Role of Peroxisome Proliferator–Activated Receptor-γ in Vascular Muscle in the Cerebral Circulation

T. Michael De Silva, Mary L. Modrick, Pimonrat Ketsawatsomkron, Cynthia Lynch, Yi Chu, Christopher J. Pelham, Curt D. Sigmund, Frank M. Faraci

Abstract—Although peroxisome proliferator–activated receptor-γ (PPARγ) is thought to play a protective role in the vasculature, its cell-specific effect, particularly in resistance vessels, is poorly defined. Nitric oxide (NO) plays a major role in vascular biology in the brain. We examined the hypothesis that selective interference with PPARγ in vascular muscle would impair NO-dependent responses and augment vasoconstrictor responses in the cerebral circulation. We studied mice expressing a dominant negative mutation in human PPARγ (P467L) under the control of the smooth muscle myosin heavy chain promoter (S-P467L). In S-P467L mice, dilator responses to exogenously applied or endogenously produced NO were greatly impaired in cerebral arteries in vitro and in small cerebral arterioles in vivo. Select NO-independent responses, including vasodilation to low concentrations of potassium, were also impaired in S-P467L mice. In contrast, increased expression of wild-type PPARγ in smooth muscle had little effect on vasmotor responses. Mechanisms underlying impairment of both NO-dependent and NO-independent vasodilator responses after interference with PPARγ involved Rho kinase with no apparent contribution by oxidative stress–related mechanisms. These findings support the concept that via effects on Rho kinase–dependent signaling, PPARγ in vascular muscle is a major determinant of vascular tone in resistance vessels and, in particular, NO-mediated signaling in cerebral arteries and brain microvessels. Considering the importance of NO and Rho kinase, these findings have implications for regulation of cerebral blood flow and the pathogenesis of large and small vessel disease in brain. (Hypertension. 2014;64:1088–1093.)

Online Data Supplement

Key Words: cerebral arteries ▪ microcirculation ▪ nitric oxide ▪ small vessel disease

The nuclear receptor peroxisome proliferator–activated receptor-γ (PPARγ) is widely expressed, including in vascular cells. Through several mechanisms, PPARγ is thought to exert protective effects on the vasculature in models of disease and in patients with diabetes mellitus and atherosclerosis.1–4 Concepts on the biological effect of PPARγ are based predominately on studies using high-affinity agonists [thiazolidinediones (TZDs)] for the ligand-binding domain of the molecule. Although this approach has value experimentally, TZDs also exhibit off-target effects, lack cell specificity, and do not provide insight into the effect of PPARγ when activated by endogenous ligands.1,5

Genetic approaches offer an alternative to the use of TZDs to define the importance of PPARγ. Mutations in PPARγ have been described, some of which alter the transcriptional activity of wild-type PPARγ.6 Patients with dominant negative mutations in the molecule (eg, P467L) exhibit early-onset hypertension,7,8 consistent with possible vascular effects. The use of transgenic mice expressing these same dominant negative molecules9,10 provides a novel approach to interfere with wild-type PPARγ, allowing insight into the importance of PPARγ without TZD treatment. Importantly, these models mimic reductions in expression or activity of PPARγ described in disease and in the presence of select genetic variants.6,7,11

Although PPARγ regulates transcription of target genes, resulting effects are often cell-specific. We initially found that cell-specific interference with PPARγ in smooth muscle impaired responses to endothelium-derived nitric oxide (NO) in the aorta in vitro.10 Because the importance of PPARγ in smaller resistance vessels was unclear, we next examined the effect of PPARγ in smooth muscle in small mesenteric arteries. We found that PPARγ normally inhibits myogenic tone in these arteries via effects on regulator of G protein signaling 5 and Ca2+-activated potassium channels.12 NO plays a fundamental role in cerebrovascular biology, influencing vascular structure and local blood flow and protecting against thrombosis.13–15 Loss of NO-mediated signaling is thought to contribute to the pathogenesis of both large and small vessel disease, with resulting hypoperfusion, cognitive impairment, and stroke.13,16 Because the effect of NO...
is prominent in the cerebral circulation,\textsuperscript{13,15} we focused the present study on cerebral blood vessels. We hypothesized that interference with PPAR\(\gamma\) in smooth muscle would impair NO-dependent responses and augment select vasoconstrictor responses. Our primary finding was that genetic interference with PPAR\(\gamma\) in vascular muscle affects NO-dependent and select NO-independent responses in resistance vessels in brain via effects on Rho kinase–dependent signaling.

**Materials and Methods**

Details about the experimental procedures are presented in the online Data Supplement. In brief, we studied transgenic mice expressing either a dominant negative mutation in human PPAR\(\gamma\) (P467L) or human wild-type PPAR\(\gamma\) under the control of the smooth muscle myosin heavy chain promoter (S-P467L or S-WT, respectively). We quantified (1) changes in diameter of cannulated, pressurized cerebral (basilar) and small mesenteric arteries in vitro; (2) changes in isometric tension in rings of carotid arteries; and (3) changes in diameter of arterioles in the pial microcirculation in vivo using anesthetized mice.

**Results**

**Interference With PPAR\(\gamma\) in Vascular Muscle Impairs Vasodilator Responses to NO**

We first verified that the transgene of interest was expressed in cerebral arteries of S-P467L mice using quantitative real-time polymerase chain reaction. Similar to results in mesenteric arteries,\textsuperscript{12} we detected human PPAR\(\gamma\) mRNA in cerebral arteries in transgenic mice (\(C\) values ranging from 21 to 25) but not nontransgenic (non-Tg) littermates (\(C\) was undetectable; data not shown).

Baseline diameter of the basilar artery was similar in non-Tg and S-P467L mice, respectively (Figure 1A). Dilation of the basilar artery to the NO donor nitroprusside was greatly reduced in S-P467L mice compared with non-Tg controls (Figure 1A). For example, increases in vessel diameter in response to 100 \(\mu\)mol/L nitroprusside in control and S-P467L mice were 78±5\% and 32±5\% (\(P\leq0.01\) versus non-Tg), respectively.

To test whether vascular phenotypes in S-P467L mice were due to dominant negative effects of the P467L mutation as opposed to overexpression of PPAR\(\gamma\), we examined responses in transgenic mice expressing wild-type human PPAR\(\gamma\) specifically targeted to smooth muscle (S-WT).\textsuperscript{10} Baseline diameter of the basilar artery was similar in S-WT compared with non-Tg (Figure 1A). In contrast to the reduced effects of nitroprusside in S-P467L mice, effects of nitroprusside were similar in S-WT and controls (Figure 1B).

We next determined whether responses to endogenously produced NO are also affected in S-P467L mice using endothelium-dependent agonists. Dilation of the basilar artery to acetylcholine is prevented by inhibition of NO synthase.\textsuperscript{17}

In the current experiments, vasodilation to angiotensin 1-7 (Ang 1-7) was greatly reduced by a mas receptor antagonist (A779; 1 \(\mu\)mol/L) and was completely inhibited by \(N^\cdot\)-nitro-L-arginine (100 \(\mu\)mol/L; Figure S1 in the online-only Data Supplement), demonstrating that this response was also mediated by NO. Dilation of the basilar artery to both acetylcholine and Ang 1-7 was reduced substantially in S-P467L mice (Figure 1C and 1D). In both strains of mice, responses to acetylcholine were similar in male and female mice (Figure S2). Responses to Ang 1-7 in arteries from S-WT were not impaired and were increased moderately at the highest concentration tested (Figure 1D).

**Interference With PPAR\(\gamma\) in Smooth Muscle Impairs Cerebral Microvascular Responses to NO**

Considering the importance of microvascular NO and small vessel disease in brain, we examined the effect of PPAR\(\gamma\) in the microcirculation in vivo. Baseline diameters of small cerebral (pial) arterioles in non-Tg and S-P467L mice were similar (34±1 and 35±2 \(\mu\)m, respectively; \(P>0.05\)). Vasodilation to acetylcholine (which is mediated by NO in brain microvessels)\textsuperscript{18} and responses to nitroprusside were reduced by \(\approx50\%\) to 65\% in S-P467L mice compared with non-Tg controls (Figure 2A and 2B).

**Regional Differences in the Effect of PPAR\(\gamma\) in Vascular Muscle**

Our initial study found that vasodilation to NO is impaired in aorta from S-P467L mice.\textsuperscript{19} To determine whether impaired responses to NO in S-P467L mice in the present study were unique to the cerebral circulation, we studied carotid arteries and small mesenteric arteries. In contrast to the marked reduction in responses in cerebral arteries in S-P467L mice, vasodilation to nitroprusside was impaired modestly in carotid arteries and was not impaired in small mesenteric arteries (Figure S3).

**Interference With PPAR\(\gamma\) in Vascular Muscle Impairs Select NO-Independent Responses in the Cerebral Circulation**

To determine whether other vasodilator mechanisms were affected in cerebral arteries in S-P467L mice, we examined...
responses to stimuli that act independent of NO. Depending on the concentration, changes in extracellular potassium (K+) can produce vasodilation or vasoconstriction. By activating inward rectifier K+ channels (KIR) in vascular muscle, modest elevations in extracellular K+ is a potent vasodilator stimulus. In control mice, low concentrations of K+ produced vasodilation, a response that was significantly reduced by barium (30 μmol/L), an inhibitor of KIR (Figure 3A). Dilation of the basilar artery to K+ was greatly impaired in S-P467L mice (Figure 3B), but was not altered in S-WT compared with non-Tg controls (Figure 3B). To determine whether the vasodilator effect of other K+ channels were affected, we tested responses to cromakalim, which activates ATP-sensitive K+ channels, and arachidonic acid, which activates calcium-dependent K+ channels in cerebral vessels. Dilation of the basilar artery to both agonists was impaired in S-P467L mice (Figure 3C and 3D). In contrast to NO and these select vasodilators, cerebral arteries dilated normally to the calcium channel blocker nifedipine and the endothelium-independent agonist papaverine (Figure 3E and 3F). Constriction of the basilar artery to Bay K 8644, an L-type calcium channel activator, was similar in both groups of mice (data not shown).

**Figure 2.** Changes in diameter of cerebral arterioles in response to acetylcholine (A) and nitroprusside (B) in nontransgenic (non-Tg; n=15) and S-P467L (n=12) mice. *P<0.05 vs non-Tg.

**Figure 3.** Dilation of the basilar artery to KCl in nontransgenic (non-Tg) mice in the absence or presence of barium (A; n=3–6). Effects of Y-27632 on dilation of the basilar artery to KCl (B; n=6–9) and responses to KCl in S-WT mice (C; n=6). Responses of the basilar artery to arachidonic acid (D; n=6), cromakalim (E; n=5–8), and nifedipine (F; n=5–10) in non-Tg and S-P467L mice, along with responses to papaverine (F) in non-Tg (n=25), S-P467L (n=21), and S-WT (n=6) mice. *P<0.01 vs non-Tg.

**Figure 4.** Effects of Y-27632 on dilation of the basilar artery to nitroprusside (A; n=5) and angiotensin (Ang) 1-7 (B; n=6) in S-P467L mice and effects of Y-27632 on Ang 1-7 responses in nontransgenic (non-Tg; n=5) mice. *P<0.01 vs non-Tg.

Vascular Dysfunction in S-P467L Mice Is Mediated by Rho Kinase

We next examined mechanisms that account for impaired responses in S-P467L mice. The primary molecular target of NO is soluble guanylate cyclase, which synthesizes cGMP after activation. Most vascular effects of NO require activation of soluble guanylate cyclase. Consistent with this concept, dilation of the basilar artery to nitroprusside in both non-Tg and S-P467L mice were completely prevented by ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-l-one) (10 μmol/L; Figure S4), an inhibitor of soluble guanylate cyclase. Oxidative stress contributes to vascular disease through several mechanisms, including impairment of NO-mediated effects. We examined the possible role of oxidative stress using 2 approaches. Tempol (1 mmol/L), a superoxide dismutase mimetic, did not improve vasodilator responses to Ang 1-7 in S-P467L mice (Figure S5). A consequence of oxidative stress is activation of poly-ADP-ribose polymerase, a nuclear protein that plays a role in vascular dysfunction in some disease states. A selective inhibitor of poly-ADP-ribose polymerase (P34; 3 μmol/L) did not alter responses to Ang 1-7 (Figure S5).

We next considered other potential targets of PPARγ that could impair regulation of vascular tone. Rho kinase, a target of the GTPase Rho, is a major determinant of vascular tone and may be inhibited by TZD treatment. We previously obtained evidence that Rho kinase was the mediator of increased contractile responses in aorta to endothelin-1 (ET-1) in S-P467L mice. Treatment of cerebral arteries with an inhibitor of Rho kinase (Y-27632; 3 μmol/L) restored responses to nitroprusside, K+, and Ang 1-7 in S-P467L mice (Figures 3B, 4A, and 4B). In time control experiments, treatment with Y-27632 alone did not alter U46619-induced tone (n=4; data not shown). In addition, Y-27632 did not alter vasodilator responses to Ang 1-7 when tested in non-Tg mice (Figure 4C). These findings suggest that impaired vasodilator responses in S-P467L mice are mediated by Rho kinase. Expression of Rho kinase isoforms (ROCK1 and ROCK2) in cerebral arteries was similar in non-Tg and S-P467L mice, although mRNA levels of ROCK2 tended to be increased (Figure S6).

**Interference With PPARγ in Vascular Muscle Augments Cerebral Vasoconstrictor Responses**

To determine whether vasoconstrictor responses were affected in S-P467L mice, we examined effects of serotonin...
and ET-1, agonists implicated in vascular disease and stroke. Constriction of the basilar artery to both ET-1 and serotonin was increased in S-P467L mice (Figure 5A and 5B). In contrast, vasoconstriction to KCl (50 mmol/L) was not altered in S-P467L or S-WT mice compared with littermate controls (Figure 5C). Enhanced vasoconstriction to both ET-1 and serotonin in S-P467L mice was prevented by Y-27632 (Figure 5A and 5B).

Discussion
There are several major new findings in this study. First, genetic interference with PPARγ in smooth muscle impaired responses to both exogenously applied and endogenously produced NO in cerebral arteries in vitro and small cerebral microvessels in vivo. These changes in resistance vessels in brain were selective because responses of small mesenteric arteries to NO were not affected. Second, the effect of PPARγ in vascular muscle extended to select NO-independent mechanisms of vasodilation and some vasoconstrictor responses. Third, in contrast to the dominant negative form of PPARγ, increased expression of wild-type PPARγ in vascular muscle did not reduce responses to several stimuli, including NO. Fourth, mechanisms underlying impairment of both NO-dependent and NO-independent vasodilator responses after interference with PPARγ involved Rho kinase with no apparent contribution by oxidative stress–related mechanisms. These findings support the concept that PPARγ in vascular muscle is a major determinant of vascular tone, particularly NO-mediated signaling, in cerebral arteries and microvessels. Considering the importance of NO and Rho kinase in resistance vessels, these findings have implications for mechanisms that regulate cerebral blood flow and the pathogenesis of large and small vessel disease (Figure S7).

PPARγ and the Vasculature
PPARγ was initially thought to predominantly influence adipocytes function in addition to having effects on glucose and lipid metabolism.12,24 More recent findings mostly based on studies using TZD treatment suggest that PPARγ can exert effects in multiple cell types. PPARγ is expressed in vascular cells.2,11,12,25 Using TZDs as an exogenous ligand, effects of PPARγ activation on vascular structure, vasomotor tone, and vascular permeability have been described.1,2,26–28 An underly-
ing assumption of most TZD-based studies is that the effects observed are mediated by PPARγ acting within vascular cells. Because systemic administration of TZDs affects all cells, distinguishing vascular-specific effects is difficult. In addition to activating PPARγ, TZDs can also exert off-target or PPARγ-independent effects.3,5

Patients with select mutations in the ligand-binding domain of PPARγ (eg, P467L) exhibit early-onset hypertension and insulin resistance.7 Heterozygous knockin mice expressing this mutation in all cells share features of human disease, including abnormal fat distribution.8 To avoid such systemic effects and to study the effect of cell-specific manipulation in vivo, we used S-P467L mice.10,29 Global knockout of PPARγ is lethal, and cell-specific knockout of PPARγ would be extremely rare.5,12 A major advantage of using a model with specific alterations in PPARγ in smooth muscle is that it avoids metabolic effects seen with global genetic manipulation of PPARγ or whole body TZD treatment. In S-P467L mice, there is no change in body weight or adipose tissue depots and no effect on plasma glucose, insulin, or leptin.10,29 S-P467L mice exhibit a modest elevation in systemic blood pressure (∼12 mm Hg).

PPARγ in Vascular Muscle Exerts Prominent Effects on NO-Mediated Vasodilation in Brain
Baseline diameter of cerebral arteries and responses to high concentrations of KCl or papaverine were not affected by transgenic expression of either human P467L or wild-type PPARγ in smooth muscle. Thus, these genetic alterations did not produce nonspecific changes in vasoconstrictor or vasodilator capacity.

Although our previous study demonstrated that genetic interference with PPARγ in smooth muscle inhibited NO-mediated relaxation in aorta,10,29 the importance of PPARγ in resistance vessels and the microcirculation in vivo was not defined. In brain, cerebral arteries and arterioles are important resistance vessels.13 Because of its prominent role in both large and small vessels in brain, we performed the present study with a focus on NO. We found that dilation of basilar arteries to an NO donor was markedly reduced in S-P467L mice. Similarly, responses to agonists that produce NO-mediated vasodilation (acetylcholine and Ang 1–7) were also greatly impaired.

Consistent with the results for the isolated basilar artery, we found that responses of small cerebral arteries to NO were substantially reduced in S-P467L mice in vivo. Thus, the effect of PPARγ in smooth muscle extends along the vascular tree in brain and is functionally important in vivo. A recent study found that genetic deletion of PPARγ in smooth muscle did not affect responses to NO in small mesenteric arteries.26 This finding is consistent with our results in S-P467L mice, where responses to an NO donor were not altered in these same arteries. Overall, these findings suggest that in the mesenteric vasculature, PPARγ does not influence NO-mediated signaling in vascular muscle. The finding that the effect of PPARγ in vascular muscle exhibits such regional heterogeneity in relation to NO was unexpected, but highlights the importance of this molecule in the cerebrovasculature.
Influence of PPARγ on NO-Independent Vasodilation

To determine whether mechanisms other than those involving NO were affected by interference with PPARγ, we examined effects of stimuli that act independently from NO in vascular muscle. Modest elevations in extracellular K+ activate inward rectifier K+ channels in vascular muscle, producing marked vasodilation.9,20 We found that vasodilation to K+ was greatly impaired in S-P467L mice. Because K+-induced vasodilation may contribute to neurovascular coupling,9 these findings implicate PPARγ as a potential determinant of functional hyperemia. To evaluate whether the effect of vascular PPARγ extended to other K+ channels, we examined effects of cromakalim and arachidonic acid. Responses to these agonists were substantially reduced in S-P467L mice. In contrast, vasodilation to both papaverine and nifedipine were unaffected. These findings suggest that interference with PPARγ in smooth muscle has effects on both NO-dependent and select NO-independent mechanisms that regulate cerebrovascular tone.

Effects of PPARγ Interference in Vascular Muscle: Role of Rho Kinase

Because interference with PPARγ in smooth muscle affected several vasodilator responses, it seemed likely that underlying mechanisms might involve pathways that more broadly influence vasomotor tone. In this context, we considered the possibility that oxidative stress was involved. However, neither a superoxide dismutase mimetic nor a poly-ADP-ribose polymerase inhibitor affected responses to NO in S-P467L mice.

A second candidate to explain reduced vasodilation in S-P467L mice was Rho kinase.21,22 Because of the effects on calcium sensitivity and the phosphorylation state of the regulatory myosin light chain, Rho kinase in vascular muscle is a major determinant of vascular tone.21,22 Our initial study in aorta provided evidence that Rho kinase signaling is altered after interference with PPARγ in vascular muscle.19 Rho kinase plays a key role in the vasculature, but its effect varies regionally and is cell specific.21,22 Thus, the effect within resistance vessels was hard to predict. We observed a similar role for Rho kinase in resistance vessels in the current experiments. We found that inhibition of Rho kinase restored responses to NO and low concentrations of K+ while preventing hyperresponsiveness to ET-1 and serotonin. In endothelium, NO-mediated signaling can be regulated via effects of Rho kinase on endothelial NO synthase phosphorylation.31 Because the model used in the present study expressed a dominant negative form of PPARγ specifically in smooth muscle, we assume effects on responses to NO are mediated within vascular muscle. In addition, we found previously that level of total and phospho-Ser177 endothelial NO synthase protein was similar in aortic tissue from control and S-P467L mice.29

Perspectives

NO has diverse effects within the cerebral vasculature, mediating responses to neurotransmitters, shear forces, metabolic factors, and therapeutic agents while inhibiting vascular hypertrophy and metabolism of β-amyloid precursor protein.13,15 Thus, loss of NO-mediated signaling is thought to be an important contributor to cerebrovascular disease. The present study suggests that PPARγ in smooth muscle is part of previously unrecognized mechanism that influences NO-mediated signaling and regulation of tone in resistance vessels in brain. Rho kinase contributes to vascular disease,21,22 but mechanisms that regulate this pathway and interactions with NO are poorly defined, particularly at the cell-specific level. In addition to effects on vascular tone, Rho kinase has been implicated in atherosclerosis, small vessel disease, development of cerebral cavernous malformations and aneurysms, as well as vasospasm after subarachnoid hemorrhage.21,32 Activity of Rho kinase is positively associated with cardiovascular events, including stroke.33 Thus, the finding that PPARγ in vascular muscle is an important regulator of NO- and Rho kinase–dependent effects has diverse implications. Therapeutic approaches or other strategies to target PPARγ or its downstream effectors in vascular muscle may be beneficial in preventing or slowing the progression of large and small vessel disease in brain.

Acknowledgments

We thank Dale Kinzenbaw for technical assistance.

Sources of Funding

This work was supported by the National Institutes of Health (NS-24621, HL-62984, HL-48058, HL-61446, and HL-113863), the Department of Veterans Affairs (BX001399), and the Fondation Leducq (Transatlantic Network of Excellence). Post-doctoral Fellowship support was provided by the American Heart Association (12POST9150027 and 11POST5720021) and the National Health and Medical Research Council of Australia (1053786). We acknowledge the generous research support of the Roy J. Carver Trust.

Disclosures

None.

References

9. Beyer AM, Baumback GL, Halabi CM, Modrick ML, Lynch CM, Gerhold TD, Ghoneim SM, de Lange WJ, Keen HL, Tsai YS, Maeda N, Sigmund...
Novelty and Significance

What Is New?

- Our findings suggest that peroxisome proliferator–activated receptor-γ (PPARγ) in vascular muscle is a major determinant of tone in resistance vessels in brain, particularly in relation to nitric oxide (NO)-mediated signaling. This influence was apparent under normal conditions, in the absence of treatment with pharmacological activators of PPARγ.
- Interference with PPARγ in smooth muscle had prominent effects in cerebral vessels, but no detectable effect in relation to NO in small mesenteric arteries.
- These findings support the concept that PPARγ in vascular muscle is part of a previously unrecognized mechanism that inhibits Rho kinase–dependent signaling, thus affecting vasoconstrictor responses.

What Is Relevant?

- Although NO is considered to be a key molecule in relation to its influence on cerebrovascular structure and function, mechanisms that regulate these influences at the cell-specific level are poorly defined.
- Considering the importance of NO and Rho kinase in cerebral vessels, these findings have implications for mechanisms that regulate brain perfusion and the pathogenesis of large and small vessel disease.

Summary

This study provides genetic evidence that interference with PPARγ in smooth muscle impairs cerebrovascular responses to NO both in vitro and in vivo. Similar effects were not seen in small mesenteric arteries. The effect of PPARγ in vascular muscle extended to select NO-independent mechanisms of vasodilation and some vasoconstrictor responses. Mechanisms underlying these effects involved Rho kinase. These findings support the concept that PPARγ in vascular muscle is a major determinant of vascular tone and, in particular, NO-mediated signaling in cerebral arteries and brain microvessels. The results further suggest that PPARγ in vascular muscle normally protects against excessive Rho kinase–dependent signaling in resistance vessels.


Role of Peroxisome Proliferator–Activated Receptor-γ in Vascular Muscle in the Cerebral Circulation

T. Michael De Silva, Mary L. Modrick, Pimonrat Ketsawatsomkron, Cynthia Lynch, Yi Chu, Christopher J. Pelham, Curt D. Sigmund and Frank M. Faraci

Hypertension. 2014;64:1088-1093; originally published online September 2, 2014; doi: 10.1161/HYPERTENSIONAHA.114.03935

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
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:
http://hyper.ahajournals.org/content/64/5/1088

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/09/02/HYPERTENSIONAHA.114.03935.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
ONLINE SUPPLEMENT

Role of PPARγ in Vascular Muscle in the Cerebral Circulation

T. Michael De Silva, Mary L. Modrick, Pimonrat Ketsawatsomkron, Cynthia Lynch, Yi Chu, Christopher J. Pelham, Curt D. Sigmund, and Frank M. Faraci

Departments of Internal Medicine and Pharmacology
Francois M. Abboud Cardiovascular Center
Carver College of Medicine
University of Iowa
Iowa City Veterans Affairs Healthcare System
Iowa City, IA 52242
SUPPLEMENTAL MATERIALS AND METHODS

Experimental animals. The animal protocols used were approved by the University of Iowa Animal Care and Use Committee. We used male and female transgenic mice expressing a dominant negative mutation in human PPARγ (P467L) under the control of the smooth muscle myosin heavy chain promoter (S-P467L) as described previously. In a subset of studies, we used transgenic mice expressing human wild-type PPARγ (at a level similar to P467L PPARγ) specifically targeted to smooth muscle (denoted S-WT). Because we observed no apparent sex-related differences in these experiments, results from male and female mice were combined. Littermates were used as controls. Mice were fed standard chow and water ad libitum and studied at ~4-6 months of age. Body weight was similar in both strains ranging from ~20-35 grams. Care of mice met the standards set forth by the NIH for the care and use of experimental animals.

Studies of vascular function. Methods used to measure vascular responses have been described in detail previously. Briefly, mice were euthanized with pentobarbital (~100 mg/kg IP) followed by removal of brain, carotid arteries, or small mesenteric arteries. Basilar arteries were isolated, cannulated, and pressurized to 60 mmHg so lumen diameter could be measured. To examine dilator responses, arteries were first constricted by ~30% (~60% of the response to 50 mM KCl) with U46619. The level of preconstriction was similar in control and S-P467L mice. For studies of carotid arteries, vessels were removed, cut into rings, and connected to force transducers to measure isometric tension in organ baths. Carotid arteries were contracted submaximally (~50-60% of maximum) using U46619, prior to testing effects of vasodilators.

For studies of small cerebral arterioles in vivo, mice were anesthetized with pentobarbital sodium (75–90 mg/kg IP), supplemented at ~20 mg/kg per hour. Animals were ventilated mechanically and a cranial window was prepared, and arterial blood pressure and blood gases were monitored as described. Arteriolar diameter was measured under control conditions and during topical application of drugs.

Quantitative real-time RT-PCR. RNA from cerebral arteries was prepared using the RNAeasy (Qiagen, Germantown, MD) method following extraction with TRIzol reagent (Invitrogen, Carlsbad, CA). RNA concentrations were determined using a NanoDrop spectrophotometer, with an OD260/OD280 ratio of greater than 1.9 (indicating very high quality RNA). Identical amounts of RNA (300 ng) were used for reverse transcription reaction (RT). Identical amounts of RT product were used for real-time PCR with a single well of a 96-well plate containing both TaqMan probes/primers (Applied Biosystems) for genes of interest (with FAM fluorophor,) and using β-actin (with VIC fluorophor) as a house-keeping gene. Expression levels were normalized to β-actin (4352341E). Relative expression levels were obtained using the ΔΔCt method as described for endothelial NO synthase (eNOS, Mm00435204_m1), ROCK1 (Mm00485745_m1) and ROCK2 (Mm00485771_m1) by quantitative real-time RT-PCR using the TaqMan method. For detection of human PPARγ in cerebral arteries from mice, a custom made Taqman probe that specifically detects human PPARγ (described elsewhere) was used.
Drugs. All agonists and inhibitors used were obtained from Sigma (St Louis) unless otherwise noted. U46619 (Cayman Chemical, Ann Arbor, MI) was dissolved in ethanol with subsequent dilutions made in saline.

Statistical analysis. All data are expressed as means±SE. Responses to KCl in the basilar artery and responses of cerebral arterioles in vivo were calculated as percent change in vessel diameter from the baseline level. For vasodilator responses in isolated arteries, results are expressed as percent dilation or relaxation (% of induced tone), with 100% representing the difference between the resting value under basal conditions and the constricted value with U-46619. Data were evaluated using analysis of variance followed by Student-Neumann Kuels post hoc test, or Student unpaired t test, as appropriate. Statistical significance was accepted at P<0.05.
References


Figure S1. Dilation of the basilar artery to Ang 1-7 in non-Tg mice in the absence or presence of A779 (A) (n=5) or L-NNA (B) (n=5). * P<0.01 vs non-Tg.
Supplemental Figure 2

Figure S2. Dilation of the basilar artery to acetylcholine in male (n=8-13) and female (n=12-13) non-Tg and S-P467L mice. * P<0.01 vs non-Tg.
Figure S3. Relaxation of carotid arteries (A) (n=7 non-Tg, n=7 S-P467L) and dilation of small mesenteric arteries (B) in response to nitroprusside in non-Tg and S-P467L mice. Baseline diameter of mesenteric arteries was 179±10 and 165±12 µm in non-Tg and S-P467L mice (n=5 for each), respectively. * P<0.05 vs non-Tg.
Supplemental Figure 4

**Figure S4.** Dilation of the basilar artery to nitroprusside in non-Tg and S-P467L mice in the absence and presence of ODQ (n=6-8). *P<0.01 vs non-Tg.*
Figure S5. Effects of tempol (n=5) and PJ34 (n=3) on dilation of the basilar artery to Ang 1-7 in S-P467L mice. * P<0.01 vs non-Tg.
Figure S6. Expression of mRNA in cerebral arteries from non-Tg and S-P467L mice. Compared to non-Tg mice, there were no significant changes in expression of eNOS, ROCK1 or ROCK2, although levels of ROCK2 tended to increase (0.05<P<0.1)(n=9 in each group).
Figure S7. Schematic summary of the major findings from this study. Activation of Rho kinase in cerebrovascular muscle produces contraction (increases in vascular tone). PPARγ normally exerts an inhibitory effect on activation of this pathway in vascular muscle. Interference with PPARγ signaling by expression of a dominant negative mutation in PPARγ (DN-PPARγ) prevents the inhibitory effects of wild type PPARγ resulting in increased Rho kinase dependent effects. As a consequence, vasodilation to NO or activation of K+ channels is impaired, and vasoconstriction to endothelin-1 (ET-1) and serotonin (5-hydroxytryptamine, 5-HT) are enhanced.