Pregnancy Reverses Hypertensive Remodeling of Cerebral Arteries

Marilyn J. Cipolla, Jeremiah Smith, Nicole Bishop, Lisa V. Bullinger, Julie A. Godfrey

Abstract—Previous studies have shown that pregnancy prevents hypertensive remodeling of cerebral arteries. In the present study, we sought to determine whether pregnancy could reverse preexisting remodeling. Nonpregnant virgin Sprague-Dawley rats were treated with the NO synthase inhibitor nitro-L-arginine (0.5 g/L in drinking water) for 2 weeks before mating, after which treatment continued until late gestation for a total of 5 weeks. Pregnant animals with preexisting hypertension (n=6) were compared with nonpregnant animals that were treated with nitro-L-arginine for either 2 (n=8) or 5 (n=9) weeks and compared with nontreated controls (n=8). Blood pressure, passive and active diameters, wall thickness, media thickness, and passive distensibility of cerebral arteries were compared between groups. Treatment with nitro-L-arginine caused a significant increase in mean arterial pressure in all of the groups compared with controls that was sustained for the entire study: 103±3 versus 137±2, 141±4, and 140±7 mm Hg (P<0.01). Both 2 and 5 weeks of hypertension caused inward eutrophic remodeling in nonpregnant animals, characterized by decreased inner and outer lumen diameters and no change in media thickness. Pregnancy reversed this remodeling, because late-pregnant animals with preexisting hypertension had inner and outer diameters similar to controls. Passive distensibility was significantly less, and active myogenic tone increased in all of the hypertensive animals, independent of pregnancy. These results demonstrate that pregnancy reverses preexisting hypertensive remodeling of cerebral arteries without a decrease in blood pressure. This reversal of protective remodeling during hypertension in pregnancy may be detrimental by lowering the upper limit of autoregulation, whereas blood pressure remains elevated. (Hypertension. 2008;51:1052-1057.)

Key Words: hypertension pregnancy cerebral arteries remodeling

Chronic hypertension is associated with significant remodeling and/or medial hypertrophy of cerebral arteries, which are thought to have a protective function.1,2 Both remodeling and hypertrophy normalize wall stress that is elevated because of increased blood pressure and shift the autoregulatory curve to the higher range of pressures.1–4 These processes are also protective of the microcirculation by attenuating increases in downstream pressure. For example, spontaneously hypertensive and stroke-prone spontaneously hypertensive rats, known to undergo medial hypertrophy, were less susceptible to blood-brain barrier (BBB) disruption during acute hypertension than normotensive Wistar Kyoto rats that did not undergo medial hypertrophy.5–7 In addition, medial hypertrophy is thought to stiffen the vascular wall, making cerebral arteries more resistant to forced dilatation during acute hypertension, thereby protecting the BBB from disruption.1,2

Hypertension during pregnancy is one of the most common complications of pregnancy, occurring in ≈8% of all pregnancies.8 It is a unique form of hypertension, somewhere between acute and chronic. In a previous study, it was shown that pregnancy prevented hypertension-induced hypertrophy of cerebral arteries, suggesting that this form of hypertension is indeed unique.9 Understanding how hypertension during pregnancy affects the cerebral circulation is important because of the risk of hypertension leading to neurologic complications and eclampsia.10 Eclampsia is thought to be similar to hypertensive encephalopathy in which an acute elevation in pressure causes forced dilatation of cerebral arteries, hyperperfusion, and BBB disruption.10–12 Therefore, prevention of hypertension-induced medial hypertrophy or remodeling during pregnancy may be detrimental and promote forced dilatation at lower pressures than in the nonpregnant state.

Preexisting chronic hypertension is a known risk factor for developing eclampsia.13 If pregnancy is a state that prevents protective hypertensive remodeling of cerebral arteries, we surmised that it may also reverse preexisting remodeling. This effect of pregnancy may be a mechanism by which preexisting hypertension is a risk factor for developing neurologic complications by making the cerebral circulation more susceptible to forced dilatation and autoregulatory breakthrough, ie, the upper limit of autoregulatory breakthrough would be lowered, whereas blood pressure remains elevated. In the present study, we measured structural (passive diameter, wall thickness, and wall:lumen ratio) and functional (active myogenic reactivity and tone) properties of
cerebral arteries from animals that were hypertensive by NO synthase inhibition with nitro-L-arginine (L-NAME) for 2 weeks before pregnancy and then remained on L-NAME for the remaining 3 weeks of gestation. We compared this group to animals that were nonpregnant and either normotensive or hypertensive by L-NAME treatment for either 2 weeks (to determine how much remodeling occurred before pregnancy) or 5 weeks (to compare to how much remodeling occurred by the end of gestation).

Methods

Animal Model
A rat model of pregnancy was used for all of the experiments. All of the 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. Animals were virgin nonpregnant control Sprague-Dawley rats that were either normotensive (NP-CTL; n=8) or treated with L-NAME (0.5g/L) in their drinking water to raise blood pressure for 2 weeks (NP-HTN-2; n=8) or 5 weeks (NP-HTN-5; n=8). To determine whether pregnancy could reverse hypertensive remodeling of cerebral arteries, a separate group of nonpregnant animals was treated with L-NAME for 2 weeks, after which the animals were mated and pregnancy confirmed by vaginal smear. These animals were kept on L-NAME throughout pregnancy and experiments performed on day 18 of a 22 day gestation (LP-HTN; n=8). A schematic of the experimental plan is shown in Figure 1. A group of late-pregnant normotensive animals was purposely omitted from the remaining 3 weeks of gestation. A group of nonpregnant animals were not treated and considered normotensive controls (NP-CTL; dashed line). Arrows indicate when animals were compared.

Experimental Protocol
Arteries were equilibrated for 1 hour at 25 mm Hg, after which the active response to pressure was determined by increasing pressure to 200 mm Hg in 25-mm Hg increments. Lumen diameter and wall thickness were measured at each pressure once stable, at ~10 minutes. To obtain fully relaxed diameter and wall measurements, papaverine (0.1 mmol/L) was added to the bath to fully relax the smooth muscle. In the presence of papaverine, pressure was lowered from 200 mm Hg to 1 mm Hg in 25-mm Hg increments until 50 mm Hg, after which pressure was lowered in 10-mm Hg increments. Diameter and wall thickness were measured at each pressure. After passive measurements were obtained, vessels were chemically fixed with glutaraldehyde for measurement of media thickness by transmission electron microscopy. Briefly, at the end of an experiment, fully relaxed arteries were pressurized to 75 mm Hg, and 1 mL of 25% glutaraldehyde was added to the bath. The arteries were fixed on the cannulas for 30 minutes and then carefully removed and placed in 2.5% glutaraldehyde for an additional 30 minutes. Arteries were then stored in PBS until processed. Arteries were processed for transmission electron microscopy in a usual manner, as described previously. All of the electron micrographs were taken at ×8000. The images were digitized and imported into an image analysis software (Metamorph) for measurement of media thickness, as described previously.

Data Calculations
Wall:lumen ratio was calculated from the inner diamater (ID) and wall thickness measurements from the video dimension analyzer by the equation:

\[ \frac{\varphi_{\text{wall}}}{\omega} \]

where \( \omega \) is wall thickness and \( \varphi_{\text{wall}} \) is the ID of the artery. Outer diameter (OD) was calculated by the equation \( \varphi_{\text{wall}} + 2\omega \).

Distensibility was calculated at each pressure, fully relaxed in papaverine, by determining diameter changes as a function of pressure and calculated by the following equation:

\[ \left[ \frac{\varphi_{\text{pap}}}{\varphi_{\text{max, pap}}} - 1 \right] \times 100 \]

where \( \varphi_{\text{pap}} \) is the diameter at that particular pressure and \( \varphi_{\text{max, pap}} \) is the diameter at 5 mm Hg. Distensibility for each artery was normalized to the diameter at 5 mm Hg, because arteries often collapse at lower pressures. Percent tone was calculated as a percentage decrease in diameter from the fully relaxed diameter in papaverine at each intravascular pressure by the following equation:

\[ 1 - \left( \frac{\varphi_{\text{tone}}}{\varphi_{\text{pap}}} \right) \times 100\% \]

where \( \varphi_{\text{tone}} \) is the diameter of vessels with tone and \( \varphi_{\text{pap}} \) is the diameter in papaverine.

Statistical Analysis
All of the results are presented as means±SEMs. Differences in blood pressure, passive structural measurements, percentage of

Figure 1. Schematic of experimental design. Nonpregnant animals were treated with L-NAME for either 2 weeks (NP-HTN-2) or 5 weeks (NP-HTN-5) and compared with nonpregnant animals that were treated with L-NAME for 2 weeks, mated, and then continued on L-NAME for ~3 weeks during gestation (LP-HTN). A group of nonpregnant animals were not treated and considered normotensive controls (NP-CTL; dashed line). Arrows indicate when animals were compared.
distensibility, and active diameters were determined by 1-way ANOVA with a posthoc Student-Newman-Keuls test for multiple comparisons. Differences were considered significant at P<0.05. Nonstatistical differences were considered at P>0.05.

Results

Blood Pressures and Animal Characteristics

Table 1 shows blood pressures for all of the groups of animals, measured weekly starting at the end of week 1. L-NAME treatment caused a significant increase in systolic, diastolic, and mean arterial pressure in all of the groups that was sustained during the duration of the study (either 2 or 5 weeks). This rise in pressure was similar in all of the groups and was significantly greater than NP-HTN-2 or NP-HTN-5 animals. (P<0.01). Importantly, the LP-HTN animals that were nonpregnant for weeks 1 to 2 did not have a drop in pressure during weeks 3 to 5 when they were pregnant.

Preexisting hypertension did not appear to have an adverse effect on the animals that became pregnant, although this was not a primary outcome of the study. All of the LP-HTN animals carried gestation until experimentation on day 18, except for 1 animal that died unexpectedly on day 12 of gestation. Only data from surviving animals were used for statistical analysis. A necropsy of the animal that died on day 12 revealed no unusual findings of pregnancy; however, inspection of the brain revealed significant edema formation. Brain water content in that animal, measured by the difference between wet and dry brain weights, was 79.5%. This result is considerably greater than what we have found previously in normal pregnant animals, suggesting that this animal died from cerebral edema formation.

One animal that was mated was not pregnant on the day of the experiment and, therefore, was used as a nonpregnant-treated animal for 5 weeks. Animal numbers and statistical analyses were adjusted to reflect the 1 death and 1 failed mating in the LP-HTN group and the additional animal in the NP-HTN-5 group. In addition, 1 animal in the NP-HTN-2 group had a cerebral artery that did not develop active tone and, therefore, was included in the passive measurements only.

Passive Measurements

Figure 2 shows passive pressure versus ID curves for all of the groups of animals. Both groups of nonpregnant hypertensive animals (NP-HTN-2 and NP-HTN-5) had significantly smaller lumen diameters at all of the pressures studied, demonstrating inward remodeling. There was no difference whether the animals were hypertensive for 2 versus 5 weeks, because their diameters were similar. However, pregnancy had a profound effect on hypertensive remodeling. LP-HTN animals had lumen diameters that were significantly greater than either NP-HTN-2 or NP-HTN-5 and were similar to the normotensive control animals. Because the LP-HTN animals were hypertensive for 2 weeks before pregnancy, this demonstrates that pregnancy reversed the remodeling that occurred during that time, which

Table 1. Weekly Blood Pressures (mm Hg) From All of the Groups of Animals

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Blood Pressure, mm Hg</th>
<th>NP-CTL (n=8)</th>
<th>NP-HTN-2 (n=8)</th>
<th>NP-HTN-5 (n=9)</th>
<th>LP-HTN (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Systolic</td>
<td>128±2</td>
<td>158±3*</td>
<td>159±3*</td>
<td>168±4*</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>90±2</td>
<td>119±3*</td>
<td>121±3*</td>
<td>129±4*</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>102±2</td>
<td>132±3*</td>
<td>131±3*</td>
<td>142±4*</td>
</tr>
<tr>
<td>Week 2</td>
<td>Systolic</td>
<td>127±5</td>
<td>164±1*</td>
<td>163±4*</td>
<td>165±5*</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>88±4</td>
<td>124±3*</td>
<td>126±3*</td>
<td>126±4*</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>102±4</td>
<td>137±2*</td>
<td>140±3*</td>
<td>139±5*</td>
</tr>
<tr>
<td>Week 3</td>
<td>Systolic</td>
<td>130±3</td>
<td>NA</td>
<td>128±5*</td>
<td>128±6*</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>103±3</td>
<td>NA</td>
<td>143±5*</td>
<td>139±6*</td>
</tr>
<tr>
<td>Week 4</td>
<td>Systolic</td>
<td>128±2</td>
<td>NA</td>
<td>166±4*</td>
<td>160±7*</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
<td>92±3</td>
<td>NA</td>
<td>128±4*</td>
<td>121±7*</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>105±3</td>
<td>NA</td>
<td>140±4*</td>
<td>134±7*</td>
</tr>
<tr>
<td>Week 5</td>
<td>Systolic</td>
<td>129±2</td>
<td>NA</td>
<td>165±4*</td>
<td>169±4*</td>
</tr>
<tr>
<td></td>
<td>Diastolic</td>
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<td>NA</td>
<td>129±3*</td>
<td>128±5*</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>106±2</td>
<td>NA</td>
<td>141±3*</td>
<td>141±4*</td>
</tr>
</tbody>
</table>

NA indicates not applicable.

*P<0.01 vs NP-CTL.

Figure 2. Passive lumen diameters vs pressure of all of the groups studied. NP-CTL animals had lumen diameters significantly greater than NP-HTN-2 or NP-HTN-5 animals. LP-HTN animals had lumen diameters similar to normotensive controls. **P<0.05 and ***P<0.01 NP-CTL vs NP-HTN-2 and NP-HTN-5; ****P<0.01 LP-HTN vs NP-HTN-2 and NP-HTN-5.
would have been similar to the NP-HTN-2 animals, because they also had 2 weeks of hypertension. The reversal of remodeling by pregnancy was not because of a drop in blood pressure, because all of the animals treated with l-NAME, both nonpregnant and pregnant, had significantly increased pressures that were similar (Table 1).

Table 2 shows passive structural remodeling in all of the groups of animals at 75 mm Hg. Both groups of hypertensive nonpregnant animals had significantly smaller IDs and ODS compared with controls (P<0.01), demonstrating that 2 and 5 weeks of hypertension caused inward remodeling. Similar to Figure 2, there was no difference in remodeling, whether hypertension was for 2 versus 5 weeks. However, pregnancy reversed this remodeling and had IDs and ODS similar to NP-HTN-2 and NP-HTN-5 animals (P>0.05). Table 2 also shows that wall thickness, as measured using video microscopy, was decreased in both nonpregnant l-NAME-treated groups but not in LP-HTN animals. Interestingly, this was not because of a change in media thickness that was measured by transmission electron microscopy. Media thickness was similar between all of the groups regardless of pregnancy or hypertension. Therefore, the remodeling associated with l-NAME treatment of nonpregnant animals could be considered inward eutrophic because there was a decrease in ID and OD, with no change in media thickness.²

Unlike passive diameter measurements, all of the groups of l-NAME–treated animals had decreased passive distensibility compared with normotensive animals (Figure 3). In fact, although pregnancy reversed hypertensive remodeling of cerebral arteries, it did not reverse changes in distensibility.

Because passive distensibility is an indirect measure of the collagen:elastin ratio in the vascular wall,¹⁶ these results suggest that hypertension affects acellular components similarly, regardless of pregnancy.

Active Measurements
Cerebral arteries from all of the groups of animals demonstrated considerable myogenic tone and responded myogenically to increases in pressure. Figure 4 shows the active pressure versus diameter curves for all of the groups of animals over the entire pressure range studied (25 to 200 mm Hg). All of the groups of animals treated with l-NAME, both nonpregnant and pregnant, had active diameters that were smaller than NP-CTL animals (P<0.01 versus all of the groups). At pressures between 75 and 150 mm Hg, LP-HTN animals had active diameters that were significantly increased compared with NP-HTN-2 and NP-HTN-5 animals (P<0.01) but not as great as NP-CTL animals. The fact that LP-HTN animals had active diameters that were in between normotensive and hypertensive NP-HTN-2 and NP-HTN-5 animals is likely because the LP-HTN animals had arteries with a similar increase in myogenic tone (Figure 5) but had inward remodeling that was reversed, whereas NP-HTN-2 and NP-HTN-5 animals had a combination of inward remodeling and increased myogenic tone (Table 2 and Figure 5).

Discussion
The major findings of this study were that nonpregnant animals exposed to either 2 or 5 weeks of l-NAME hyper-

![Figure 3. Passive distensibility (%) vs pressure for all of the groups of animals. NP-CTL had significantly greater distensibility than NP-HTN-2, NP-HTN-5, or LP-HTN animals. *P<0.05 and **P<0.01 vs NP-HTN-2, NP-HTN-5, and LP-HTN.](image1)

![Figure 4. Active diameter vs pressure for all of the groups of animals. NP-CTL animals had active diameters that were significantly greater than any of the hypertensive animals regardless of pregnancy. However, at pressures between 75 and 150 mm Hg, LP-HTN animals had active diameters that were significantly greater than either NP-HTN-2 or NP-HTN-5 animals. *P<0.01 vs NP-HTN-2, NP-HTN-5, and LP-HTN; **P<0.01 vs NP-HTN-2 and NP-HTN-5.](image2)
tension had posterior cerebral arteries that underwent inward eutrophic remodeling, as shown by the significant decrease in IDs and ODs without a change in media thickness. Importantly, pregnancy reversed the inward remodeling and had cerebral arteries with both IDs and ODs that were similar to normotensive nonpregnant control animals (Figure 2). Because the pregnant animals were hypertensive for 2 weeks before pregnancy and would have had remodeling similar to that of the NP-HTN-2 animals, these results demonstrate that the pregnant state reversed the remodeling. However, this reversal appeared independent of changes in pressure, because it occurred although pressures remained elevated to similar levels as both nonpregnant hypertensive groups for the duration of the study.

Although passive diameters from LP-HTN animals were similar to normotensive control animals, passive distensibility was decreased and more similar to nonpregnant hypertensive animals. It therefore seems that whereas pregnancy reversed cerebrovascular remodeling induced by L-NAME hypertension, it did not affect the change in distensibility. Changes in distensibility of cerebral arteries and arterioles have been noted previously in response to chronic hypertension, with studies finding increased or no change depending on the type of remodeling and the size of the vessel studied.4,17–20 In 2 previous studies, we demonstrated that normal pregnancy did not affect passive distensibility in normotensive animals compared with nonpregnant animals.9,14 Therefore, the fact that LP-HTN animals had decreased distensibility suggests that this effect was related to NO synthase inhibition and hypertension and not the pregnant state.

Wall thickness was measured under passive conditions using video microscopy that is accurate within ±1 μm.21 This methodology cannot distinguish hypertrophy of different layers within the vascular wall, and, therefore, we used transmission electron microscopy to visualize and measure the medial layer. Interestingly, we found that, whereas wall thickness was decreased in nonpregnant groups treated with L-NAME compared with untreated controls, this was not because of a change in media thickness. Because animals treated with L-NAME had decreased wall thickness and distensibility, it is possible that the change in wall thickness is a reflection of a change in the nonecular layers of the wall, such as the internal elastic laminae, and that this contributed to the wall thickness of those animals.

Although remodeling and hypertension of cerebral arteries during hypertension have been well documented, the mechanism by which this occurs, or its reversal, is less understood. One mechanism of interest to this study is the effect of the renin-angiotensin system and the role of angiotensin II (Ang II) in vascular remodeling. In stroke-prone spontaneously hypertensive rats, angiotensin-converting enzyme inhibition reversed the remodeling of cerebral22–24 and mesenteric arteries,25 although an effect of blood pressure lowering could not be ruled out. A similar effect was noted with Ang II type 1 (AT1) receptor antagonism,26,27 suggesting that this receptor is involved in hypertension-induced hypertrophy and remodeling. In pregnancy, circulating levels of Ang II are significantly elevated with a concomitant refractoriness to the vasoconstrictor effects of infused Ang II,28,29 suggesting the pregnancy alters Ang II receptor density and/or affinity. Numerous studies have examined AT1 and Ang II type 2 (AT2) receptor numbers on systemic and uteroplacental vessels and have shown that they are altered in pregnancy, with most studies finding a decrease in receptor number with no change in affinity.30–32 Although no studies have investigated how pregnancy affects Ang II receptors on the cerebral circulation, Faraci et al33 demonstrated a significant gender effect of Ang II on the cerebral circulation. Basilar arteries from male mice constricted to Ang II, whereas arteries from female mice were relatively unresponsive. One possible explanation for this difference is a gender-induced decrease in AT1 receptors (the receptor thought to be responsible for Ang II–induced vasoconstriction), which were not measured in that study. Pregnancy may have a similar effect on cerebral artery Ang II type receptors, and if so, may be one mechanism by which pregnancy reverses hypertensive remodeling independent of blood pressure lowering.

In addition to measuring structural changes induced by hypertension, active responses to pressure were also determined. Cerebral arteries from all of the groups of animals had considerable myogenic tone (Figure 5) and responded to pressure myogenically (Figure 4). These active responses are thought to contribute to segmental vascular resistance in the brain and autoregulation of cerebral blood flow, respectively.34 NO synthase inhibition caused a significant increase in myogenic tone in all of the groups treated, independent of pregnancy. The increase in tone is likely because of the lack of vasodilatory NO in those animals, because there is considerable basal NO produced in cerebral vessels that mitigates tone35 and likely contributed to the considerably smaller active diameters in the L-NAME-treated animals (Figure 4). In fact, it is likely that the combination of smaller passive diameters, due to remodeling and increased tone, caused the substantial decrease in active diameters of nonpregnant hypertensive animals. Because pregnancy reversed remodeling, but did not affect the increase in tone, this likely explains why pregnant hypertensive animals had diameters that were in between normotensive control and hypertensive nonpregnant animals.
Perspectives
Pregnancy has a profound effect on cerebrovascular structure and function. In the present study, we used a model of hypertension that has been used previously to investigate hypertensive remodeling of cerebral arteries.\(^1\)\(^7\)\(^-\)\(^9\) Although this model of hypertension in pregnancy is also considered a model of preeclampsia,\(^\text{10}\) we were not specifically studying this disease state but used this model of hypertension to determine the effect of preexisting hypertension on cerebrovascular remodeling. The importance of finding that pregnancy both prevents and reverses hypertensive remodeling may be related to the development of neurologic complications during preeclampsia and eclampsia. Eclampsia is thought to be similar to hypertensive encephalopathy, in which an acute elevation in pressure causes forced dilation of cerebral arteries and arterioles, hyperperfusion, and BBB disruption.\(^\text{10}\)\(^-\)\(^\text{12}\) The reversal of hypertension-induced inward remodeling during pregnancy would shift the upper limit of autoregulation to lower pressures whereas blood pressure remains elevated. This scenario could promote autoregulatory breakthrough, hyperperfusion, BBB disruption, and the neurologic complications of eclampsia.

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Disclosures
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References
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