PPARγ Activation Prevents Hypertensive Remodeling of Cerebral Arteries and Improves Vascular Function in Female Rats

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Background and Purpose—Previous studies have shown that peroxisome proliferator-activated receptor γ (PPARγ), a ligand-activated transcription factor expressed in vascular cells, is protective of the vasculature. We hypothesized that activation of PPARγ could prevent hypertensive remodeling of cerebral arteries and improve vascular function.

Methods—Ten female Sprague-Dawley rats were treated with the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) for 5 weeks, 8 were treated with L-NAME plus the PPARγ activator rosiglitazone, and 8 received no treatment and served as controls. Blood pressure, myogenic activity, passive diameters and wall thickness of cerebral arteries, and brain capillary density were compared between the groups.

Results—Treatment with L-NAME caused an increase in arterial blood pressure that was sustained with rosiglitazone treatment. L-NAME also caused inward hypertrophic remodeling and enhanced myogenic reactivity of cerebral arteries that was reversed by rosiglitazone. In addition, L-NAME hypertension caused rarefaction of brain capillaries by approximately 12%, whereas treatment with rosiglitazone increased capillary density by approximately 20%.

Conclusion—PPARγ activation may be an effective and clinically relevant way to prevent hypertensive remodeling of cerebral arteries and capillary rarefaction as well as improving vascular function without affecting blood pressure. (Stroke. 2010;41:1266-1270.)

Key Words: capillary density ■ cerebral arteries ■ L-NAME hypertension ■ myogenic tone ■ PPARγ ■ remodeling

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcription factor involved in a diverse array of biological processes. PPARγ agonists such as rosiglitazone and pioglitazone are thiazolidinediones used extensively to treat Type 2 diabetes. More recently, PPARγ agonists have been shown to be protective of cardiovascular disease, including hypertension, atherosclerosis, and metabolic disorders, through potent anti-inflammatory and antioxidant effects. In the brain, PPARγ agonists also have marked anti-inflammatory and antioxidant effects and have been shown to be protective against cerebrovascular disease. For example, treatment with PPARγ agonists prevents damage from ischemic and hemorrhagic stroke and Alzheimer disease through reduced superoxide production, decreased macrophage infiltration, and suppression of proinflammatory cytokines.

In addition to its anti-inflammatory and antioxidant properties, PPARγ has been shown to have a significant role in maintaining vascular structure and function. Specific interference of PPARγ in endothelium or vascular muscle causes reduced endothelium-dependent vasodilation, enhanced vasoconstriction, and systolic hypertension. In addition, interference of PPARγ causes inward remodeling and medial hypertrophy of cerebral arterioles independent of a change in blood pressure. Together, these findings suggest that the role of PPARγ in the vasculature is to counteract the damaging effects of disease in the brain and elsewhere, including endothelial dysfunction and structural remodeling.

Hypertension is a major risk factor for cerebrovascular disease that has significant effects on the structure and function of cerebral vessels. Hypertension causes inward remodeling that limits vasodilator reserve, promotes hyperfusion, and causes brain infarction and hemorrhage. In addition, hypertension causes capillary rarefaction that can increase cerebrovascular resistance and decrease oxygen and glucose transport. Given the protective nature of PPARγ, we hypothesized that PPARγ activation during hypertension could improve the structure and function of cerebral arteries and capillaries. We therefore compared structural remodeling, capillary density changes, and active myogenic responses in cerebral arteries from rats that were hypertensive by nitric oxide synthase inhibition to a group of rats that were ischemic and hemorrhagic stroke and Alzheimer disease through reduced superoxide production, decreased macrophage infiltration, and suppression of proinflammatory cytokines.

In addition to its anti-inflammatory and antioxidant properties, PPARγ has been shown to have a significant role in maintaining vascular structure and function. Specific interference of PPARγ in endothelium or vascular muscle causes reduced endothelium-dependent vasodilation, enhanced vasoconstriction, and systolic hypertension. In addition, interference of PPARγ causes inward remodeling and medial hypertrophy of cerebral arterioles independent of a change in blood pressure. Together, these findings suggest that the role of PPARγ in the vasculature is to counteract the damaging effects of disease in the brain and elsewhere, including endothelial dysfunction and structural remodeling.

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cation can prevent remodeling and improve vascular function, including myogenic responses. Female rats were used for this study because few studies have examined how hypertension affects the cerebral circulation in this gender despite the prevalence of hypertensive and cerebrovascular disease in women.

Materials and Methods

Animals and Treatment Groups
All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animals were virgin female nonpregnant Sprague-Dawley rats (230 to 250 g; Charles River, Canada) that were either normotensive (CTL; n=8) or treated with L-NAME (0.5 g/L) in their drinking water to raise blood pressure for 5 weeks (HTN; n=10). To determine if PPARγ activation could prevent hypertensive remodeling and improve vascular function of cerebral arteries, a separate group of animals was treated with L-NAME for 5 weeks, but after 2 weeks on L-NAME were given rosiglitazone (20 mg/kg in food) for the remaining 3 weeks of L-NAME treatment (HTN + Rosi; n=8).

Blood Pressure Measurements
Blood pressure was measured noninvasively each week by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail cuff (Coda 8 System; Kent Scientific, Torrington, Conn) as previously described.15

Vessel Preparation and Pressurized Arteriograph System
Isolated and pressurized third-order branches of the posterior cerebral artery were studied for changes in lumen diameter and wall thickness, as previously described.15 All experimental protocols for obtaining active myogenic responses and passive structural measurements have been previously described.15

Brain Histology and Capillary Density Measurements
Capillary density of the posterior cerebral cortex was determined by immunohistological staining for von Willebrand factor and morphometric analysis. Brain sections were paraffin-embedded and processed for immunohistochemistry in a typical manner as previously described.16 A polyclonal rabbit antihuman von Willebrand factor primary antibody (dilution 1:400; Dako, Glostrup, Denmark) and an Ultravision LP horseradish peroxidase kit (Thermoscientific, Fremont, Calif) were used according to specifications. Sections of cerebral cortex from the posterior brain region were imaged at 20×, imported into Metamorph, and used to count capillaries per area. Four images from each section and 2 sections from each animal were counted blinded to group. Images were counted twice and the results averaged to obtain a measure of capillary density.

Statistical Analysis
All results are presented as mean±SEM. Differences were determined by one-way analysis of variance with a post hoc Student-Newman-Keuls test for multiple comparisons. Differences were considered significant at P<0.05.

Results

Animals and Blood Pressures
L-NAME treatment caused a significant increase in arterial pressure in both the HTN and HTN + Rosi groups that was sustained during the duration of the study and greater than CTL animals (P<0.01; Table). PPARγ activation for the last 3 weeks of the 5 weeks on L-NAME did not decrease blood pressure in the HTN + Rosi group.

Table. Weekly Blood Pressures From Control (CTL), Hypertensive (HTN), and Hypertensive Treated With Rosiglitazone (HTN + Rosi) Animals

<table>
<thead>
<tr>
<th>Blood Pressures, mm Hg</th>
<th>CTL (n=8)</th>
<th>HTN (n=10)</th>
<th>HTN + Rosi (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>126±4</td>
<td>151±5†</td>
<td>154±4†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>94±4</td>
<td>117±4†</td>
<td>123±3†</td>
</tr>
<tr>
<td>Mean</td>
<td>105±4</td>
<td>128±4†</td>
<td>133±3†</td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>132±3</td>
<td>158±4†</td>
<td>163±5†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>95±3</td>
<td>124±4†</td>
<td>125±3†</td>
</tr>
<tr>
<td>Mean</td>
<td>107±3</td>
<td>135±5†</td>
<td>137±3†</td>
</tr>
<tr>
<td>Week 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>129±2</td>
<td>152±3†</td>
<td>155±3†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>95±2</td>
<td>121±4†</td>
<td>119±3†</td>
</tr>
<tr>
<td>Mean</td>
<td>106±1</td>
<td>131±3</td>
<td>130±3†</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>130±2</td>
<td>152±5†</td>
<td>158±4†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>96±2</td>
<td>120±5†</td>
<td>120±4†</td>
</tr>
<tr>
<td>Mean</td>
<td>107±2</td>
<td>130±5†</td>
<td>133±4†</td>
</tr>
<tr>
<td>Week 5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>134±2</td>
<td>157±5†</td>
<td>158±3†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>100±2</td>
<td>122±5†</td>
<td>120±3†</td>
</tr>
<tr>
<td>Mean</td>
<td>111±2</td>
<td>134±5†</td>
<td>132±2†</td>
</tr>
</tbody>
</table>

*Values are mean±SE. †P<0.01 versus CTL.

Passive Artery Measurements
One posterior cerebral artery from CTL animals was excluded for technical reasons and therefore only data with complete active and passive measurements were used for statistical comparison. Hypertension induced by L-NAME caused a significant decrease in inner diameter and an increase in wall thickness over the entire pressure range compared with CTL (Figure 1). Treatment of hypertensive animals with rosiglitazone prevented inward remodeling and the increase in wall thickness, because this group of animals had lumen diameters and arterial walls that were similar to controls.

Active Artery Measurements
Cerebral arteries from all groups of animals had considerable pressure-dependent tone and responded myogenically to increases in pressure (Figure 2). CTL animals developed myogenic reactivity within the autoregulatory pressure range from 75 to 125 mm Hg as demonstrated by the relatively flat curve within this pressure range. HTN animals had considerably smaller active diameters than CTL animals over the entire pressure range and underwent forced dilatation at higher pressures (Figure 2A). The smaller lumen diameters in HTN animals actively was partially due to inward remodeling, but also likely due to an increase in myogenic tone (Figure 2B). Treatment of hypertensive animals with rosiglitazone partially restored the active diameters and myogenic activity to CTL levels. HTN + Rosi animals had posterior cerebral arteries that were actively larger than HTN animals and similar to CTL animals at pressures within the autoregulatory range (75 to 125 mm Hg). However, at pressures above and below this range, active diameters of arteries from HTN + Rosi animals were more
similar to HTN animals and underwent forced dilatation at higher pressures compared with CTL animals.

### Capillary Density

Figure 3A–Cs show representative micrographs of brain sections stained for visualization of capillaries with von Willebrand factor. Figure 3D shows the average capillary density/mm² for all groups of animals. Notice that 5 weeks of hypertension caused rarefaction of brain capillaries demonstrated by a 12% decrease in capillary density in the HTN group versus CTL ($P < 0.01$). Treatment of hypertensive animals with rosiglitazone prevented this effect and had capillary densities that were 20% greater than HTN animals ($P < 0.01$) and similar to CTL animals.

### Discussion

The major findings of this study were that animals exposed to 5 weeks of L-NAME hypertension had posterior cerebral arteries that underwent inward remodeling as shown by the significant decrease in inner diameters and increased wall thickness. PPARγ treatment for the last 3 of 5 weeks of L-NAME hypertension prevented the inward remodeling and had cerebral arteries with both inner diameters and wall thicknesses that were similar to normotensive control animals (Figure 1). In addition, PPARγ activation during hypertension prevented brain capillary rarefaction, suggesting a protective effect of PPARγ on brain capillaries as well as larger cerebral arteries. It is worth noting that HTN+Rosi animals were hypertensive for 2 weeks before treatment with rosiglitazone and may have already undergone inward remodeling. A previous study showed similar remodeling and hypertrophy in animals that were hypertensive for 2 versus 5 weeks by nitric oxide synthase inhibition, suggesting that remodeling was likely to be present in hypertensive animals in this study before rosiglitazone treatment and that PPARγ activation may actually reverse hypertensive remodeling and capillary rarefaction. However, the effect of rosiglitazone on the

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**Figure 1.** Passive lumen diameter (A) and wall thickness (B) versus intravascular pressure of posterior cerebral arteries from nonpregnant control (CTL, open circles), hypertensive animals treated with L-NAME for 5 weeks (HTN, closed circles), and hypertensive animals treated with L-NAME for 5 weeks plus the PPARγ agonist rosiglitazone for the last 3 of 5 weeks on L-NAME (HTN+Rosi, closed squares). Hypertension for 5 weeks caused inward remodeling and increased wall thickness that was completely reversed by rosiglitazone treatment. *$P < 0.05$ versus CTL; **$P < 0.01$ versus CTL; ‡‡$P < 0.01$ versus HTN.

**Figure 2.** A, Active lumen diameters versus intravascular pressure of posterior cerebral arteries from nonpregnant control (CTL, open circles), hypertensive animals treated with L-NAME for 5 weeks (HTN, closed circles), and hypertensive animals treated with L-NAME for 5 weeks plus the PPARγ agonist rosiglitazone for the last 3 of 5 weeks on L-NAME (HTN+Rosi, closed squares). L-NAME hypertension caused a significant decrease in lumen diameters and increased the pressure at which forced dilatation occurred. Treatment with rosiglitazone partially reversed the increase in active diameters at pressures within the myogenic pressure range (75 to 125 mm Hg). B, Percent myogenic tone at 75 mm Hg in posterior cerebral arteries from CTL, HTN, and HTN+Rosi animals. There was a nonsignificant increase in myogenic tone in HTN animals that likely contributed to the decrease in active diameters in that group. **$P < 0.01$ versus CTL; ‡‡$P < 0.01$ versus HTN.
vascular wall appeared independent of changes in blood pressure because it occurred in the HTN+Rosi animals although pressures were similarly elevated as the HTN animals for the duration of the study.

The effect of PPARγ activation on hypertensive remodeling and improvement of vascular function is strikingly similar to what has been shown to occur in models of hypertension during pregnancy, that is, pregnancy reverses hypertensive remodeling of cerebral arteries and improves vascular function independent of blood pressure lowering.15 Hypertension during pregnancy affects up to 8% of all pregnancies17 and impacts many organs, including the brain, in the form of pre-eclampsia/eclampsia.18 In addition, pregnancy is a state of high PPARγ activation,19 suggesting that this may be an underlying mechanism by which pregnancy reverses hypertensive remodeling without lowering blood pressure.

Although the present study demonstrated that activation of PPARγ is an underlying mechanism by which remodeling and hypertrophy of cerebral arteries was prevented, the specific target(s) of PPARγ that affects these structural changes in the cerebrovasculature is not clear. There are several factors known to be involved in cerebral artery remodeling and medial hypertrophy that are also regulated by PPARγ, including the renin–angiotensin system. Both angiotensin-converting enzyme inhibition and angiotensin II Type I receptor antagonism reverse hypertensive remodeling of cerebral arteries.20,21 PPARγ activation decreases expression of angiotensin II Type I receptors in vascular smooth muscle and prevents medial hypertrophy of mesenteric arteries in angiotensin II-dependent hypertension.22,23 Although the present study did not assess changes in the renin–angiotensin system or angiotensin II Type I receptors, remodeling was independent of changes in mean arterial pressure and nitric oxide. Alternatively, the actions of PPARγ on medial hypertrophy may not be related to transcription. PPARγ signaling inhibits cell cycle progression and growth in smooth muscle, suggesting that activation of PPARγ could inhibit medial hypertrophy in cerebral arteries.24

Another structural effect of PPARγ activation was on the cerebral microcirculation to prevent rarefaction that occurred during hypertension. Similar to the peripheral microcirculation, hypertension causes rarefaction of brain capillaries and impairs microvessel formation that can increase vascular resistance.3,13 In the present study, there was a significant decrease in brain capillary density with L-NAME hypertension, suggesting rarefaction of the cerebral microcirculation occurs in this model of hypertension. PPARγ activation prevented the decrease in capillary density in hypertensive animals. Because animals were hypertensive for 2 weeks before rosiglitazone treatment, it is possible that rarefaction already occurred and that PPARγ activation caused angiogenesis and reversed this effect in the cerebral microcirculation, as has been shown in several studies.25–27 It is also worth noting that PPARγ activation has been shown to have significant neuroprotective effects and improve outcome in models of brain injury.3–5 The finding that PPARγ activation prevents or reverses capillary rarefaction in the brain may contribute to these neuroprotective effects.

In addition to an effect of PPARγ on structural remodeling, rosiglitazone treatment also partially restored functional responses of cerebral arteries, including myogenic reactivity and tone. Previous studies showed that PPARγ activation improves endothelium-dependent vasodilator production in carotid and cerebral arteries.3,28 Although it is possible that rosiglitazone improved myogenic tone through an effect on the endothelium, it is not likely through improving nitric oxide production given that nitric oxide synthase was inhibited with L-NAME. The improvement in myogenic reactivity and tone in hypertensive animals with rosiglitazone suggests that this treatment could affect cerebral hemodynamics, including autoregulation and vascular resistance, that are known to be significantly affected by hypertension.8
Summary
In the present study, we show that activation of PPARγ with rosiglitazone prevented hypertensive remodeling and hypertrophy of cerebral arteries, improved vascular function, and prevented capillary rarefaction independent of a change in blood pressure. Whether or not prevention of remodeling by PPARγ activation is beneficial or detrimental to the brain is not clear. Remodeling and medial hypertrophy of cerebral arteries in response to hypertension is thought to be protective of the microcirculation by increasing cerebrovascular resistance and limiting transmission of damaging hydrostatic pressure that can cause blood–brain barrier disruption and edema formation. PPARγ activation prevented remodeling and medial hypertrophy, whereas blood pressure remained elevated, suggesting that this protective mechanism was lost. In addition, the increase in capillary density that occurred in rosiglitazone-treated animals could cause overperfusion and increase transcapillary exchange during persistent hypertension and under conditions that decrease vascular resistance such as acute hypertension and eclampsia. However, the effect of PPARγ on arterial diameter and capillary density could also be considered beneficial by increasing vasodilator reserve and perfusion and may be a mechanism by which PPARγ is protective during cerebral ischemia and Alzheimer disease.

Sources of Funding
We gratefully acknowledge the continued support of the National Institute of Neurological Disorders and Stroke grants NS045940 and NS043316, the American Heart Association grant EI 0540081N, and the Toorn Medical Research Trust.

Disclosures
None.

References
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Stroke. 2010;41:1266-1270; originally published online April 15, 2010; doi: 10.1161/STROKEAHA.109.576942

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