PPAR-γ agonist rosiglitazone reverses increased cerebral venous hydraulic conductivity during hypertension

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PPAR-γ agonist rosiglitazone reverses increased cerebral venous hydraulic conductivity during hypertension. Am J Physiol Heart Circ Physiol 297: H1347–H1353, 2009. First published August 7, 2009; doi:10.1152/ajpheart.00630.2009.— Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists have been shown to protect the cerebral vasculature, including the blood-brain barrier. In the present study, we investigated the effect of the PPAR-γ agonist rosiglitazone on changes in venous permeability during chronic hypertension induced by nitric oxide synthase inhibition. Female Sprague-Dawley rats were either treated with NO3-nitro-l-arginine methyl ester (l-NAME; 0.5 g/l in drinking water) for 5 wk (HTN; n = 8), l-NAME for 5 wk plus the PPAR-γ agonist rosiglitazone (20 mg/kg in food) for the last 3 wk (HTN + Rosi; n = 5), l-NAME for 5 wk plus the superoxide dismutase mimetic Tempol (1 mmol/l in drinking water) for the last 3 wk (HTN + Tempol; n = 8), or were untreated controls (n = 9). Fluid filtration (Jv/S) and hydraulic conductivity (Lp) of cerebral veins were compared in vitro between groups after a step increase in pressure from 10 to 25 mmHg to mimic the change in hydrostatic pressure during acute hypertension. Hypertension increased Jv/S by 2.2-fold and Lp by 3.2-fold. Rosiglitazone treatment after 2 wk of hypertension completely reversed the increased Jv/S and Lp that occurred during hypertension, whereas Tempol had no effect. These results demonstrate that rosiglitazone was effective at reversing changes in venous permeability that occurred during chronic hypertension, an effect that does not appear to be related to its antioxidant properties. Our findings suggest that PPAR-γ may be a key regulator of blood-brain barrier permeability and a potential therapeutic target during hypertension.

A primary event in response to acute hypertension is the rapid formation of pinocytotic vesicles that can promote passage of albumin and other proteins into the brain parenchyma, which can then damage neurons and cause neurological complications, including seizure (9, 30, 36, 49). Several studies have investigated BBB disruption during acute hypertension using a cranial window preparation (23–26). Although these studies suggested that veins are an important site of BBB disruption, little is known regarding the mechanisms that regulate changes in BBB permeability during hypertension.

Unlike measures of solute or tracer permeability, Lp is the critical transport parameter that relates water flux to hydrostatic pressure (18). Together with transvascular filtration (per surface area; Jv/S), Lp is an important determinant of the movement of water into the brain (18). Lp is also a characteristic parameter of convective fluid motion and can, therefore, influence the mass transport of solutes and other molecules through the vascular wall (4, 5, 45). Although Lp has been commonly studied in peripheral vessels (4, 5, 45), less is known about how Lp and Jv/S are regulated in cerebral vessels that have unique barrier properties.

The present study had several objectives. First, we developed methodology to measure Lp and Jv/S of cerebral veins in vitro. We used a pressurized arteriograph system to measure Lp and Jv/S (14). This system facilitated the study of effects of elevated hydrostatic pressure on filtration, similar to what is experienced during acute hypertension. The second objective of this study was to compare Lp and Jv/S in veins from chronically hypertensive animals to normotensive controls. To do this, we used a model of nitric oxide synthase (NOS) inhibition to induce hypertension. This model of chronic hypertension is commonly used and promotes renal damage, vascular inflammation, and activation of the renin-angiotensin system (52, 53). In addition, this model of hypertension has increased BBB permeability to tracers (1, 17, 21). However, to our knowledge, it is not known how chronic hypertension affects Lp and Jv/S. The third objective of this study was to determine whether increases in venous permeability during hypertension could be reversed by rosiglitazone, an activator of peroxisome proliferator-activated receptor-γ (PPAR-γ). PPAR-γ is a nuclear receptor and transcription factor expressed in vascular cells and has been recently shown to protect the vasculature, including the cerebral vasculature (7, 11, 31, 32, 39, 43). In addition, several studies demonstrated a protective effect of PPAR-γ agonists on the BBB (15, 51). However, to our knowledge, no studies have investigated the effect of PPAR-γ on venous permeability or if activators of PPAR-γ could reverse increased BBB permeability during hypertension. We, therefore, treated rats with NO3-nitro-l-arginine methyl ester (l-NAME) for 5 wk, but, after 2 wk on l-NAME,
METHODS

Animal model. All animals were virgin female Sprague-Dawley rats (Charles River, Canada). Animals were housed in the University of Vermont Animal Care Facility, an Association for Assessment and Accreditation of Laboratory Care-accredited facility. All procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drug treatments. The effects of chronic hypertension on J/S and Lp were determined by treating animals with the NOS inhibitor L-NAME (0.5 g/l; Sigma, St. Louis, MO) in drinking water for 5 wk (HTN; n = 10). To determine whether the effect of L-NAME hypertension on J/S and Lp could be reversed by PPAR-γ activation, a separate group of animals was treated with L-NAME for 5 wk, but, after 2 wk on L-NAME, the animals were treated with the PPAR-γ agonist rosiglitazone (20 mg/kg in food; HTN + Rosi; n = 7; Cayman Chemical, Ann Harbor, MI) for the remaining 3 wk on L-NAME. Similarly, to determine whether the effect of L-NAME hypertension on J/S and Lp could be reversed by scavenging of superoxide, a group of animals was treated with 4-hydroxy-TEMPO (Tempol; Sigma, St. Louis, MO), a superoxide dismutase mimetic that has been shown to increase the antioxidative stress in the vasculature (35). Rats were treated with Tempol (1 mmol/l in drinking water; HTN + Tempol; n = 8), similar to the treatment with rosiglitazone, starting 2 wk after the start of L-NAME treatment and continuing for 3 wk. A control group that was untreated was also included (CTL; n = 10).

Determination of blood pressure. Blood pressures (mean, systolic, and diastolic) were determined noninvasively by measuring the tail blood volume with a volume pressure recording sensor and an occlusion tail-cuff (Coda 8 System, Kent Scientific, Torrington, CT), as described previously (10). All animals had their blood pressures measured weekly.

Determination of J/S and Lp of cerebral veins. Lp reflects the specific permeability of the vessel to water and is determined by Eq. 1, in which S represents the surface area of the vessel and J, the transcapillary volume flux (27, 33). The transcapillary hydrostatic pressure difference (∆P) was the intravascular pressure in these experiments. The transcapillary osmotic pressure (∆Π) was assumed to be 0, because the intraluminal fluid was identical to the fluid surrounding the cannulated vessel. Therefore, in these experiments, Lp was defined as J/s per S per ∆P (Eq. 2).

Lp = J/s × (∆P − ∆Π))
(1)

Jp = J/s × ∆P
(2)

The J/S was determined by Eq. 3, in which ∆V represents the volume flux, and ∆t is the time interval.

J/S = ∆V/(∆t × S)
(3)

The S of the vessels was determined using the vessel length (L) and luminal diameter (D), assuming the vessels were open-ended right cylinders (Eq. 4).

S = π × L × D
(4)

Pressurized arteriograph system and vessel preparation. The modified Landis technique by Kimura (19) used the movement of an oil drop inside the lumen of an isolated cerebral arteriole to determine the volume flux across the vessel wall. In contrast to this technique, we took advantage of a servo system to adjust and measure the intravascular pressure of an isolated vein to measure fluid flux and determine filtration. A pressure-servo controller and a peristaltic pump (Living Systems Instrumentation, Burlington, VT) measured and maintained the intravascular pressure in isolated veins. When the pressure-servo controller and peristaltic pump were disconnected from the transducer, the intravascular pressure was allowed to decrease over time due to the volume flux across the vessel wall. The drop in intravascular pressure was recorded continuously during the experiment.

A leak in the pressurized system would influence the results because there would be a greater decrease in intravascular pressure not due to filtration. Therefore, the system was tested for leaks during setup for each experiment by disconnecting the pressure-servo controller and peristaltic pump from the transducer and measuring the decrease in pressure with the stopcock before the cannula closed. If a leak was detected, all connections were tightened until no leak was observed.

Cerebral veins were used for all experiments because they have been shown to be a major site of BBB disruption during acute hypertension (24–26). Pial veins are highly branched, making cannulation and pressurization very difficult. Therefore, the vein of Galen, a relatively long and unbranched vein, was used. Animals were anesthetized with isoflurane, decapitated, and the brain was quickly removed and placed in ice-cold physiological saline solution (PSS). The vein of Galen was carefully dissected, and the proximal end of the vessel was mounted on a glass cannula perfused with HEPES-buffered PSS (HEPES-PSS; Sigma) in an arteriograph chamber. The distal end of the vein was tied off with a nylon suture. The arteriograph chamber was connected to a 100-ml reservoir and a heat exchanger that recirculated HEPES-PSS at a constant temperature of 37.0 ± 0.5°C and pH of 7.40 ± 0.05.

Experimental protocol for measuring J/S and Lp. After mounting in the arteriograph, veins were equilibrated at 10.0 ± 0.2 mmHg for 60 ± 5 min, after which intravascular pressure was increased to 25.0 ± 0.2 mmHg. This level of intravascular pressure is similar to what has been measured in the superior sagittal sinus, a similarly sized cerebral vein, during acute hypertension (3). The pressure-servo controller and peristaltic pump were disconnected from the transducer immediately, and the intravascular pressure decreased over time due to filtration and was measured for 40 min (Fig. 1). Cerebral veins were excluded from results if the decrease in intravascular pressure was >10 mmHg during the first 2 min of the experiment, or if there was a visible leakage site, not due to filtration. Therefore, the system was tested for leaks during setup for each experiment by disconnecting the pressure-servo controller and peristaltic pump from the transducer and measuring the decrease in pressure with the stopcock before the cannula closed. If a leak was detected, all connections were tightened until no leak was observed.

Conversion of the decrease in intravascular pressure per minute (mmHg/min) to actual volume flux across the vessel wall (µm/s) was necessary to determine J/S and Lp. To do this, a conversion graph of pressure vs. volume was made (Fig. 2). Because of the low volume of fluid flux at these low pressures, the volume of fluid for different decreases in pressure was determined. The amount of HEPES-PSS that flowed out of the tip of the cannula after a decrease in pressure without a vessel connected to the cannula was captured in a capillary tube. The length and diameter of the fluid in the capillary tube were measured using video microscopy, and the volume of HEPES-PSS was calculated, assuming the capillary tube was a right cylinder.

Statistical analysis. All results are presented as means ± SE. Differences in blood pressure, intravascular pressure drop, J/S, and Lp were determined by one-way ANOVA with a post hoc Student-Newman-Keuls test for multiple comparisons. Differences were considered significant at P < 0.05.

RESULTS

Blood pressure. Blood pressures of HTN, HTN + Rosi, and HTN + Tempol animals were all significantly higher during
the study than those of CTL animals (P < 0.01 vs. CTL). Treatment with rosiglitazone or Tempol for the last 3 wk out of a total of 5 wk of L-NAME treatment did not decrease blood pressure, consistent with previous studies that have shown that L-NAME hypertension is persistent after 4–6 wk of NOS inhibition, even if L-NAME is discontinued (12, 28, 38, 52). The systolic, diastolic, and mean blood pressures of the last week of the study (wk 5) are shown in Table 1.

Intravascular pressure. A total of five animals, two from the HTN + Rosi group, two from the HTN group, and one from the CTL group, were excluded from the data due to leaks in the vessels.

Figure 1A shows the decrease in intravascular pressure vs. time in CTL, HTN, and HTN + Rosi animals. The decrease in intravascular pressure of the HTN animals was significantly greater than in CTL and HTN + Rosi animals (P < 0.05), suggesting an increase in venous permeability during chronic hypertension and reversal by rosiglitzone treatment. Figure 1B shows the intravascular pressure decrease of the HTN + Tempol animals compared with the HTN and CTL animals. Unlike in the HTN + Rosi animals, there was no significant difference in intravascular pressure decrease between the HTN and the HTN + Tempol animals, suggesting that Tempol did not reverse the increased venous permeability in hypertension.

Jv/S. Changes in Jv/S vs. time are shown in Fig. 3A for the CTL, HTN, and HTN + Rosi animals. As suggested by the increased drop in intravascular pressure (Fig. 1), Jv/S was significantly higher in the HTN animals compared with the CTL and HTN + Rosi animals (P < 0.05), indicating that rosiglitazone effectively reversed the increase in venous filtration caused by hypertension. Figure 3B shows Jv/S for the HTN + Tempol animals compared with the HTN and CTL animals. As with the intravascular pressure decrease, the HTN + Tempol animals were similar to the HTN animals and also significantly different from CTL animals, suggesting that Tempol did not reverse the increased filtration during chronic hypertension. In all cases, however, Jv/S did not change with time over the 40 min of the measurements during the experiment.

Lp. Changes in Lp vs. time of the CTL, HTN, and HTN + Rosi animals are shown in Fig. 4A. Lp of HTN animals was

### Table 1. Body weights and blood pressures for all groups of animals

<table>
<thead>
<tr>
<th></th>
<th>CTL</th>
<th>HTN</th>
<th>HTN + Rosi</th>
<th>HTN + Tempol</th>
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<tr>
<td>n</td>
<td>9</td>
<td>8</td>
<td>5</td>
<td>8</td>
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<td>Body weight, g</td>
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<td>316±8</td>
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<td>158±3*</td>
<td>163±4*</td>
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<tr>
<td>Systolic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
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<td>122±5*</td>
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<td>127±3*</td>
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<tr>
<td>Mean</td>
<td>111±2</td>
<td>134±5*</td>
<td>132±2*</td>
<td>138±3*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. CTL, untreated controls; HTN, rats treated with Nω-nitro-l-arginine methyl ester (L-NAME; 0.5 g/l in drinking water) for 5 wk; HTN + Rosi, rats treated with L-NAME for 5 wk plus the peroxisome proliferator-activated receptor-γ agonist rosiglitzone (20 mg/kg in food) for the last 3 wk; HTN + Tempol, rats treated with L-NAME for 5 wk plus the superoxide dismutase mimetic Tempol (1 mmol/l in drinking water) for the last 3 wk. *P < 0.05 vs. CTL.
significantly higher than that of CTL and HTN + Rosi animals (P < 0.05), demonstrating that rosiglitazone effectively reversed the increase in Lp induced by chronic hypertension. Figure 4B shows the effect of Tempol treatment (HTN + Tempol) on Lp compared with HTN and CTL. In contrast to the rosiglitazone treatment, Tempol treatment did not reverse the increase in Lp by L-NAME hypertension. However, unlike Jv/S, Lp increased over time in the HTN and HTN + Tempol animals.

DISCUSSION

The major findings of the present study were that chronic hypertension induced by L-NAME significantly increased both Jv/S and Lp in cerebral veins, and that treatment with rosiglitazone completely reversed this increase in venous permeability (Figs. 3A and 4A). In contrast to rosiglitazone treatment, Tempol had no effect on the increase in Jv/S and Lp induced by chronic hypertension (Figs. 3B and 4B), suggesting that the reversal of venous permeability by rosiglitazone was not likely due to antioxidant effects. While other studies have shown that PPAR-γ activation can protect the BBB from disruption during conditions such as traumatic brain injury and ischemic stroke (48, 51), the major novel finding from the present study was that PPAR-γ activation may protect the BBB from disruption during chronic hypertension. In addition, because rosiglitazone treatment was given 2 wk after the onset of hypertension, it appears that PPAR-γ activation effectively reversed changes in venous permeability induced by hypertension.

The results from this study demonstrate that chronic hypertension produced by L-NAME decreased barrier properties of the cerebral endothelium, as demonstrated by the significant increase in Lp and Jv/S compared with normotensive CTL (Figs. 3 and 4). This effect on venous permeability is likely due to effects of chronic hypertension and not NOS inhibition per se. For example, acute NOS inhibition reduces Lp of rat mesenteric veins, suggesting that nitric oxide (NO) directly enhances permeability (42). Mayhan (24) showed that administration of the NOS inhibitor N(G)-methyl-L-arginine before acute hypertension decreased permeability to albumin and protected the BBB. In another study by Mayhan (23), the NO

Fig. 3. The effect of L-NAME hypertension, Rosi treatment, and Tempol treatment on water filtration in cerebral veins after an acute elevation in pressure from 10 to 25 mmHg. A: transvascular filtration per surface area (Jv/S) vs. time for CTL (●), HTN (○), and HTN + Rosi (▲). HTN animals had an increase in filtration that was reversed by Rosi treatment. *P < 0.05 vs. CTL and HTN + Rosi. B: Jv/S vs. time for CTL, HTN, and HTN + Tempol (○). HTN + Tempol and HTN animals had increased filtration that was not affected by Tempol. *P < 0.05 vs. CTL.

Fig. 4. The effect of L-NAME hypertension, Rosi treatment, and Tempol treatment on hydraulic conductivity (Lp) in cerebral veins after an acute elevation in pressure from 10 to 25 mmHg. A: Lp vs. time for CTL (●), HTN (○), and HTN + Rosi (▲). HTN increased Lp that was reversed by Rosi treatment. *P < 0.05 vs. CTL and HTN + Rosi. B: Lp vs. time for CTL, HTN, and HTN + Tempol (○). Tempol treatment had no effect on the increase in Lp during hypertension. *P < 0.05 vs. CTL.
donors 3-morpholinosydnonimine and S-nitroso-N-acetyl-penicillamine were shown to increase BBB permeability, an effect that was prevented by pretreatment with the superoxide scavenger tiron. These results suggest that it is the combination of NO and superoxide to form peroxynitrite that increases BBB permeability and not NO itself. In the present study, chronic NOS inhibition with sustained hypertension produced decreased barrier properties, suggesting that this effect was related to the hypertensive condition and not to the lack of NO.

Other studies have also shown that chronic NOS inhibition that causes hypertension enhances BBB permeability (1, 17, 21). Chronic L-NAME-induced hypertension promotes numerous complications, including renal damage (glomerulosclerosis and ischemia), vascular inflammation, oxidative stress, and activation of both the renin-angiotensin system and sympathetic nervous system (52). It is likely that one or all of these events contribute to the persistent nature of L-NAME hypertension that remains, even if NOS inhibition is discontinued (12, 28, 38). In this study, treatment with rosiglitazone or Tempol had no effect on elevated blood pressure induced by L-NAME. Although both of these compounds have been shown to lower blood pressure in other models (43, 44), they were given after animals had been on L-NAME for 2 wk in this study, further demonstrating the persistent nature of L-NAME hypertension that is the likely cause of increased venous permeability.

One of the major findings from this study was that, despite chronic, persistent hypertension, rosiglitazone reversed the increase in venous permeability that occurred during hypertension (Figs. 3A and 4A). There are several mechanisms by which L-NAME-induced hypertension could affect BBB properties that may also be altered by PPAR-γ activation. L-NAME hypertension has been shown to cause dysregulation of tight junction proteins, including zona occludens 1 and occludin (1). Tight junctions are an essential component of the BBB that restrict the movement of most molecules into the brain parenchyma (20, 41). Tight junctions are composed of several transmembrane proteins, including occludin, claudins, and zona occludens 1 (20). Decreased protein expression and/or localization of these tight junction proteins have been shown to have a profound effect on barrier properties and are associated with increases in permeability (2, 22). PPAR-γ activation has been shown to attenuate dysregulation of tight junction proteins in the BBB in other models through an effect on matrix metalloproteinase and protease activity (15). It is, therefore, possible that the protective effect of rosiglitazone on venous permeability induced by chronic L-NAME hypertension was due to a beneficial effect at the level of tight junctions.

PPAR-γ activation has been shown to have protective effects on the cerebral endothelium of spontaneously hypertensive stroke-prone rats (SHRSP), a model of chronic hypertension with significant cerebrovascular dysfunction (31). Hypertension in SHRSP can progress to hypertensive encephalopathy and develop similar neurological complications, including BBB disruption and hemorrhage (31, 47). Treatment of SHRSP with the PPAR-γ activator pioglitazone for 4 wk protected against hypertension-induced cerebrovascular injury, including suppression of remodeling of large cerebral blood vessels, improving endothelial function, and decreasing superoxide levels through inhibition of NADPH oxidase activity (31). Although changes in BBB permeability were not specifically investigated in that study, pioglitazone decreased infiltration of macrophages into the brain, suggesting that PPAR-γ activation improved other aspects of BBB properties during chronic hypertension. Similar to the present study, pioglitazone improved vascular function in SHRSP, independent of blood pressure lowering.

Several studies have shown that PPAR-γ agonists are protective of the cerebral endothelium, possibly by reducing superoxide production through decreased NADPH oxidase activity (7, 11, 31, 32, 39, 43). While most studies have assessed the effect of PPAR-γ activation on endothelium-dependent regulation of vascular tone, other indexes of the health of the cerebral endothelium are barrier properties. Oxidative stress has been shown to be a significant contributor to BBB disruption via several mechanisms, including lipid peroxidation, tight junction protein expression and/or phosphorylation, cytoskeletal reorganization, and matrix metalloproteinase activation (37). In the present study, we did not detect any effect of Tempol on venous permeability (Figs. 3B and 4B), suggesting that superoxide is not likely to be the cause of the increases in venous permeability in this model. In addition, our results also suggest that the mechanism by which rosiglitazone reversed the increase in venous permeability was not via antioxidant effects related to superoxide. Although it is possible that the dose of Tempol used was not high enough to sufficiently decrease superoxide in these animals, we chose a dose that has been shown to be effective in chronic L-NAME hypertensive animals (44), making this possibility less likely. It is worth noting that we measured venous permeability in response to an acute elevation in pressure to mimic what occurs during acute hypertension. Acute hypertension has been shown to increase superoxide levels in the brain (50); however, one study showed that disruption of the BBB was not affected by either acute or chronic treatment with antioxidants (34). Together, these results suggest enhanced venous permeability during hypertension was not due to superoxide; however, a role for other oxidants, such as hydrogen peroxide (H₂O₂), cannot be ruled out.

In the present study, we used the vein of Galen to measure $L_p$ and $J_v/S$ during hypertension, with and without different treatments. We specifically focused on veins, because they have been shown to be a major site of disruption of the BBB during acute hypertension (23–26). Although studies on BBB disruption during acute hypertension have found that pial venules disrupt (23–26), larger veins, such as the superior sagittal sinus, also have severe elevations in pressure during acute hypertension (3). Thus, similar to the arterial side of the circulation, both large and small veins are significantly affected by acute hypertension and experience a large increase in hydrostatic pressure.

Identifying mechanisms that contribute to disruption of the BBB during acute hypertension has been difficult. In an effort to better define these mechanisms, we developed a new model that focuses on changes in permeability in the venous portion of the circulation, but that may also allow more detailed mechanistic and translational studies. Because our methodology to measure $L_p$ and $J_v/S$ required an unbranched segment of vessel, pial vessels could not be used (preliminary studies found that pial veins and venules were highly branched and very difficult to cannulate and pressurize). Using this technique, we measured $L_p$ and $J_v/S$ in response to an acute elevation in intravascular pressure from 10 to 25 mmHg. These
values were chosen because they are in the range that the venous circulation experiences during acute hypertension that causes BBB disruption and edema formation, but is not high enough to cause rupture (3). We found that veins from CTL and HTN + Rosi animals had significantly less transvascular filtration than that of untreated HTN animals or HTN + Tempol (Fig. 3). In addition to lower water permeability, CTL and HTN + Rosi animal $L_p$ values were constant over time, whereas, in the other groups, $L_p$ increased over the 40 min of measurement, suggesting that barrier properties were degrading over time (Fig. 4). Therefore, not only was water permeability enhanced during hypertension, this dysfunction appeared to progress over time.

Our results showed that the mean $L_p$ of cerebral veins in CTL animals was $19 \pm 1 \times 10^{-11} \text{cm}^2\cdot\text{s}^{-1}\cdot\text{cmH}_2\text{O}^{-1}$, a value that is lower than that previously measured in rat brain arterioles ($13 \pm 4 \times 10^{-9} \text{cm}^2\cdot\text{s}^{-1}\cdot\text{cmH}_2\text{O}^{-1}$) (19) and in frog cerebral microvessels ($2 \pm 0.7 \times 10^{-9} \text{cm}^2\cdot\text{s}^{-1}\cdot\text{cmH}_2\text{O}^{-1}$) (13). However, our data also show that mesenteric veins had an approximately threefold increase in $L_p$ (data not shown), which is in agreement with previous studies (19), and suggests that differences in methodology for measuring $L_p$ may account for our different values. One major difference between our methodology and others that used modified Landis techniques to measure $L_p$ and $J_r/s$ was that we had the same fluid (HEPES-PSS) on both the luminal and abluminal sides of the vessel. We did this not just to simplify our calculation, but to compare $L_p$ and $J_r/s$ under similar conditions without the influence of different plasma constituents found in vivo that would influence the measurements. This is particularly important when considering conditions such as pregnancy that have profoundly different ionic and oncotic pressures in plasma (6).

In summary, the present study used a new technique to compare $J_r/s$ and $L_p$ in cerebral veins from control and hypertensive rats. We found that chronic NOS inhibition increased $J_r/s$ and $L_p$ in cerebral veins, and that this effect was reversed by rosiglitazone, but not Tempol. These results suggest that PPAR-γ may have a major role in changes in BBB permeability during hypertension and that activators of PPAR-γ may protect against vasogenic edema during acute hypertension.

**GRANTS**

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