PPAR γ. This model would also imply that the genes effecting blood pressure are ones actively repressed by PPAR γ in the absence of ligand and induced in their presence. The model is also a cautionary tale that genetic deficiency caused by genetic knockout can sometimes result in an apparent gain of function.

Perspectives

In total, the data obtained from TZD treatment of humans and animals, along with genetic studies interfering or ablating PPAR γ in the vasculature, are consistent with a cardioprotective and vasculoprotective role. The data are consistent with a model of vascular protection in which induction of PPAR γ by free fatty acids or TZD induces an anti-inflammation, antioxidant, and prometabolic state (Figure 2B). This facilitates normal NO release by the endothelium and NO-cGMP action by the vascular muscle to promote vasodilation, all the while antagonizing the contractile state induced by agonists such as ET-1, which act through the Rho kinase pathway. Antagonizing PPAR γ renders the cells resistant to endogenous and exogenous PPAR γ ligands and promotes a pro-oxidant and proinflammatory environment that, along with abnormalities in cellular metabolism, impairs NO-mediated vasodilation and promotes ET-1 contractile activity by inducing Rho kinase among other signaling pathways (Figure 2B). Some of these effects may be more pronounced under conditions such as a high-fat diet. Because PPAR γ is a transcription factor, these changes should occur by reprogramming the gene expression profile of the endo-

thelial and VSMC. Consequently, understanding the spectrum of gene and pathways induced (and repressed) by PPAR γ becomes of paramount importance.

Acknowledgments

The author is indebted to the outstanding graduate students, post-
doctoral fellows, research assistants, and research scientists who

have worked on this project over the years. The author also

acknowledges the collaboration of Dr Frank M. Faraci and Dr

Thomas Casavant for their insights and friendship during the tenure

of this project.

Sources of Funding

This work was supported by National Institutes of Health grants

HL048058, HL061446, HL084207, NS024621, and HL062984, the

Roy J. Carver Trust, and The Novartis Award in Hypertension

Research.

Disclosures

None.
Vascular PPARγ in Blood Pressure Regulation

References


Endothelial and Vascular Muscle PPARγ in Arterial Pressure Regulation: Lessons From Genetic Interference and Deficiency

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*Hypertension*. 2010;55:437-444; originally published online December 28, 2009;
doi: 10.1161/HYPERTENSIONAHA.109.144170

*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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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