Cerebral artery reactivity changes during pregnancy and the postpartum period: a role in eclampsia?

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HYPERTENSION IS ONE OF THE MOST COMMON MEDICAL CONDITIONS OF PREGNANCY that affects both maternal and fetal health, often with life-threatening consequences (2, 37). A number of maternal organs are affected by hypertension in pregnancy including the brain in the form of eclampsia (12, 46). Eclampsia is a leading cause of maternal death with classic neurological features that include headache, nausea, visual disturbances, vomiting, and convulsions (11, 13, 27, 44, 48).

Clinical findings of eclampsia include varying degrees of hemorrhage, cerebral edema, and vasculopathy (15, 37, 42, 48). The reversibility of clinical neurological signs and neuroimaging lesions within a few days or weeks postpartum in most cases argues against the existence of true cerebral ischemic necrosis. In fact, the clinical and neuroimaging findings are more consistent with edema (15, 21, 25, 23, 48). For example, the neuroimaging hallmarks of eclampsia are reversible abnormalities that appear hypodense on computed tomography (CT) studies and hyperintense on T2-weighted magnetic resonance imaging (MRI; Refs. 15, 25, 33, 39), both of which are suggestive of edema. Additional studies using diffusion-weighted MRI found that these hyperintense areas had a high apparent diffusion-coefficient value, which is indicative of vasogenic edema (23, 38, 40, 41).

The primary explanation for the pathogenesis of neurological symptoms and edema formation during eclampsia is that they represent a form of hypertensive encephalopathy, which is thought to arise from an rapid rise in blood pressure (i.e., acute hypertension) that overcomes the myogenic vasoconstriction and thereby causes loss of autoregulatory capacity in the veins, venules, precapillary arteries, and arterioles (21, 23, 25, 46, 48). This explanation has arisen from numerous similarities in clinical presentations including comparable imaging findings on CT and MRI (15, 41, 46), the same neurological features (headache, nausea, seizures; Refs. 13, 34, 35, 47), and the prompt reversal of symptoms after blood pressure is restored to normal (13, 47).

During hypertensive encephalopathy, acute and excessive intravascular pressure causes forced dilatation (FD) of intrinsic myogenic tone of cerebral arteries that decreases cerebrovascular resistance (CVR) and increases pressure on the microcirculation thereby causing vasogenic edema formation (10). Because women who develop eclampsia are in general normotensive before pregnancy, we have hypothesized that pregnancy and/or the postpartum period predisposes the cerebral arteries to FD that leads to the symptoms of eclampsia when blood pressure is elevated. In the present study, we used isolated and pressurized resistance-sized (≤200 μm) posterior cerebral arteries (PCAs) to investigate reactivity changes and myogenic activity during pregnancy and the postpartum period, when eclampsia is most likely to occur (37).

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (Harlan; body wt 240–280 g) were used for all experiments. All procedures were approved by the Institutional Animal Care and Use Committee at the University of
Vermont. Animals were housed in the Animal Care Facility, which is an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. We compared animals that were either nonpregnant (NP, \( n = 7 \)), late pregnant (LP, day 19, \( n = 10 \)), or postpartum (PP, day 3, \( n = 7 \)).

Preparation of arterial segments and pressurized arteriograph system. After receiving anesthesia with halothane-oxygen, the animals were decapitated, and the brains were quickly removed and placed in cold physiological salt solution (HEPES buffer) at pH 7.4 ± 0.03. A third-order branch of the PCA was carefully dissected and mounted on glass cannulas in an arteriograph chamber and secured with nylon suture as previously described (7). The proximal cannula was attached to an in-line pressure transducer and controller that allowed intravascular pressure to be maintained at a set pressure or increased at a variable rate. The distal cannula was closed off so there was no flow through the arteries. The entire chamber was placed on an inverted microscope for measurement of lumen diameter through an optical window in the bottom of the chamber. An attached video camera and monitor that were connected to the microscope were used to measure diameter electronically with the aid of a video dimension analyzer (VDA). The output of the VDA was sent to a computer via a data-acquisition system that provided visualization of dynamic responses in pressure and diameter, similar to a chart recorder.

Experimental protocol. After a 1-h equilibration at 25 mmHg, pressure was increased to 175 mmHg in 25-mmHg steps. Diameter measurements were recorded at each pressure once stable (~10 min). The pressure at which FD occurred, which was easily determined by a large increase in lumen diameter, was recorded. After the pressure vs. diameter curves were obtained, a single dose of the nitric oxide (NO) inhibitor \( N^\ominus \) -nitro-l-arginine (l-NNA, 0.1 mM) was added to the bath, and the amount of constriction that occurred in response to NO inhibition was used as a measure of basal NO production. A single concentration of the cyclooxygenase inhibitor indomethacin (10 \(^{-5}\) M) was then added to the bath, and any further change in diameter was used as a measure of basal prostaglandin production. In the presence of both NO and cyclooxygenase inhibition, 5-hydroxytryptamine (serotonin) was cumulatively added to the bath at a rate of 0.01–10 mM, and the diameter at each concentration was measured once stable (~10 min).

The concentration response to serotonin was performed in the presence of l-NNA and indomethacin so that serotonin receptors on the endothelium, which are known to cause vasodilation (14), would not interfere with the contractile response. Last, a single concentration (0.1 mM) of papaverine was added to the bath to obtain fully relaxed diameter measurements and to calculate passive distensibility.

Data calculations. Percent tone was calculated as the percent decrease in diameter from the fully relaxed measurement obtained with papaverine at each intravascular pressure by the equation \( 1 - (\phi_{\text{vaso}}/\phi_{\text{baseline}}) \times 100 \), where \( \phi_{\text{vaso}} \) and \( \phi_{\text{baseline}} \) are the diameters of vessels with tone and in papaverine, respectively. Contraction in response to l-NNA and indomethacin was calculated as a percent decrease in diameter from baseline. The response to serotonin was determined as a percent change in diameter by the equation \( (\phi_{\text{max}} - \phi_{\text{baseline}})/\phi_{\text{baseline}} \times 100 \), where \( \phi_{\text{max}} \) and \( \phi_{\text{baseline}} \) are the vessel inner diameters in the presence of serotonin and at baseline, respectively. Distensibility was calculated at each pressure with the vessels fully relaxed in papaverine by determining diameter changes as a function of pressure. Distensibility was calculated by the equation \( (\phi_{\text{pressure}}/\phi_{\text{baseline}} - 1) \times 100 \), where \( \phi_{\text{pressure}} \) and \( \phi_{\text{baseline}} \) are the vessel diameters at that particular pressure and at 5 mmHg of pressure, respectively. Distensibility for each arteriole was normalized to the diameter at 5 mmHg of pressure because arterioles often collapse at pressures below this value.

Drugs and solutions. HEPES, serotonin, indomethacin, l-NNA, and papaverine were all purchased from Sigma. Serotonin was made fresh each day as a 10 \(^{-3}\) M stock solution; l-NNA, indomethacin, and papaverine were made fresh each week as 10 \(^{-5}\) M stock solutions and were stored at 4°C. Vessel-reactivity experiments were conducted in physiological salt solution, the composition of which was (in mM) 142.0 NaCl, 4.7 KCl, 1.71 MgSO\(_4\), 0.50 EDTA, 2.8 CaCl\(_2\), 1.0 HEPES, 1.2 KH\(_2\)PO\(_4\), and 5.0 glucose.

Statistical analysis. Results are presented as means ± SE. Differences between gestational groups were determined using one-way ANOVA with a post hoc Bonferroni correction for multiple comparisons and were considered significant at \( P < 0.05 \). Differences at various pressures within a gestational group were determined by repeated-measures ANOVA. The animal number was used as the \( n \) value; only one artery was taken per animal. The presence of different \( n \) values on several figures occurs because several arteries did not respond to papaverine and therefore did not produce relaxed diameters for measurement. Without relaxed diameters, certain calculations could not be performed (e.g., percent tone).

RESULTS

Reactivity to pressure and myogenic activity. The responses of all artery groups to stepwise increases in pressure are shown in Fig. 1. Notice that arteries from NP and PP animals dilated when pressure was increased from 25 to 50 mmHg and then constricted and developed myogenic tone when pressure was increased to 75 mmHg. Arteries from LP animals developed spontaneous tone and constricted when pressure was increased from 25 to 50 mmHg. In addition, tone was maintained in the NP arteries at all pressures studied; however, both LP and PP arteries dilated at higher intravascular pressures. The pressure at which FD occurred was >175 mmHg for NP animals but decreased to 146 ± 6.5 mmHg (\( P < 0.01 \) vs. NP) for LP and 162 ± 7.7 mmHg for PP (\( P < 0.01 \) vs. NP) animals. When percent tone was compared at a normal pressure of 75 mmHg vs. a high pressure of 175 mmHg (one that might be experienced during eclampsia), there was a significant loss of tone at higher pressures in LP and PP arteries compared with NP arteries (Fig. 2). The amount of tone in the NP arteries actually increased when pressure was increased from 20 ± 2.7 at 75 mmHg to 35 ± 2.2% at 175 mmHg. Both LP and PP arteries had diminished tone at the higher pressure. The amount of tone in LP and PP animals at 75 vs. 175 mmHg was 34 ± 3 vs. 11 ± 3% (\( P < 0.01 \) vs. NP) and 30 ± 7.6 vs. 20 ± 7.2% (\( P < 0.05 \) vs. values at 75 mmHg).
Reactivity to inhibition of NO and cyclooxygenase and to serotonin. Arteries from all groups of animals constricted in response to NO inhibition with L-NNA thereby demonstrating significant basal NO release (Fig. 3). Interestingly, arteries from PP animals had an increased contraction to L-NNA and constricted arteries 25\% compared with 18\% for NP and 14\% for LP animals (P < 0.05). Addition of indomethacin caused little change in diameter in any of the groups. In the presence of NO and prostaglandin inhibition, LP and PP animals contracted in a dose-dependent manner to serotonin (Fig. 4). In contrast, NP arteries dilated to most concentrations of serotonin, demonstrating release of endothelial hyperpolarizing factor (EDHF). Although the composition of EDHF is unknown, dilation in the presence of NO and cyclooxygenase inhibition is used as a means of identifying it (19). To test the endothelial dependence of the dilation in response to serotonin in NP arteries, several PCAs (n = 3) were denuded of endothelium by gently pulling the vessel on and off of a human hair (30). Once denuded of endothelium, NP PCAs contracted in response to serotonin similar to LP and PP animals. Figure 4 also shows the percent change in diameter in response to serotonin in NP animals without endothelium. Notice that arteries without endothelium contracted in response to serotonin as shown by the decreased diameter measurements.

Passive distensibility. The changes in diameter in response to changes in pressure in fully relaxed arteries were used to calculate passive distensibility; these values provide an indication of extracellular matrix remodeling. Distensibility values were similar between NP and LP animals; however, they were significantly diminished in the PP animals at pressures ≥100 mmHg (Fig. 5).
DISCUSSION

The neurological complications of eclampsia (headaches, nausea, visual disturbances, and convulsions) are thought to be similar to those of hypertensive encephalopathy, whereby an acute, excessive rise in blood pressure causes FD of cerebral arteries and arterioles, diminished CVR, hyperperfusion, and edema formation (11, 13, 15, 27, 44, 48). Because women who develop eclampsia are in general normotensive before pregnancy, we hypothesized that LP or PP, two states in which eclampsia is known to occur most often (37), predisposes the cerebral arteries to FD. In the present study, we demonstrated that PCAs from LP and PP animals dilated at significantly lower pressures than those from NP animals (Fig. 1). NP arteries maintained significant tone at all pressures \( \leq 175 \) mmHg, whereas arteries from LP and PP animals dilated at 146 and 162 mmHg, respectively. In addition, the level of myogenic tone was significantly less at higher pressures in PCAs from LP and PP animals; this result could decrease CVR and promote hyperperfusion. Therefore, it appears that pregnancy and the PP state predispose the cerebral circulation to forced dilatation at lower pressures. Because FD only occurs at pressures beyond the myogenic or autoregulatory pressure range, it is possible that during normal pregnancy, when blood pressure is normal, there is no consequence of attenuated pressure-induced reactivity, and that only during hypertension in pregnancy, when pressure is pathologically elevated, there is FD and edema formation that leads to eclampsia.

Several studies have examined changes in cerebral hemodynamics in patients with preeclampsia and eclampsia and have demonstrated that both conditions are associated with altered cerebrovascular reactivity and autoregulatory failure that is consistent with decreased CVR and hyperperfusion (36, 45, 46). For example, in a study where transcranial Doppler ultrasound was used (36) to examine the cerebrovascular reactivity of 45 normotensive and 36 preeclamptic women, it was found that preeclamptic women had a higher baseline perfusion pressure but a lower resistance index and reduced vasodilation to CO\(_2\) inhalation, which suggests that although perfusion pressure is higher during preeclampsia, CVR and reactivity are diminished. A similar study that compared six patients with severe preeclampsia to three with eclampsia found that cerebral perfusion pressure was higher but CVR was decreased to a greater extent in the eclamptic patients (46). These results demonstrate that eclampsia is associated with an altered cerebral hemodynamic status that includes loss of autoregulation, diminished CVR, and hyperperfusion.

Very few studies have examined cerebral hemodynamics in women with normal pregnancy; however, it has been suggested that normal pregnancy is also associated with a progressive increase in cerebral perfusion pressure and decreased CVR (3), as well as an autoregulatory curve that is shifted to the lower range of pressures (47, 48). This suggests that pregnancy alone is associated with alterations in the cerebral circulation that makes the brain more susceptible to FD and hyperperfusion during acute hypertension. The results from the present study, that pregnancy lowers the pressure at which FD occurs, support this concept. There are several known contributors to FD that may be altered during pregnancy. First, activation of smooth muscle Ca\(^{2+}\)-activated K\(^+\) (K\(_{Ca}\)) channels has been shown to regulate arterial tone and the pressure at which FD occurs (4, 32). A study by Paterno et al. (32) demonstrated that when K\(_{Ca}\) channels were inhibited by tetraethylammonium in cerebral arteries, the pressure at which FD occurred significantly increased, which suggests that K\(_{Ca}\)-channel activation is involved in attenuating vasoconstriction at higher pressures (i.e., causing FD). It is possible that pregnancy alters the expression of cerebral artery smooth muscle K\(^+\) channels to lower the pressure at which FD occurs. In fact, gestation-induced changes in smooth muscle K\(^+\) channels have been reported in myometrium (5, 22). Second, the state of actin polymerization in smooth muscle has also been shown to be involved in FD of cerebral arteries (7). It is possible that pregnancy alters the state of actin polymerization in cerebrovascular smooth muscle to lower the pressure at which FD occurs.

Alternatively, the effects of pregnancy and the PP state on vascular tone and the pressure at which FD occurs may be due to altered endothelium-dependent vasodilator production. Although myogenic activity is intrinsic to smooth muscle, vascular tone is modulated by several endothelium-derived compounds including NO and prostacyclin (prostacyclin I\(_2\) or PGI\(_2\); Refs. 17, 28, 31). In the cerebral circulation, there is considerable basal NO production that mitigates myogenic tone as is demonstrated by significant constriction in response to NO inhibition with L-NNA. It is interesting that PCAs from PP animals had significantly increased constriction in response to L-NNA, which suggests increased basal NO in that gestational group. It is possible that increased NO production during the PP period diminishes tone and lowers the pressure at which FD occurs. Increased constriction to L-NNA was not found in LP; this suggests that enhanced NO production did not occur. However, the suggestion that endothelium-derived vasodilators are altered during pregnancy is not new. Regarding the peripheral circulation, pregnancy has been shown to be a high-NO state, a result that is thought to contribute to the decreased peripheral vascular resistance that is necessary to accommodate the large increase in plasma volume (1, 43). Because we only noted augmented L-NNA-induced constriction in PCAs during the PP state and not during LP, it is possible that the effects on myogenic activity and FD are more due to an effect of pregnancy on smooth muscle and not endothelium. In any case, there does appear to be a shift in endothelium-dependent vasodilator production to increased NO during the PP period.

A shift in vasodilator production is clearly demonstrated in the responses to serotonin (Fig. 4). Serotonin is a complex compound (8) that acts on both smooth muscle (to produce contraction) and endothelium (to cause dilation). In the present study, the response to serotonin was measured in the presence of both NO and cyclooxygenase inhibition so that differences in endothelial vasodilators would not interfere with the reactivity. To our surprise, PCAs from NP animals dilated at most concentrations of serotonin. This dilation may demonstrate EDHF release because it occurred in the presence of NO and prostaglandin inhibition (which is the current definition of EDHF; Ref. 19). In addition, endothelium removal abolished the dilation in response to serotonin, so that NP arteries contracted similarly to arteries from LP and PP animals. However, EDHF is thought to involve endothelial K\(_{Ca}\) channels and smooth muscle cell hyperpolarization (19), and until these factors are tested in NP animals, we cannot be certain of the role of EDHF in mediating dilations in response to serotonin. Given the results from this study, it appears that in the
NP state. EDHF may be responsible for the dilation, but in LP and PP animals, other vasodilators dominate (e.g., NO during the PP period).

There are several explanations for the different responses to serotonin between gestational groups. First, the endothelium may be more sensitive to Ca\(^{2+}\) in the NP state. EDHF release has been shown to be Ca\(^{2+}\) dependent, similar to NO; however, there is evidence that a greater level of intracellular Ca\(^{2+}\) must be reached before EDHF can be released (26). It therefore appears that serotonin is capable of releasing EDHF in the cerebral endothelium of NP but not LP or PP animals, which suggests that pregnancy and the PP state alter Ca\(^{2+}\) signaling in endothelium. Second, the composition of serotonin receptors on the endothelium may be different in PCAs from LP and PP animals and may cause a differential response. Third, expression of K\(^{+}\) channels on the smooth muscle that mediate the EDHF dilation may be altered during pregnancy, as suggested above. Although additional studies are needed to determine the mechanism of the differential serotonin dilation, it is clear that gestational effects on endothelium-dependent vasodilator production occur in the cerebral circulation, and this effect could significantly affect diameter regulation when mean arterial pressure is increased beyond the myogenic pressure range.

We also determined that passive distensibility was diminished in PCAs from PP animals. This suggests that structural remodeling occurs along with functional alterations in the cerebral circulation during the PP state. Because distensibility was determined in the presence of papaverine to inactivate the contractile apparatus, the change indirectly represents a change in the extracellular matrix composition, e.g., the collagen-elastin ratio. It is well known that pregnancy is associated with significant vascular remodeling of the uterine and systemic circulations to accommodate the large increases in plasma volume and blood flow to the uterus (24, 29). However, this is the first study to demonstrate vascular remodeling of the cerebral circulation during the PP period.

In summary, the present study demonstrates that the LP and PP periods, two states in which eclampsia occurs most often, are associated with diminished myogenic tone and a lower pressure at which FD occurs. In addition, there appears to be a shift in cerebral endothelial vasodilator production from greater amounts of EDHF release in NP animals to increased NO values in LP and the PP state. These data suggest that normal pregnancy and the PP state have significant influence on the regulation of cerebral artery diameter. Although this result may not have a deleterious effect on women who are normotensive, it may promote FD and edema formation during hypertension when mean arterial pressure is elevated beyond the normal autoregulatory range.

GRANTS

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REFERENCES


