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Cerebral Blood Flow Autoregulation and Edema Formation During Pregnancy in Anesthetized Rats

Anna G. Euser, Marilyn J. Cipolla

Abstract—Eclampsia is considered a form of hypertensive encephalopathy in which an acute elevation in blood pressure causes autoregulatory breakthrough, blood–brain barrier disruption, and edema formation. We hypothesized that pregnancy predisposes the brain to eclampsia by lowering the pressure of autoregulatory breakthrough and enhancing cerebral edema formation. Because NO production is increased in pregnancy, we also investigated the role of NO in modulating autoregulation. Cerebral blood flow autoregulation was determined by phenylephrine infusion and laser Doppler flowmetry. Four groups were studied: untreated nonpregnant (n=7) and late-pregnant (days 19 to 21; n=8) Sprague–Dawley rats and nonpregnant (n=8) and late-pregnant (n=8) animals treated with an NO synthase inhibitor (N^G-nitro-L-arginine methyl ester; 0.5 to 0.7 g/L). Brain water content and blood–brain barrier permeability to sodium fluorescein were determined after breakthrough. Pregnancy caused no change in autoregulation or the pressure of breakthrough. However, treatment with the NO synthase inhibitor significantly increased the pressure of autoregulatory breakthrough (nonpregnant: 183.6±3.0 mm Hg versus 212.0±2.8 mm Hg, *P*<0.05; late-pregnant: 180.8±3.2 mm Hg versus 209.3±4.7 mm Hg, *P*<0.05). After autoregulatory breakthrough, only late-pregnant animals showed a significant increase in cerebral edema formation, which was attenuated by NO synthase inhibition. There was no difference in blood–brain barrier permeability between nonpregnant and late-pregnant animals in response to acute hypertension, suggesting that pregnancy may predispose the brain to eclampsia by increasing cerebral edema through increased hydraulic conductivity. (*Hypertension*. 2007;49:334-340.)

Key Words: autoregulation ■ eclampsia ■ L-NAME ■ laser Doppler flowmetry ■ NO synthase ■ pregnancy

Eclampsia is a hypertensive disorder of pregnancy that occurs when hypertension in pregnancy presents with neurologic complications, including headache, nausea, vomiting, visual disturbances, and death.^{1,2} This disease remains a leading cause of maternal and fetal mortality worldwide.^{3–5} In fact, it is estimated that 40% of eclamptic deaths are due to cerebral involvement.¹

Eclampsia is thought to be a form of hypertensive encephalopathy.^{6–8} This acute syndrome occurs from a sudden and excessive elevation of blood pressure that causes forced dilatation of the cerebrovasculature, autoregulatory breakthrough, and hyperperfusion that leads to disruption of the blood–brain barrier (BBB) and vasogenic edema formation.^{6,9,10} There is considerable evidence to suggest that eclampsia and hypertensive encephalopathy are similar, including similar symptoms (headache, nausea, vomiting, visual disturbances, and, in the most severe cases, convulsions)^{2,6,9} and comparable findings on imaging that indicate white matter edema and evidence of localized BBB disruption.^{6,11,12} In addition, clinical reports demonstrate increased cerebral blood flow (CBF) both before and after the onset of eclamptic seizures,^{10,13–16} further supporting eclampsia as a hyperperfusion disorder.

Our previous studies on isolated and pressurized posterior cerebral arteries demonstrated a significant decrease in the pressure at which force dilatation occurred in late-pregnant (LP) versus nonpregnant (NP) rats.¹⁷ These data suggest that CBF autoregulatory breakthrough may occur at lower pressures in pregnancy, perhaps predisposing women to the neurologic complications of eclampsia during episodes of hypertension. Therefore, this study sought to understand how pregnancy alone affects cerebral hemodynamics, including autoregulation, which may be an important step to preventing and treating eclampsia.

In addition to pregnancy, CBF autoregulation may be modulated by NO. It has been shown that NO synthase (NOS) inhibition during acute hypertension extended the autoregulatory range,¹⁸ suggesting that NO may play an active role in mediating the pressure of autoregulatory breakthrough. Because NO production is significantly increased during pregnancy,^{19–21} it is possible that this is a mechanism by which autoregulation is shifted during pregnancy.

The goal of the present study was to examine CBF autoregulation during pregnancy and to determine the upper limit of autoregulation in LP and NP rats. We also investigated the contribution of NOS to CBF autoregulation. Auto-

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Characteristics of Groups Studied

Group	Average Litter Size	Animals With Fetal Resorption	Body Weight, g	ABP, mm Hg	PAB, rCBF=2.0, mm Hg
NP (n=7)			288.6±7.2	114.4±2.6	183.6±3.0
LP (n=8)	12.4	1	323.1±8.3*	111.1±3.3	180.8±3.2
NP+L-NAME (n=8)			291.3±7.1	149.5±4.6*	212.0±2.8*
LP+L-NAME (n=8)	13.0	1	322.5±7.8‡	120.4±7.1‡	209.3±4.7‡

* $P < 0.05$ vs NP control; † $P < 0.05$ vs LP control; ‡ $P < 0.05$ vs NP+L-NAME.

regulatory curves were determined in LP and NP Sprague–Dawley rats using a model of acute hypertension in vivo with and without NOS inhibition using N^G-nitro-L-arginine methyl ester (L-NAME). In addition, because eclampsia has been shown to be associated with increased cerebral edema,^{6,9,11} we also determined cerebral edema formation and BBB permeability after autoregulatory breakthrough, a potential mechanism of edema formation.

Methods**Animal Model**

All of the experiments used a rat model of pregnancy and were conducted using Sprague–Dawley female rats (Charles River). It has been shown that gravid rats undergo many cardiovascular changes similar to those seen in human pregnancy.²² Animals were housed in the University of Vermont Animal Care Facility, an Association for Assessment and Accreditation of Laboratory Care-accredited facility. All of the 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. Virgin animals were used for NP experiments. LP experiments were conducted on days 19 to 21 of gestation. A total of 54 animals were studied. Further animal characteristics are summarized in the Table.

NOS Inhibition and Determination of Blood Pressure

For experiments investigating the effect of NOS inhibition on CBF autoregulation, the NOS inhibitor L-NAME (Sigma) was used. L-NAME was administered to animals in their drinking water for 7 days (0.5 g/L for NP animals and 0.7 g/L for LP animals). These doses have been shown to cause similar elevations of blood pressure in NP and LP animals.²³ The time course of L-NAME treatment was chosen to mimic the last trimester of pregnancy (7 days of a 22-day gestation) when eclampsia most often occurs in gestation.³ Based on earlier studies,²³ this period of treatment was sufficient to cause mean arterial pressure elevation as determined by the noninvasive tail cuff technique (Coda 6 System, Kent Scientific). Arterial blood pressure was monitored over the 7-day treatment period, as described previously.²³ Animals treated with L-NAME had their blood pressures monitored for 1 to 3 days before the initiation of L-NAME treatment and at least 6 of the 7 days of L-NAME treatment. For each day, the average of 3 representative measurements was taken (data not shown).

In Vivo Acute Hypertension Model

For determination of CBF autoregulatory curves, a model of acute hypertension was used that allowed for continuous recording of both CBF and arterial blood pressure in vivo. Anesthesia was initiated with isoflurane ($\leq 3\%$ in O₂, inhaled), which was then tapered off and discontinued. Anesthesia was maintained with intravenous pentobarbital (≤ 60 mg kg⁻¹ h⁻¹), which was decreased, as tolerated, during the surgical preparation to minimize effects on experimental parameters. Adequate anesthesia was assessed by toe pinch and

changes in arterial blood pressure. Animals were mechanically ventilated with a mixture of compressed air and 100% O₂ via tracheostomy. Ventilation was adjusted to maintain arterial blood gases within physiological ranges (pH 7.35 to 7.45, pCO₂ 35 to 45 mm Hg, and pO₂ ≥ 100 mm Hg).

CBF was measured transcranially using laser Doppler flowmetry with a 1-mm probe (Perimed). The right side of the skull was exposed and cleared of membranes. The flow probe was affixed over a thinned area of skull posterior to the coronal suture and lateral to the sagittal suture over the middle cerebral artery perfusion domain, as described elsewhere.²⁴

A femoral arterial catheter was used to obtain blood samples for analysis (Medica), and to measure arterial blood pressures via a pressure servo transducer (Living Systems Instrumentation). A filtered solution of heparin sulfate and lactated Ringer's solution (1000 U in 6 mL) was used within the arterial catheter to prevent clotting. Two femoral venous catheters were placed to deliver pentobarbital and phenylephrine (PE, 0.01 g/10 mL of lactated Ringer's solution, Sigma) intravenously. PE dosage was increased at regular intervals starting at 0.5 μ L/min (0.5 μ g/min) to ensure a consistent rise in arterial blood pressure. After CBF autoregulatory curves were obtained and evidence of breakthrough occurred, elevated blood pressure and CBF were maintained for 10 minutes. Animals were quickly decapitated, and the brain was then removed for wet and dry weight measurements. The brain was first weighed wet followed by drying in an oven at 100°C for 24 hours, at which point the brain was weighed again dry.

Four groups of animals were studied for determination of CBF autoregulation curves: NP (n=7), LP (n=8), NP animals treated with L-NAME (NP+L-NAME, 0.5 g/L for 7 days, n=8), and LP animals treated with L-NAME (LP+L-NAME, 0.7 g/L for 7 days, n=8). In addition, 2 control groups were added for brain water content analysis to control for edema because of either pregnancy and/or the response to the surgical preparation of acute hypertension. These groups are designated as NP without (w/o) hypertension (HTN) and LP w/o HTN.

Determination of CBF Autoregulatory Curves

Autoregulatory curves were determined for each animal by analysis of CBF and pressure tracings. Tracings of CBF and arterial blood pressures were collected during PE infusion using commercially available software (Figure 1; Perisoft, Perimed). During acute hypertension, the average CBF and arterial pressure were determined for the same time point over a range of pressures from baseline to autoregulatory breakthrough. These data were used to determine pressure versus flow curves for each experimental group. The point at which the curve became vertical was taken to signify autoregulatory breakthrough.

Relative CBF

Because laser Doppler units are a relative measure of changes in CBF, the laser Doppler signal was normalized to the flow at baseline (after anesthesia had been minimized and before PE administration) to determine a relative CBF (rCBF). The following equation was used: rCBF=(CBF_x/CBF_{baseline}), where CBF_x is the flow in laser Doppler units at various pressures and doses of phenylephrine, and CBF_{baseline} is the flow in laser Doppler units at the start of the

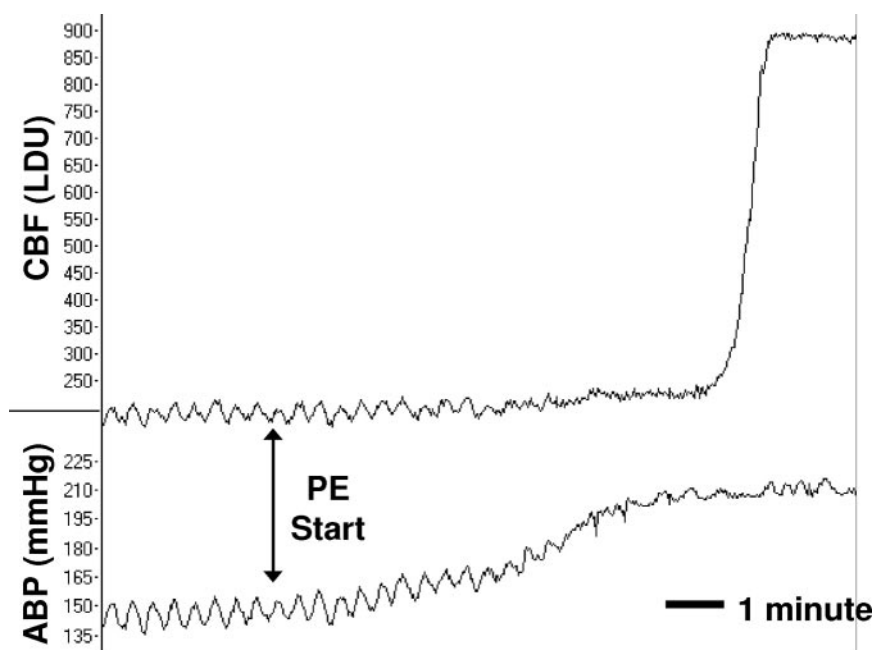


Figure 1. Tracing of CBF (in laser Doppler units) and ABP (in mm Hg) in response to increasing doses of PE. In this experiment, CBF increased 4 times greater than baseline as ABP was increased from 140 to 210 mm Hg demonstrating autoregulatory breakthrough.

experiment. For example, rCBF of 2 signifies a 2-fold increase in CBF from baseline.

Brain Water Content

The percentage of water content of the brain is a measure of cerebral edema.²⁵ Brain water content (in percentage) was determined by the following formula: $[(\text{weight}_{\text{wet}} - \text{weight}_{\text{dry}}) / \text{weight}_{\text{wet}}] \times 100\%$; where $\text{weight}_{\text{wet}}$ was the weight of the brain immediately after removal from the animal, and $\text{weight}_{\text{dry}}$ was the weight of the brain after drying.

In Vivo BBB Solute Permeability Studies

To investigate BBB permeability in response to acute hypertension, studies were conducted that combined the in vivo acute hypertension model described above with central infusion of a dye tracer, sodium fluorescein (NaFl, 376 Da; Sigma). Animals were prepared and instrumented as described for the in vivo acute hypertension model with the addition of the placement of a catheter (22-gauge) in the right common carotid artery before the infusion of PE. A solution of 0.1% NaFl in lactated Ringer's solution filled the catheter, and 0.5 mL of this solution was infused directly into the left ventricle of the heart and allowed to circulate for 10 minutes before beginning the PE infusion (starting at 0.5 $\mu\text{L}/\text{min}$ [0.5 $\mu\text{g}/\text{min}$]). Once arterial pressures had reached ≥ 180 mm Hg (sufficient to cause autoregulatory breakthrough), arterial blood pressure and CBF were maintained for 10 minutes, the same time course as the cerebral edema formation experiments. The cerebral circulation was then flushed by perfusion with 40 mL of lactated Ringer's solution through the carotid catheter, and at the same time the chest was opened and the right atrium snipped to allow for the removal of the dye from the vasculature. Animals were quickly decapitated and the brain removed. Any animals in which appropriate flushing of the vasculature was not evident on gross examination were excluded from analysis. The brain was weighed and then homogenized in 10 mL of 50% trichloroacetic acid (Sigma) 15 times in glass Dounce tissue grinders. Homogenized samples were centrifuged at 4000g at 4°C for 10 minutes. The supernatant was analyzed by fluorescence spectrophotometry (460 to 515 nm) to determine dye clearance into the brain tissue.

Four groups were studied for in vivo BBB solute permeability: NP sham (n=4), NP HTN (n=4), LP sham (n=4), and LP HTN (n=4). Sham controls were surgically prepared in an identical manner with the exception that PE was not infused; thus, no acute hypertension occurred. The time course of the sham experiments was similar to

that of an experiment with acute hypertension. Data are expressed as average fluorescence counts per second (CPS) per gram of brain tissue.

Statistical Analysis

All of the data are expressed as mean \pm SEM. Differences in arterial blood pressures at different rCBFs between NP and LP groups and between untreated control and L-NAME-treated groups were determined by an ANOVA with a posthoc Student–Newman–Keuls test for multiple comparisons. Similarly, differences in brain water content between nonsurgical controls (w/o HTN) and NP and LP groups, between nonsurgical controls (w/o HTN) and L-NAME-treated groups, and between untreated control and L-NAME-treated groups were determined by an ANOVA. Differences in BBB permeability between groups were determined by ANOVA and a posthoc Student–Newman–Keuls test for multiple comparisons. Differences were considered significant if $P < 0.05$.

Results

The Table presents data on the characteristics of each group of animals studied. L-NAME treatment during the last trimester of pregnancy did not affect average litter size, the number of animals showing fetal resorption, or the body weight of the animal. However, the body weight of pregnant animals was significantly greater than either NP group, as expected. Arterial blood pressures, determined at the time of surgery via arterial catheter, were similar between groups before PE infusion with the exception of the NP+L-NAME group, which had a significantly higher baseline blood pressure (Table and Figure 2).

All of the groups of animals showed CBF autoregulation over a range of pressures up to the pressure of autoregulatory breakthrough (PAB), as shown in Figure 2. The PAB was determined for each group at an rCBF of 2 and is reported in the Table. There was a dramatic increase in the PAB in L-NAME-treated animals versus NP and LP control groups, respectively (at rCBF of 2: NP animals, 183.6 \pm 3.0 mm Hg versus 212.0 \pm 2.8 mm Hg, $P < 0.05$; LP animals, 180.8 \pm 3.2 mm Hg versus 209.3 \pm 4.7 mm Hg, $P < 0.05$). No

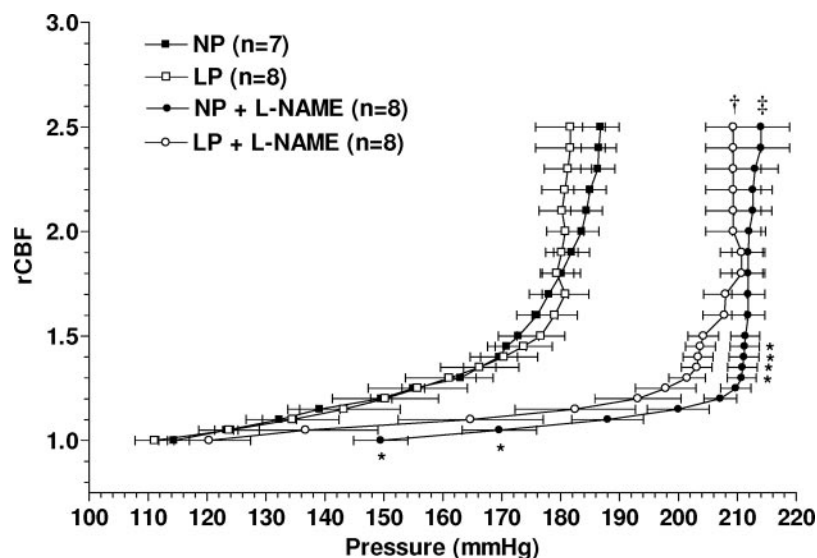


Figure 2. Graph showing autoregulatory curves for all groups of animals. All of the animals maintained CBF autoregulation (represented as rCBF) up to the PAB. There was no difference between NP and LP controls (■ and □, respectively). Treatment of animals with L-NAME caused a significant shift in the PAB to higher pressures for both NP and LP animals (● and ○, respectively). **P*<0.05 vs LP+L-NAME, †*P*<0.05 vs LP control (all points rCBF >1.15), ‡*P*<0.01 vs NP control.

differences were observed between NP and LP control groups at any point on the autoregulatory curve. However, in L-NAME-treated animals, between rCBFs of 1.0 to 1.05 and 1.30 to 1.45, there was a shift to lower pressures in LP+L-NAME versus NP+L-NAME animals (*P*<0.05).

Brain water content was used as a measure of cerebral edema formation.²⁵ After autoregulatory breakthrough had occurred because of acute hypertension, brain water content was determined for each animal (Figure 3). Brain water content was also determined for additional control groups that did not undergo surgery or acute hypertension (NP w/o HTN and LP w/o HTN) to control for any effects of pregnancy or the surgical preparation. There was no difference in brain water content between any of the NP groups, regardless of acute hypertension or L-NAME treatments (NP w/o HTN 77.84±0.22%, NP acute HTN 77.70±0.11%, and NP L-NAME+acute HTN 77.60±0.15%). However, there was a significant increase in brain water content after autoregulatory breakthrough in LP animals that underwent acute hyper-

tension versus NP animals. In addition, L-NAME treatment significantly attenuated the rise in brain water content because of autoregulatory breakthrough in LP animals (LP w/o HTN 77.86±0.05%, LP acute HTN 78.56±0.10%, and LP L-NAME+acute HTN 78.28±0.08%). Lastly, increased cerebral edema was not due to pregnancy alone, because only those animals that underwent autoregulatory breakthrough had increased edema formation.

To investigate the mechanism by which brain water content was increased in LP animals in response to acute hypertension, the permeability of the BBB to a small solute under the same conditions was determined. The passage of NaFl into cerebral brain tissue was determined in response to acute hypertension as shown in Figure 4. Acute hypertension caused an increase in permeability in both NP and LP animals compared with sham controls, although this was not statistically significant. However, there was no difference in BBB permeability between NP and LP animals under either sham or acute hypertensive conditions (NP sham 9930.0±3056.7

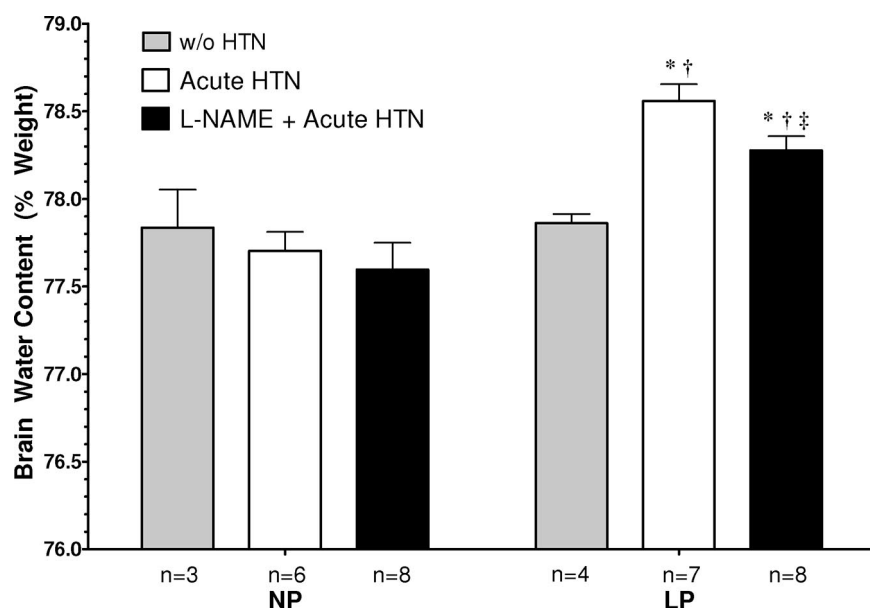


Figure 3. Graph showing percentage of brain water content for all groups of animals. Controls (■, w/o HTN) did not undergo acute hypertension or autoregulatory breakthrough. There was no difference in brain water content between NP groups. LP animals (□ and ■) had increased brain water content after autoregulatory breakthrough (acute HTN) compared with animals that did not undergo acute hypertension (LP w/o HTN, □). Treatment with L-NAME attenuated brain water content in LP animals. **P*<0.01 vs respective NP control, †*P*<0.01 vs LP w/o HTN, ‡*P*<0.01 vs LP acute HTN group.

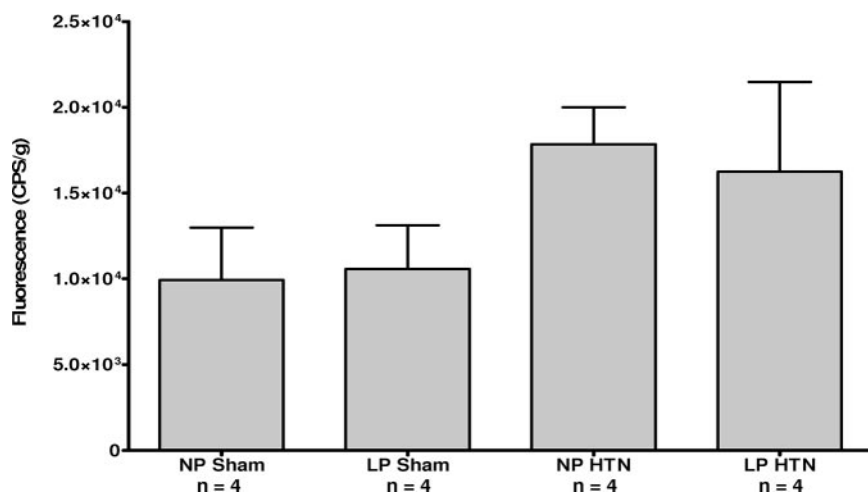


Figure 4. Graph showing average fluorescence (CPS/g) of sodium fluorescein in the brain as a measure of BBB permeability in response to acute hypertension. Sham controls did not undergo phenylephrine infusion to cause acute hypertension. No difference in permeability was seen between NP and LP groups.

CPS/g versus LP sham $10\,568.5 \pm 2564.4$ CPS/g; NP HTN $17\,855.0 \pm 2151.8$ CPS/g versus LP HTN $16\,245.8 \pm 5237.9$ CPS/g).

Discussion

There were several major findings in this study that examined CBF autoregulation, cerebral edema, and BBB permeability during pregnancy. Both NP and LP animals demonstrated CBF autoregulation up to ≈ 180 mm Hg that was similar between gestational groups. Likewise, the PAB was not different between untreated NP and LP animals. However, treatment with the NOS inhibitor L-NAME shifted the autoregulatory curve to significantly higher pressures in both NP and LP animals, suggesting that NOS has an active role in modulating the PAB. In addition, edema formation after autoregulatory breakthrough was significantly increased in LP versus NP animals, demonstrating that pregnancy alone promotes cerebral edema formation when pressure is elevated. There was no difference in BBB permeability to NaFl between LP and NP groups, suggesting that the increase in cerebral edema formation was not primarily because of increased solute permeability. Lastly, NOS inhibition attenuated edema formation after autoregulatory breakthrough in the LP+L-NAME group, further suggesting that increased NO production during pregnancy may contribute to the enhanced edema formation.

The present study is the first to examine CBF autoregulatory breakthrough and edema formation during pregnancy. Establishing the autoregulatory pressure range in female animals and the effect of pregnancy on this cerebrovascular parameter is important because of the hyperperfusory nature of eclampsia. Clinical reports have demonstrated increased CBF in the maternal brain both preceding and after the onset of eclamptic seizures,^{10,13,14,16} as well as in severe preeclampsia.^{15,16} Clinical evidence also suggests that the autoregulatory curve is shifted to a lower range of pressures during pregnancy as evidenced by the onset of seizures at relatively low mean arterial pressures when compared with cases of hypertensive encephalopathy.^{1,13} In addition, our *in vitro* data demonstrated that the pressure of force dilatation was lower during pregnancy, also suggesting that PAB would be lower.¹⁷ However, the results of the present study did not show a

difference in CBF autoregulation or PAB between NP and LP animals. This discrepancy with our *in vitro* studies may be because of the fact that CBF autoregulation is a complex interaction of endothelial, neuronal, and metabolic influences that cannot be mimicked *in vitro*. In addition, our *in vitro* studies examined the posterior cerebral artery, whereas this *in vivo* study used laser Doppler to measure changes in flow in the middle cerebral artery perfusion domain, and it is possible that different regions of the brain have differing autoregulatory capabilities. Although autoregulation may not differ with normal gestation, it remains possible that circulating factors and/or oxidative damage as part of eclampsia could cause greater endothelial dysfunction that affects either the PAB or edema formation. This would agree with clinical reports of neurologic complications and seizures at lower pressures in settings of endothelial dysfunction.^{11,13,26}

An important finding of the present study was the effect of NOS inhibition on CBF autoregulation. Treatment with L-NAME for 7 days shifted the autoregulatory curve to higher pressures in both groups of animals, suggesting that NO has an active role in determining autoregulation and the PAB. In addition, arterial forced dilatation before autoregulatory breakthrough seems to be an active process that involves the pressure-dependent production of NO rather than a mechanical dilation. Studies by Talman and Dragon¹⁸ also suggest that NO has an active role in autoregulatory breakthrough in male rats, because NOS inhibition prevented autoregulatory breakthrough. This concept is further supported by work that showed that potassium channel inhibition shifted autoregulatory curves to higher pressures.²⁷ Taken together, these findings suggest that autoregulatory breakthrough is an active process that is mediated by both NO and possibly potassium channels.

An alternative explanation for the shift in autoregulation because of NOS inhibition is vascular adaptation due to either hypertension-induced vascular remodeling or L-NAME-induced vasoconstriction. However, studies have shown that while L-NAME treatment for just 7 days caused medial hypertrophy and an increased wall:lumen ratio in NP animals, these same vascular adaptations were not seen in LP animals.²³ A similar lack of remodeling was seen in pregnant Dahl salt-sensitive hypertensive animals,²⁸ suggesting that autoregulation is shifted

to higher pressures after NOS inhibition for reasons other than structural vascular adaptations. An effect of L-NAME on the contractile state and increased cerebrovascular resistance cannot be ruled out from these studies.

Cerebral edema is one of the hallmark pathologies of the eclamptic brain and is tied to the hyperperfusible nature of the disease. It has been shown that 93% of eclamptic women studied with diffusion-weighted MRI had evidence of cerebral vasogenic edema.¹¹ Other classic imaging findings in eclampsia are subcortical white matter edema with evidence of localized BBB disruptions, particularly in parietal–occipital locations.^{6,7,12} The results of the present study demonstrate that LP animals had increased cerebral edema formation after autoregulatory breakthrough that was not evident in NP animals. Because enhanced BBB permeability can cause edema formation, we determined permeability to NaFl in response to acute hypertension in NP and LP animals. Our results show that whereas there was a nonsignificant increase in permeability in response to acute hypertension, there was not a difference in permeability between NP and LP animals, suggesting that the mechanism by which pregnancy enhances edema is not because of enhanced solute permeability.

An alternative mechanism by which pregnancy may be affecting edema formation is through aquaporin expression in the brain. Aquaporin 4, located in astrocytic end feet^{29,30} and cerebral endothelium,²⁹ has been shown to be significantly upregulated in the brain during pregnancy.³¹ This gestational effect may influence the formation and management of cerebral edema during acute hypertension. Because this study did not find a difference in BBB permeability despite the increased cerebral edema formation after acute hypertension, it is possible that in the first 10 minutes after autoregulatory breakthrough, there is a specific movement of water across the BBB independent of an increase in solute permeability. We hypothesize that pregnancy acts to increase water permeability (hydraulic conductivity) by increased aquaporin expression leading to significantly enhanced cerebral edema formation. Interestingly, L-NAME treatment decreased cerebral edema formation in response to acute hypertension, possibly because of increased cerebrovascular resistance and reduced microvascular pressure that protects the microvessels from the increased hydrostatic pressure associated with autoregulatory breakthrough.

Perspectives

The pathogenesis of eclampsia seems to begin with an acute elevation in blood pressure leading to autoregulatory breakthrough and hyperperfusion of the brain. Subsequent vasogenic edema formation likely contributes to the clinical symptoms of eclampsia. The results from this study suggest that changes during normal pregnancy may predispose women to the occurrence of eclampsia when arterial blood pressure is acutely elevated above the reference range. In particular, the enhancement of cerebral edema formation without a change in autoregulation could potentiate the neurologic complications of eclampsia. Because we found that pregnancy did not increase BBB permeability, it seems that increased cerebral edema is because of an increase in

hydraulic conductivity (possibly by an increase in aquaporin expression). In addition, it seems that NO contributes to the PAB and edema formation. These data lend further insight into the process of autoregulatory breakthrough, which is an important component in the pathogenesis of eclampsia.

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Disclosures

None.

References

1. Donaldson JO. *Neurology of Pregnancy*. London, United Kingdom: W.B. Saunders Company; 1989.
2. Sibai BM. Diagnosis, prevention, and management of eclampsia. *Obstet Gynecol*. 2005;105:402–410.
3. Mattar F, Sibai BM. Eclampsia. VIII. Risk factors for maternal morbidity. *Am J Obstet Gynecol*. 2000;182:307–312.
4. Duley L. Maternal mortality associated with hypertensive disorders of pregnancy in Africa, Asia, Latin America and the Caribbean. *Br J Obstet Gynaecol*. 1992;99:547–553.
5. MacKay AP, Berg CJ, Atrash HK. Pregnancy-related mortality from preeclampsia and eclampsia. *Obstet Gynecol*. 2001;97:533–538.
6. Schwartz RB, Feske SK, Polak JF, DeGirolami U, Iaia A, Beckner KM, Bravo SM, Klufas RA, Chai RY, Repke JT. Preeclampsia-eclampsia: Clinical and neuroradiographic correlates and insights into the pathogenesis of hypertensive encephalopathy. *Radiology*. 2000;217:371–376.
7. Engelter ST, Provenzale JM, Petrella JR. Assessment of vasogenic edema in eclampsia using diffusion imaging. *Neuroradiology*. 2000;42:818–820.
8. Williams KP, Wilson S. Persistence of cerebral hemodynamic changes in patients with eclampsia: a report of three cases. *Am J Obstet Gynecol*. 1999;181:1162–1165.
9. Wityk RJ, Pessin MS. Hypertensive encephalopathy. In: Batjer HH, ed. *Cerebrovascular Disease*. Philadelphia, PA: Lippincott–Raven; 1997:97–102.
10. Oehm E, Reinhard M, Keck C, Els T, Spreer J, Hetzel A. Impaired dynamic cerebral autoregulation in eclampsia. *Ultrasound Obstet Gynecol*. 2003;22:395–398.
11. Zeeman GG, Fleckenstein JL, Twickler DM, Cunningham FG. Cerebral infarction in eclampsia. *Am J Obstet Gynecol*. 2004;190:714–720.
12. Schwartz RB, Jones KM, Kalina P, Bajakian RL, Mantello MT, Garada B, Holman BL. Hypertensive encephalopathy: findings on CT, MR imaging, and SPECT imaging in 14 cases. *Am J Roentgenol*. 1992;159:379–383.
13. Ohno Y, Wakahara Y, Michiyasu K, Arii Y. Cerebral hyperperfusion in patient with eclampsia. *Acta Obstet Gynecol Scand*. 1999;78:555–556.
14. Belfort MA. Is high cerebral perfusion pressure and cerebral flow predictive of impending seizures in preeclampsia? A case report. *Hypertens Pregnancy*. 2005;24:59–63.
15. Zeeman GG, Hatab MR, Twickler DM. Increased cerebral blood flow in preeclampsia using magnetic resonance imaging. *Am Journal Obstet Gynecol*. 2004;191:1425–1429.
16. Zunker P, Happe S, Georgiadis AL, Louwen F, Georgiadis D, Ringelstein EB, Holzgreve W. Maternal cerebral hemodynamics in pregnancy-related hypertension. A prospective transcranial doppler study. *Ultrasound Obstet Gynecol*. 2000;16:179–187.

17. Cipolla MJ, Vitullo L, McKinnon J. Cerebral artery reactivity changes during pregnancy and the postpartum period: a role in eclampsia? *Am J Physiol Heart Circ Physiol*. 2004;286:H2127–H2132.
18. Talman WT, Dragon DN. Inhibition of nitric oxide synthesis extends cerebrovascular autoregulation during hypertension. *Brain Res*. 1995;672:48–54.
19. Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. *Am J Physiol*. 1997;272:R441–R463.
20. Salas SP. Role of nitric oxide in maternal hemodynamics and hormonal changes in pregnant rats. *Biol Res*. 1998;31:243–250.
21. Boccardo P, Soregaroli M, Aiello S, Noris M, Donadelli R, Lojacono A, Benigni A. Systemic and fetal-maternal nitric oxide synthesis in normal pregnancy and pre-eclampsia. *Br J Obstet Gynaecol*. 1996;103:879–886.
22. Gilson GJ, Mosher MD, Conrad KP. Systemic hemodynamics and oxygen transport during pregnancy in chronically instrumented, conscious rats. *Am J Physiol*. 1992;263:H1911–H1918.
23. Cipolla MJ, DeLance N, Vitullo L. Pregnancy prevents hypertensive remodeling of cerebral arteries: a potential role in the development of eclampsia. *Hypertension*. 2006;47:619–626.
24. Smeda JS, VanVliet BN, King SR. Stroke-prone spontaneously hypertensive rats lose their ability to auto-regulate cerebral blood flow prior to stroke. *J Hypertens*. 1999;17:1697–1705.
25. Schwab M, Bauer R, Zwiener U. The distribution of normal brain water content in Wistar rats and its increase due to ischemia. *Brain Res*. 1997;749:82–87.
26. Hinchey J, Chaves C, Appignani B, Breen J, Pao L, Wang A, Pessin MS, Lamy C, Mas JL, Caplan LR. A reversible posterior leukoencephalopathy syndrome. *N Engl J Med*. 1996;334:494–500.
27. Paterno R, Heistad DD, Faraci FM. Potassium channels modulate cerebral autoregulation during acute hypertension. *Am J Physiol Heart Circ Physiol*. 2000;278:H2003–H2007.
28. Aukes AM, Vitullo L, Zeeman GG, Cipolla MJ. Pregnancy prevents hypertensive remodeling and decreases myogenic reactivity in posterior cerebral arteries in Dahl salt-sensitive rats: Role in eclampsia? *Am J Physiol Heart Circ Physiol*. DOI: 10.1152/ajpheart.00980.2006.
29. Amiry-Moghaddam M, Xue R, Haug FM, Neely JD, Bhardwaj A, Agre P, Adams ME, Froehner SC, Mori S, Ottersen OP. Alpha-syntrophin deletion removes the perivascular but not endothelial pool of aquaporin-4 at the blood-brain barrier and delays the development of brain edema in an experimental model of acute hyponatremia. *FASEB J*. 2004;18:542–544.
30. Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P, Ottersen OP. Specialized membrane domains for water transport in glial cells: High-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci*. 1997;17:171–180.
31. Quick AM, Cipolla MJ. Pregnancy-induced up-regulation of aquaporin-4 protein in brain and its role in eclampsia. *FASEB J*. 2005;19:170–175.