Effect of Pregnancy and Nitric Oxide on the Myogenic Vasodilation of Posterior Cerebral Arteries and the Lower Limit of Cerebral Blood Flow Autoregulation

Reproductive Sciences
20(9) 1046-1054
© The Author(s) 2013
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719112473661
rs.sagepub.com

Abbie C. Chapman, BS^{1,2}, Marilyn J. Cipolla, PhD^{2,3,4}, and Siu-Lung Chan, PhD²

Abstract

Hemorrhage during parturition can lower blood pressure beyond the lower limit of cerebral blood flow (CBF) autoregulation that can cause ischemic brain injury. However, the impact of pregnancy on the lower limit of CBF autoregulation is unknown. We measured myogenic vasodilation, a major contributor of CBF autoregulation, in isolated posterior cerebral arteries (PCAs) from nonpregnant and late-pregnant rats ($n = 10/\text{group}$) while the effect of pregnancy on the lower limit of CBF autoregulation was studied in the posterior cerebral cortex during controlled hemorrhage ($n = 8$). Pregnancy enhanced myogenic vasodilation in PCA and shifted the lower limit of CBF autoregulation to lower pressures. Inhibition of nitric oxide synthase (NOS) prevented the enhanced myogenic vasodilation during pregnancy but did not affect the lower limit of CBF autoregulation. The shift in the autoregulatory curve to lower pressures during pregnancy is likely protective of ischemic injury during hemorrhage and appears to be independent of NOS.

Keywords

CBF autoregulation, hypotension, myogenic vasodilation, nitric oxide, pregnancy

Introduction

Cerebral blood flow (CBF) autoregulation is an intrinsic property of the brain that maintains relatively constant blood flow despite fluctuations in blood pressure (BP).^{1,2} In normotensive adults, CBF autoregulation operates within the arterial pressure range of approximately 60 to 160 mm Hg, outside of which autoregulation is lost and CBF becomes dependent on pressure in a linear fashion.²⁻⁵ A drop in BP within this autoregulatory range results in insignificant clinical symptoms as brain perfusion is maintained by autoregulatory mechanisms.² However, when BP falls below the lower limit of CBF autoregulation, CBF decreases with pressure, potentially causing loss of consciousness and hypoxic-ischemic brain injury.⁶⁻⁸ During pregnancy, hemorrhage occurs with parturition. In some pregnancies, hemorrhage may be severe (>1500 mL blood loss) and cause an acute drop in maternal BP potentially below the lower limit of CBF autoregulation.⁹⁻¹¹ However, whether pregnancy alters the lower limit of CBF autoregulation is not known, but is important to understand. For example, a shift in CBF autoregulation to lower pressures during pregnancy may be protective to the brain, allowing maintenance of blood flow in the face of acute hypotension. Alternatively, a shift of the lower limit of CBF autoregulation to higher pressures could increase the susceptibility of the brain to injury during parturition.

The myogenic response of cerebral arteries and arterioles is a major contributor to CBF autoregulation.¹² Myogenic vasodilation occurs as the BP decreases, contributing to the maintenance of blood flow to the brain.⁵ If BP decreases below the lower limit of CBF autoregulation, maximal dilation of cerebral vessels occurs and this vascular contributor to CBF autoregulation becomes insufficient to maintain brain perfusion.⁸ Several mechanisms may be involved in the relaxation of vascular smooth muscle (VSM) to decrease intravascular pressure, including endothelial vasodilators such as nitric oxide (NO).^{13,14} Pregnancy has been shown to increase the expression of endothelial-NO synthase (eNOS) in several vascular

¹ Neuroscience Graduate Program, University of Vermont College of Medicine, Burlington, VT, USA

² Department of Neurological Sciences, University of Vermont College of Medicine, Burlington, VT, USA

³ Department of Obstetrics, Gynecology and Reproductive Sciences, University of Vermont College of Medicine, Burlington, VT, USA

⁴ Department of Pharmacology, University of Vermont College of Medicine, Burlington, VT, USA

Corresponding Author:

Marilyn J. Cipolla, Department of Neurological Sciences, University of Vermont College of Medicine, 149 Beaumont Ave., HSRF 416A, Burlington, VT 05405, USA.

Email: Marilyn.Cipolla@uvm.edu

Table 1. Physiological Parameters of Nonpregnant (NP), Late-Pregnant (LP) and LP Rats Infused With L-NAME During Hemorrhagic Hypotension to Assess the Lower Limit of CBF Autoregulation.

	NP (n = 8)	LP (n = 8)	LP + L-NAME (n = 7)
Weight, g	342 ± 6	442 ± 11	389 ± 12
Arterial pH	7.40 ± 0.01	7.33 ± 0.01	7.42 ± 0.01
Arterial P _{CO} ₂	40.1 ± 1.6	40.3 ± 1.5	40.2 ± 1.4
Arterial P _O ₂	128 ± 8	139 ± 14	121 ± 6

Abbreviation: L-NAME, N^ω-nitro-L-arginine methyl ester hydrochloride.

beds.^{15,16} However, the involvement of NO in the myogenic vasodilatory response of cerebral arteries and CBF autoregulation during pregnancy is yet to be investigated.

In the present study, *in vitro* methodology was used to investigate the myogenic vasodilatory response to decreased intravascular pressure of posterior cerebral arteries (PCAs) from nonpregnant (NP) and late-pregnant (LP) rats. The contribution of NO to myogenic vasodilation during pregnancy was also assessed. We found that pregnancy enhanced myogenic vasodilation in response to decreased pressure that was NO dependent. To test whether this enhanced myogenic vasodilation translates to a shift of CBF autoregulation, the effect of pregnancy on the lower limit of CBF autoregulation was measured using an *in vivo* model of hemorrhagic hypotension and measuring changes in CBF in the posterior cerebral cortex. We further investigated the role of NO on CBF autoregulation during acute hypotension by infusing a NOS inhibitor.

Materials and Methods

Animal Model

All experiments were conducted using virgin NP female (14–16 weeks) or timed-pregnant Sprague Dawley rats (Charles River, Canada). Rats were housed individually in the University of Vermont Animal Care Facility. The NP females were chosen randomly and timed-pregnant rats were studied during LP on days 19 to 21 of a 22-day gestation. All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Isolated Vessel Preparation and Pressurized Arteriograph System

The NP and LP animals were anesthetized with isoflurane (3% in oxygen) and decapitated. Brains were promptly removed and placed in cold, oxygenated physiological saline solution (PSS). The PCAs were carefully dissected and cleared of connective tissue. Third-order PCAs were mounted and secured onto glass cannulas in an arteriograph chamber as previously described.¹⁷ Briefly, the proximal cannula was connected to an in-line pressure transducer and a servo-null pressure control system (Living Systems Instrumentation, Inc, Burlington, Vermont).

The distal cannula remained closed throughout the experiment to avoid flow-mediated responses. The vessel diameters were measured via video microscopy. The PSS was aerated with 5% CO₂, 10% O₂, and 85% N₂ to maintain pH at 7.40 ± 0.05. Temperature within the arteriograph chamber was maintained at 37.0 ± 0.1°C throughout the experiments.

Determination of Myogenic Vasodilation in Isolated PCA

The vessels were equilibrated at 50 mm Hg for 1 hour, after which the pressure was increased to 125 mm Hg in 25 mm Hg increments to allow for tone development. Myogenic vasodilation was measured by decreasing pressure from 125 to 5 mm Hg in a step-wise manner and recording active luminal diameter at each pressure once stable. Myogenic vasodilation was measured in PCA from NP and LP rats in PSS alone (n = 10/group) and in the presence of the NOS inhibitor N^ω-nitro-L-arginine (L-NNA, 0.1 mmol/L, n = 7/group). Pressure steps were then repeated in zero calcium PSS to obtain passive diameter measurements.

In Vivo Measurement of CBF During Hemorrhagic Hypotension in the Posterior Cerebral Cortex

A separate set of NP and LP rats (n = 8/group) were anesthetized initially with isoflurane (3% in O₂), which was then lowered to 1.5% to 2.0% in O₂ for instrumentation and tracheostomy. Anesthesia was then shifted to intravenous injections of chloral hydrate (200 mg/kg, left femoral vein). Animals were mechanically ventilated to maintain blood gases and pH within normal physiological ranges (Table 1). Body temperature was monitored and maintained with a heating pad at 37°C throughout the experiment. The CBF was measured and recorded transcranially using laser Doppler flowmetry as described previously.¹⁸ The left side of the medioposterior skull was exposed and a laser Doppler probe was affixed over a thinned area 2 mm lateral to the sagittal suture and 1 mm anterior to the lambdoid suture to measure CBF in the PCA territory. Both femoral arteries were cannulated to measure arterial BP via a pressure transducer, and to obtain blood samples for blood gas measurements and controlled blood withdrawal. Blood was slowly withdrawn through the femoral catheter at a steady rate (0.67–0.69 mL/min) to gradually decrease arterial BP from 100 to 30 mm Hg. The lower limit of CBF autoregulation was defined as the arterial pressure at which CBF decreased by 20% from baseline.^{19,20}

A separate set of LP animals (n = 7) underwent the same instrumentation as described above, with the addition of cannulation of the right femoral vein for the infusion of the NOS inhibitor N^ω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 10 mg/kg per min, 3 minutes). After drug infusion, controlled blood withdrawal was performed in the same manner as described above and the lower limit of CBF autoregulation was determined as stated above.

Real-Time Quantitative PCR of eNOS in Pial Arteries

A separate group of NP ($n = 5$) and LP ($n = 4$) rats were used for the isolation of arteries for real-time qualitative polymerase chain reaction (qPCR) for eNOS. The PCA segments from both right and left sides of the brain were pooled from each animal. The RNA was extracted using Trizol reagent (Life Technologies, Carlsbad, California) followed by purification using an RNeasy Micro Kit (Qiagen, Valencia, California) as per manufacturers' protocols. The RNA concentrations and quality were determined using an Agilent Bioanalyzer (Agilent, Santa Clara, California). Real-time qPCR was performed in a 2-step process. Total RNA was reverse transcribed using a mix of oligo dTs and random primers using the iScript cDNA Synthesis Kit (Biorad, Hercules, California). For each sample, complementary DNA (cDNA) was used to amplify the target gene eNOS and 2 housekeeping genes *Hprt1* and *Ywhaz*. One microliter of cDNA was used per reaction with 150 nmol/L of the forward and reverse primers (*eNOS*: forward CCTGAGCAGCACAAGAGTTACAA, reverse GGAGCCCAGCCCAACACA; *Hprt1*: forward CTC ATGGACTGATTATGGACAGGAC, reverse GCAGGTCAG CAAAGAACTTATAGCC; and *Ywhaz*: forward GATGAAGC CATTGCTGAACTTG, reverse GTCTCCTTGGGTATCCG ATGTC) and 12.5 μ L of Power Sybrgreen Master mix (Life Technologies) in a 25 μ L reaction. Primers were designed by the Obstetrics and Gynecology Departmental Molecular Core Facility at the University of Vermont using PrimerSelect. The reactions were performed using an initial denaturation of 3 minutes at 95°C, 40 cycles of 15 seconds at 95°C, and 60 seconds at 60°C followed by a melt curve analysis to ensure only the correct product was amplified. One set of PCR products for each gene were checked for correct size on a 2% agarose gel. Each sample was run in triplicate on the ABI 7000 Sequence Detection System (Life Technologies, Carlsbad, California). Negative water controls were run for each primer set in the real-time PCR reaction to ensure no contamination in the reagents as well as no secondary primer structures were amplified. Primers were designed over an exon–exon junction or the amplicon was designed to span an exon–exon junction to ensure genomic DNA was not amplified. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.²¹

Drugs and Solutions

All experiments were conducted using a bicarbonate-based PSS containing (mmol/L): sodium chloride 119.0, sodium bicarbonate 24.0, potassium chloride 4.7, monopotassium phosphate 1.18, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.17, calcium chloride (CaCl_2) 1.6, and EDTA 0.026. The PSS was prepared and stored without glucose at 4°C; glucose (5.5 mmol/L) was added to the PSS prior to each experiment. Zero calcium PSS was made similarly, omitting the addition of CaCl_2 . Both L-NNA and L-NAME were purchased from Sigma-Aldrich (St Louis, Missouri). The L-NNA was made weekly in a 0.01 mmol/L stock solution and stored at 4°C. The L-NAME was freshly prepared daily at 40 mg/mL in sterile lactated ringers solution.

Data Calculations and Statistical Analysis

Results are presented as mean $\pm$ standard error of the mean. Percentage tone of isolated arteries was calculated at 100 mm Hg and after addition of L-NNA as the percentage decrease in active luminal diameter from the passive diameter by the equation: $(1 - [\text{diameter}_{\text{active}}/\text{diameter}_{\text{passive}}]) \times 100\%$, where $\text{diameter}_{\text{active}}$ is the luminal diameter in PSS with or without L-NNA and $\text{diameter}_{\text{passive}}$ is the maximum luminal diameter in zero calcium PSS. To determine the pressure at which diameters of PCA from NP and LP rats differed from baseline of 125 mm Hg, repeated measures analysis of variance with a post hoc Bonferroni test was used. Differences in diameters between the presence and absence of L-NNA were determined using Student unpaired *t* test. The lower limit of CBF autoregulation, defined as when CBF decreased 20% from baseline, was determined from the laser Doppler traces for each animal. Differences in the percentage change in CBF during hemorrhagic hypotension and between the pressure at which the lower limit of CBF autoregulation was reached between NP and LP, and LP and LP + L-NAME animals were determined using Student unpaired *t* test. The differences were considered significant at $P < .05$.

Results

Myogenic Vasodilation in Response to Decreased Intravascular Pressure in PCA from NP and LP Rats

We sought to determine the effect of pregnancy on the myogenic vasodilatory response of PCA to decreased intraluminal pressure. We used PCA because they are the main blood supply to the posterior cortex.²² The PCA from NP and LP animals developed similar myogenic tone at 100 mm Hg ($33.8\% \pm 2.3\%$ and $33.7\% \pm 1.5\%$; nonsignificant [NS]). When intravascular pressure was decreased, luminal diameter of PCA from NP and LP rats remained relatively unchanged until approximately 60 mm Hg (Figure 1A). As intravascular pressure was lowered below 60 mm Hg, myogenic vasodilation occurred in PCA from both NP and LP animals. However, PCA from LP rats had significantly greater dilation compared to NP rats when pressure was lowered between 50 and 30 mm Hg. The diameter of PCA from LP rats was significantly greater than baseline diameter ($183 \pm 8 \mu\text{m}$ at 50 mm Hg vs $147 \pm 5 \mu\text{m}$ at 125 mm Hg; $P < .05$). In contrast, arteries from NP rats dilated less in response to decreased intravascular pressure, with luminal diameter never becoming statistically significantly different compared to baseline at any pressure (Figure 1A). Below 30 mm Hg, the diameter of PCA from both NP and LP animals passively decreased with pressure. Figure 1B shows that there was no difference in passive diameters of PCA from either group at any pressure studied, suggesting the difference in the magnitude of myogenic vasodilation between the groups was due to a difference in active vasodilation and not structural remodeling. Thus, the magnitude of the myogenic vasodilation in response to decreased pressure was greater in PCA from LP compared to NP rats.

Figure 1. Impact of pregnancy on myogenic vasodilation to decreased pressure in posterior cerebral arteries (PCAs). A, Graph showing active pressure–diameter relationship in PCA from nonpregnant (NP) and late-pregnant (LP) rats. Note that greater myogenic vasodilation was seen in PCA from LP animals, with diameters becoming statistically greater than baseline at 50, 40, and 30 mm Hg. B, Graph showing passive pressure–diameter relationship in PCA from NP and LP rats. There was no difference in passive diameters between PCA from NP and LP rats at any pressure studied. * $P < .05$ versus LP at 125 mm Hg by repeated measures analysis of variance (ANOVA).

Effect of NOS Inhibition on Myogenic Vasodilation to Decreased Pressure

As greater myogenic vasodilation occurred in PCA from LP compared to NP rats, we investigated NO as an underlying mechanism by which pregnancy increases myogenic vasodilation in PCA by inhibiting NOS with L-NNA and measuring myogenic vasodilation. Addition of L-NNA caused similar constriction of PCA from both groups of animals and the percent tone with NOS inhibition at 100 mm Hg was similar between PCA from NP and LP animals ($52.1 \pm 3.4\%$ and $51.8 \pm 3.2\%$; NS). In PCA from NP rats treated with L-NNA, vasodilation occurred and diameters were similar to PCA in PSS alone when pressure was decreased, becoming significantly greater than baseline at 60 mm Hg ($176 \pm 20 \mu\text{m}$ at 60 mm Hg vs $105 \pm 7 \mu\text{m}$ at 125 mm Hg; $P < .05$; Figure 2A). In contrast, vasodilation of PCA from LP rats was markedly reduced with NOS inhibition (Figure 2B). The diameters of L-NNA-treated vessels from LP animals were smaller than those in PSS alone ($P < .01$; Figure 2B). Despite this, luminal diameter of L-NNA-treated PCA from LP rats still became significantly greater than baseline at 50 mm Hg ($140 \pm 20 \mu\text{m}$ at 50 mm Hg vs $93 \pm 8 \mu\text{m}$ at 125 mm Hg; $P < .05$; Figure 2B).

Figure 2C compares vasodilation of PCA with L-NNA treatment from NP and LP rats. Vessels from both groups of animals underwent similar vasodilation with NOS inhibition as pressure was decreased. However, the dilation was shifted to lower pressure in PCA from LP compared to NP rats. When pressure was lowered to 70 mm Hg the diameter of PCA from LP rats was significantly smaller than the diameter of PCA from NP rats (88 ± 8 vs $148 \pm 22 \mu\text{m}$, respectively; $P < .05$; Figure 2C). Thus, NOS inhibition prevented the greater myogenic vasodilation of PCA from LP compared to NP rats from occurring

without eliminating myogenic vasodilation all together, and it shifted the dilation of PCA from LP rats leftward compared to PCA from NP rats.

The results above suggest that enhanced vasodilation of PCA from LP rats was NO dependent. To determine whether this was due to an effect of pregnancy on eNOS expression, real-time qPCR was performed on PCA from both groups of animals. There were no differences in relative quantity of messenger RNA (mRNA) expression of eNOS between PCA from NP and LP rats (0.86 ± 0.25 vs 1.02 ± 0.27 ; NS; Figure 2D). These results suggest that greater vasodilation of PCA from LP compared to NP rats via NO was not due to the changes in mRNA expression of eNOS during pregnancy.

The CBF Autoregulation During Acute Hypotension in NP and LP Rats

The myogenic response of cerebral arteries is a main contributor to CBF autoregulation.¹² Since we found that enhanced myogenic vasodilation in response to decreased pressure during pregnancy was NO dependent, we sought to determine the effect of pregnancy on the lower limit of CBF autoregulation and investigate the role of NOS in CBF autoregulation during acute hypotension. Table 1 shows the physiological parameters of all groups of animals used. Importantly, arterial pH and arterial gasses that can affect CBF were within physiological ranges and were not different between the groups.

Figure 3A and B shows the effect of pregnancy on CBF autoregulation during hemorrhagic hypotension in the posterior cortex. The baseline BPs were similar between LP and NP rats (100.1 ± 0.2 vs 100.0 ± 0.3 mm Hg; NS) prior to controlled blood withdrawal. The autoregulatory curve was shifted leftward to lower pressures in LP compared to NP animals during

Figure 2. Role of nitric oxide synthase (NOS) inhibition on myogenic vasodilation of posterior cerebral arteries (PCAs) during pregnancy. Graphs showing active pressure–diameter relationships of PCA from (A) nonpregnant (NP) and (B) late-pregnant (LP) animals in the presence or absence of the NOS inhibitor *N*^o-nitro-L-arginine (L-NNA). C, Graph comparing the effect of NOS inhibition on myogenic vasodilation in PCA from NP and LP rats. Despite NOS inhibition, PCA from NP and LP rats had substantial myogenic vasodilation in response to decreased pressure. D, Relative endothelial NOS (eNOS) messenger RNA (mRNA) expression in PCA from NP and LP rats. There was no difference between eNOS expression in cerebral arteries from NP and LP rats. **P* < .05 versus baseline by repeated measures analysis of variance (ANOVA); ††*P* < .01 versus physiological saline solution (PSS) and †*P* < .05 versus NP L-NNA by *t* test.

hemorrhagic hypotension (Figure 3A). The lower limit of CBF autoregulation was significantly lower in LP versus NP rats (Figure 3B). To determine the role of NO in the pregnancy-specific leftward shift in CBF autoregulation, L-NAME was infused into LP rats and the autoregulatory curve was determined. Figures 3C and D show the effect of acute NOS inhibition during pregnancy on CBF autoregulation and its lower limit during hemorrhagic hypotension, respectively. The NOS inhibition caused a rise in BP, which has been previously shown,^{23,24} with the BP of LP rats infused with L-NAME being 115.1 ± 2.0 mm Hg prior to blood withdrawal. Despite this baseline increase in BP with L-NAME infusion, CBF was maintained similarly between LP rats with and without NOS inhibition during hemorrhagic hypotension (Figure 3C). The lower limit of CBF autoregulation was also unaffected by acute NOS inhibition during pregnancy (Figure 3D). Thus, it appears that pregnancy shifts the autoregulatory curve leftward to lower pressures, and that this is unaffected by acute NOS inhibition.

Discussion

In the present study, we investigated the effect of pregnancy and NOS inhibition on the myogenic vasodilatory response of PCA to decreased intravascular pressure and the lower limit of CBF autoregulation. The PCA from LP rats dilated to a greater extent in response to decreased pressure compared to PCA from NP rats that was NO dependent. However, L-NNA did not prevent myogenic vasodilation from occurring in PCA from either NP or LP rats, but it eliminated the pregnancy-specific enhancement of vasodilation, causing the magnitude of dilation of PCA from NP and LP rats to be similar. Using an animal model of controlled hemorrhage, we found that pregnancy caused a leftward shift in the CBF autoregulatory curve during acute hypotension, with the lower limit being reached at significantly lower pressures compared to the NP state. We hypothesized that this was due to the enhanced vasodilatory response to decreased pressure seen in PCA of LP rats; however, acute NOS inhibition in pregnancy did not affect the

Figure 3. Effect of pregnancy on the lower limit of cerebral blood flow (CBF) autoregulation (CBFAR). A, Graph showing changes in CBF during hemorrhagic hypotension in the posterior cerebral cortex of nonpregnant (NP) and late-pregnant (LP) rats, and (B) the lower limit of CBF autoregulation in NP and LP rats during hypotension. C, Graph showing the effect of acute nitric oxide synthase (NOS) inhibition on changes in CBF during hemorrhagic hypotension in the posterior cerebral cortex in LP rats only, and (D) the lower limit of CBF autoregulation in LP rats with and without NOS inhibition. The lower limit is indicated by the dotted line and defined as the pressure at which CBF decreased to 20% of baseline. * $P < .05$ versus NP by t test.

lower limit of CBF autoregulation. These results suggest that CBF autoregulation is more effective in pregnancy during hemorrhagic hypotension, and that this shift in the lower limit of the CBF autoregulatory curve to lower pressure is not due to NO.

The NO appears to be responsible for the enhanced vasodilation of PCA from LP compared to NP rats. There are at least 3 possibilities by which NO may be affecting vasodilation during pregnancy. First, expression of eNOS could be increased in PCA during pregnancy, although this is unlikely as no difference in mRNA expression was seen in PCA from NP and LP rats. Second, pregnancy could enhance the sensitivity of VSM to NO. However, a previous study showed no differences in VSM sensitivity to the NO donor sodium nitroprusside between PCA from NP and LP rats.¹⁷ Finally, the activity of eNOS could be increased in endothelium from pregnant animals as intravascular pressure was lowered. Changes in phosphorylation of eNOS during pregnancy could change the activity of NO and increase NO production.²⁵ In addition, the role of

NO in myogenic vasodilation appeared to be pressure dependent because there was no difference in the magnitude of constriction of PCA with NOS inhibition between NP and LP rats at a constant pressure, as has also been shown previously.²⁶ Pregnancy increases flow-mediated vasodilation in an NO-dependent manner in mesenteric arteries from rats^{27,28} and subcutaneous arteries from humans.²⁹ Thus, it is possible that changes in response to shear stress during decreased intravascular pressure are responsible for increasing NO in PCA from LP rats.

To our knowledge, this is the first study investigating the effect of pregnancy on CBF autoregulation during acute hypotension and the involvement of NOS in the pregnant state. The NOS inhibition did not alter the lower limit of CBF autoregulation although it inhibited pregnancy-specific enhancement of myogenic vasodilation in response to decreased pressure. Thus, the response of an isolated cerebral artery may not be indicative of what is occurring during hemorrhagic hypotension that encompasses the entire brain. Our previous study showed that,

in pregnancy, brain parenchymal arterioles are significantly larger than in the NP state, an effect that may also contribute to more effective CBF autoregulation when upstream pial vessels are dilated.³⁰ Thus, it is possible that even when the NO-dependent enhancement of myogenic vasodilation of PCA in pregnancy was inhibited, CBF was better maintained in pregnancy during acute hypotension due to the vasodilation that was occurring in upstream vessels, coupled with structurally larger downstream arterioles.

Our findings of NOS inhibition having no effect on the lower limit of the autoregulatory curve are in accordance with other studies utilizing systemic NOS inhibition in the investigation of the role of NO in the lower limit of CBF autoregulation.³¹⁻³⁴ In fact, the involvement of NO in CBF autoregulation is controversial, with several studies showing contrasting findings.^{23,24,31-37} It is possible that differences in methodology, such as the method that hypotension is induced, may play a role in the outcome of a study. Our result agrees with others when hypotension was induced by hemorrhage, but not by ganglionic blockade or administration of a potassium channel activator.²⁴ Another previous study used similar methodology as the present study and found intravenous infusion of a NOS inhibitor shifted the lower limit of CBF autoregulation to higher pressure.²³ However, NOS inhibition raised BP in the present study to 115 mm Hg, compared to over 150 mm Hg in the previous study.²³ Therefore, it is possible that the difference between the 2 findings is due to the greater acute increase in BP upon NOS inhibition. It should be noted that the rise in BP in our study confirmed that eNOS was indeed inhibited by L-NAME infusion. However, it is possible that a greater degree of inhibition would have produced a shift in the autoregulatory curve seen in other studies.

Despite evidence for NO having no role in the lower end of CBF autoregulation with intravenous infusion of a NOS inhibitor,^{24,31-34} including our findings in the present study, other studies have identified a role of NO in the lower limit of CBF autoregulation when the NOS inhibitor was suffused over the cortex.^{36,37} The L-NAME suffusion over a cranial window both raised the lower limit of CBF autoregulation and depressed the height of the CBF autoregulatory curve.^{36,37} In addition to eNOS, neuronal NOS (nNOS) contributes to CBF autoregulation and increases NO production during hypotension-induced hypoxia, effectively dilating cerebral vessels.^{38,39} The nNOS mRNA and protein levels are increased in the hypothalamus of pregnant rats¹⁵ suggesting that pregnancy increases nNOS in some brain regions. Although our recent study determined there were no changes in nNOS mRNA expression in the posterior cortex between NP and LP rats, activity of nNOS was not measured and may be increased during pregnancy.⁴⁰ These data support the idea that changes in NO production by increased activation of nNOS during acute hypotension in pregnancy could contribute to the leftward shift of the CBF autoregulatory curve seen in LP rats. As inhibition of nNOS appears to take substantially longer time to achieve than that of eNOS,³⁶ it is possible that nNOS was not inhibited in the present study due to the acute nature of the L-NAME infusion.

Therefore, it is possible that NO derived from nNOS was still maintaining CBF in the face of acute hypotension.

In this study, we investigated the myogenic component of CBF autoregulation. However, there are other contributors to CBF autoregulation, including metabolic and neuronal influences in addition to myogenic responses of VSM of cerebral arteries and arterioles.^{2,8} Oxygen metabolism of the pregnant brain has been found to be similar to that of NP brain; thus, this is unlikely to be contributing to the shift in the autoregulatory curve.⁴¹ Pregnancy-induced changes in neuronal contributors, such as nNOS as previously discussed, may be contributing to the leftward shift of the autoregulatory curve. The baroreceptor reflex also influences CBF autoregulation during increases in BP, with its disruption extending the autoregulatory curve surpassing the pressure at which autoregulatory breakthrough would normally occur.⁴² The baroreflex stimulates sympathetic fibers that have been shown to affect CBF autoregulation during acute hypotension as well.⁴³ Both α -adrenergic blockade as well as sympathectomy shifts the lower limit of CBF autoregulation leftward to lower pressures.^{6,43,44} A recent study by our group measured perivascular sympathetic fiber density of PCA of NP and LP rats and found that pregnancy did not affect innervation of PCA; however, nerve activity was not measured.⁴⁰ Thus, pregnancy-induced attenuation of the baroreflex^{30,45} may be partly responsible for the leftward shift of the lower limit of CBF autoregulation seen in LP rats by decreasing activity of sympathetic nerves that innervate PCA.

In summary, investigation of the myogenic vasodilation to decreased intravascular pressure of PCA revealed greater dilation in vessels from pregnant rats that was NO dependent. Pregnancy improved CBF autoregulation during hemorrhagic hypotension by shifting the lower limit of CBF autoregulation leftward in the posterior cortex, which remained unaffected by acute NOS inhibition. This leftward shift in CBF autoregulation in the posterior cortex during pregnancy may be a protective mechanism by which the maternal brain is better prepared to maintain CBF in the face of acute hypotension that can occur during parturition.

Acknowledgments

We would like to thank Karen Oppenheimer of the Obstetrics, Gynecology and Reproductive Sciences Departmental Molecular Core Facility at the University of Vermont for her technical expertise.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: the National Institute of Neurological Disorders and Stroke (grant number RO1 NS045940 and RO1 NS045940-06S1).

References

- Harper AM. Autoregulation of cerebral blood flow: influence of the arterial blood pressure on the blood flow through the cerebral cortex. *J Neurol Neurosurg Psychiatry*. 1966;29(5):398-403.
- Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev*. 1959;39(2):183-238.
- McHenry LC Jr, West JW, Cooper ES, Goldberg HI, Jaffe ME. Cerebral autoregulation in man. *Stroke*. 1974;5(6):695-706.
- Strandgaard S, Olesen J, Skinhoj E, Lassen NA. Autoregulation of brain circulation in severe arterial hypertension. *Br Med J*. 1973;1(5852):507-510.
- MacKenzie ET, Farrar JK, Fitch W, Graham DI, Gregory PC, Harper AM. Effects of hemorrhagic hypotension on the cerebral circulation. I. Cerebral blood flow and pial arteriolar caliber. *Stroke*. 1979;10(6):711-718.
- Fitch W, MacKenzie ET, Harper AM. Effects of decreasing arterial blood pressure on cerebral blood flow in the baboon. Influence of the sympathetic nervous system. *Circ Res*. 1975;37(5):550-557.
- Brierley JB, Brown AW, Excell BJ, Meldrum BS. Brain damage in the rhesus monkey resulting from profound arterial hypotension. I. Its nature, distribution and general physiological correlates. *Brain Res*. 1969;13(1):68-100.
- Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev*. 1990;2:161-192.
- Dildy GA, 3rd. Postpartum hemorrhage: new management options. *Clin Obstet Gynecol*. 2002;45(2):330-344.
- Garrioch MA. The body's response to blood loss. *Vox Sang*. 2004;87(suppl 1):74-76.
- Kainer F, Hasbargen U. Emergencies associated with pregnancy and delivery: peripartum hemorrhage. *Dtsch Arztebl Int*. 2008;105(37):629-638.
- Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI, Patterson JL Jr. Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am J Physiol*. 1978;234(4):H371-H383.
- Gotoh F, Fukuuchi Y, Amano T, et al. Role of endothelium in responses of pial vessels to change in blood pressure and to carbon dioxide in cats. *J Cereb Blood Flow Metab*. 1987;7:S275.
- Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987;327(6122):524-526.
- Xu DL, Martin PY, St John J, et al. Upregulation of endothelial and neuronal constitutive nitric oxide synthase in pregnant rats. *Am J Physiol*. 1996;271(6 pt 2):R1739-R1745.
- Sladek SM, Magness RR, Conrad KP. Nitric oxide and pregnancy. *Am J Physiol*. 1997;272(2 pt 2):R441-R463.
- Chan SL, Chapman AC, Sweet JG, Gokina NI, Cipolla MJ. Effect of ppgamma inhibition during pregnancy on posterior cerebral artery function and structure. *Front Physiol*. 2010;1:130.
- Euser AG, Cipolla MJ. Cerebral blood flow autoregulation and edema formation during pregnancy in anesthetized rats. *Hypertension*. 2007;49(2):334-340.
- Omura-Matsuoka E, Yagita Y, Sasaki T, et al. Hypertension impairs leptomeningeal collateral growth after common carotid artery occlusion: restoration by antihypertensive treatment. *J Neurosci Res*. 2011;89(1):108-116.
- Takada J, Ibayashi S, Ooboshi H, et al. Valsartan improves the lower limit of cerebral autoregulation in rats. *Hypertens Res*. 2006;29(8):621-626.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods*. 2001;25(4):402-408.
- Hayman LA, Berman SA, Hinck VC. Correlation of ct cerebral vascular territories with function: Ii. Posterior cerebral artery. *AJR Am J Roentgenol*. 1981;137(1):13-19.
- Preckel MP, Leftheriotis G, Ferber C, Degoute CS, Banssillon V, Saumet JL. Effect of nitric oxide blockade on the lower limit of the cortical cerebral autoregulation in pentobarbital-anesthetized rats. *Int J Microcirc Clin Exp*. 1996;16(6):277-283.
- Dieguez G, Fernandez N, Sanchez MA, et al. Role of nitric oxide in the cerebral circulation during hypotension after hemorrhage, ganglionic blockade and diazoxide in awake goats. *Brain Res*. 1999;851(1-2):133-140.
- Fulton D, Gratton JP, McCabe TJ, et al. Regulation of endothelium-derived nitric oxide production by the protein kinase akt. *Nature*. 1999;399(6736):597-601.
- 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(6):H2127-2132.
- Cockell AP, Poston L. Isolated mesenteric arteries from pregnant rats show enhanced flow-mediated relaxation but normal myogenic tone. *J Physiol*. 1996;495(pt 2):545-551.
- Learmont JG, Cockell AP, Knock GA, Poston L. Myogenic and flow-mediated responses in isolated mesenteric small arteries from pregnant and nonpregnant rats. *Am J Obstet Gynecol*. 1996;174(5):1631-1636.
- Cockell AP, Poston L. Flow-mediated vasodilatation is enhanced in normal pregnancy but reduced in preeclampsia. *Hypertension*. 1997;30(2 pt 1):247-251.
- Brooks VL, Quesnell RR, Cumbee SR, Bishop VS. Pregnancy attenuates activity of the baroreceptor reflex. *Clin Exp Pharmacol Physiol*. 1995;22(2):152-156.
- Buchanan JE, Phillis JW. The role of nitric oxide in the regulation of cerebral blood flow. *Brain Res*. 1993;610(2):248-255.
- Saito S, Wilson DA, Hanley DF, Traystman RJ. Nitric oxide synthase does not contribute to cerebral autoregulatory phenomenon in anesthetized dogs. *J Auton Nerv Syst*. 1994;49(suppl):S73-S76.
- Wang Q, Paulson OB, Lassen NA. Is autoregulation of cerebral blood flow in rats influenced by nitro-l-arginine, a blocker of the synthesis of nitric oxide? *Acta Physiol Scand*. 1992;145(3):297-298.
- Thompson BG, Pluta RM, Girton ME, Oldfield EH. Nitric oxide mediation of chemoregulation but not autoregulation of cerebral blood flow in primates. *J Neurosurg*. 1996;84(1):71-78.

35. Iadecola C, Pelligrino DA, Moskowitz MA, Lassen NA. Nitric oxide synthase inhibition and cerebrovascular regulation. *J Cereb Blood Flow Metab.* 1994;14(2):175-192.
36. Jones SC, Radinsky CR, Furlan AJ, Chyatte D, Perez-Trepichio AD. Cortical NOS inhibition raises the lower limit of cerebral blood flow-arterial pressure autoregulation. *Am J Physiol.* 1999;276(4 pt 2):H1253-H1262.
37. Jones SC, Easley KA, Radinsky CR, Chyatte D, Furlan AJ, Perez-Trepichio AD. Nitric oxide synthase inhibition depresses the height of the cerebral blood flow-pressure autoregulation curve during moderate hypotension. *J Cereb Blood Flow Metab.* 2003;23(9):1085-1095.
38. Bauser-Heaton HD, Bohlen HG. Cerebral microvascular dilation during hypotension and decreased oxygen tension: A role for nNOS. *Am J Physiol Heart Circ Physiol.* 2007;293(4):H2193-H2201.
39. Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J.* 1994;298 (pt 2):249-258.
40. Cipolla MJ, Bishop N, Chan SL. Effect of pregnancy on autoregulation of cerebral blood flow in anterior versus posterior cerebrum. *Hypertension.* 2012;60(3):705-711.
41. Mc CM. Cerebral blood flow and metabolism in toxemias of pregnancy. *Surg Gynecol Obstet.* 1949;89(6):715-721.
42. Talman WT, Dragon DN, Ohta H. Baroreflexes influence autoregulation of cerebral blood flow during hypertension. *Am J Physiol.* 1994;267(3 pt 2):H1183-H1189.
43. Edvinsson L. Innervation of the cerebral circulation. *Ann N Y Acad Sci.* 1987;519:334-348.
44. Edvinsson L, MacKenzie ET, Robert JP, Skarby T, Young AR. Cerebrovascular responses to haemorrhagic hypotension in anaesthetized cats. Effects of alpha-adrenoceptor antagonists. *Acta Physiol Scand.* 1985;123(3):317-323.
45. Brooks VL, Dampney RA, Heesch CM. Pregnancy and the endocrine regulation of the baroreceptor reflex. *Am J Physiol Regul Integr Comp Physiol.* 2010;299(2):R439-R451.