Placental Ischemia Impairs Middle Cerebral Artery Myogenic Responses in the Pregnant Rat

Michael J. Ryan, Emily L. Gilbert, Porter H. Glover, Eric M. George, C. Warren Masterson, Gerald R. McLemore, Jr, Babbette LaMarca, Joey P. Granger, Heather A. Drummond

Abstract—One potential mechanism contributing to the increased risk for encephalopathies in women with preeclampsia is altered cerebral vascular autoregulation resulting from impaired myogenic tone. Whether placental ischemia, a commonly proposed initiator of preeclampsia, alters cerebral vascular function is unknown. This study tested the hypothesis that placental ischemia in pregnant rats (caused by reduced uterine perfusion pressure [RUPP]) leads to impaired myogenic responses in middle cerebral arteries. Mean arterial pressure was increased by RUPP (135±3 mm Hg) compared with normal pregnant rats (103±2 mm Hg) and nonpregnant controls (116±1 mm Hg). Middle cerebral arteries from rats euthanized on gestation day 19 were assessed in a pressure arteriograph under active (+Ca²⁺) and passive (0 Ca²⁺) conditions, whereas luminal pressure was varied between 25 and 150 mm Hg. The slope of the relationship between tone and pressure in the middle cerebral artery was 0.08±0.01 in control rats and was similar in normal pregnant rats (0.05±0.01). In the RUPP model of placental ischemia, this relationship was markedly reduced (slope=0.01±0.00; P<0.05). Endothelial dependent and independent dilation was not different between groups, nor was there evidence of vascular remodeling assessed by the wall:lumen ratio and calculated wall stress. The impaired myogenic response was associated with brain edema measured by percentage of water content (RUPP P<0.05 versus control and normal pregnant rats). This study demonstrates that placental ischemia in pregnant rats leads to impaired myogenic tone in the middle cerebral arteries and that the RUPP model is a potentially important tool to examine mechanisms leading to encephalopathy during preeclamptic pregnancies. (Hypertension. 2011;58:1126-1131.)

Key Words: pregnancy ▪ encephalopathy ▪ autoregulation ▪ preeclampsia ▪ cerebral

Hypertensive disorders during pregnancy, such as preeclampsia, are a leading cause of maternal morbidity and mortality. The cause of hypertension during preeclampsia remains unclear; however, impaired placental vascularization leading to hypoxia and ischemia is considered by many to be a key event. Although many organ systems are affected during preeclampsia, the brain is involved in ~40% of maternal deaths. Impaired cerebral vascular function may be permissive for the development of cerebral edema and injury that can progress to eclampsia, a life-threatening condition characterized by blurred vision, headaches, and seizure that is often considered to be a form of hypertensive encephalopathy. The mechanisms that promote cerebral vascular changes during preeclampsia and whether placental ischemia can lead to encephalopathies during pregnancy are not clear.

One potential factor that contributes to cerebral encephalopathies during hypertensive pregnancies is an impaired autoregulatory capacity of cerebral blood vessels in response to changes in pressure. The myogenic response is an important mechanism for maintaining constant blood flow in response to changes in blood pressure. Data from studies in experimental animal models suggest that hypertension during pregnancy impairs myogenic reactivity in cerebral vessels.

Understanding mechanisms that lead to cerebral vascular changes during preeclampsia have been difficult to address in part because of the limited number of experimental animal models that adequately mimic preeclampsia in humans. We demonstrated previously that reduced uterine perfusion pressure (RUPP) in the pregnant rat triggers many characteristics of preeclampsia observed in humans. These include hypertension and proteinuria, endothelial dysfunction, an increased total peripheral resistance, and a decreased cardiac output. In the present study, we tested the hypothesis that placental ischemia in the pregnant rat leads to impaired myogenic responses in middle cerebral arteries (MCAs).

Materials and Methods

Animals
All of the studies were performed in timed pregnant Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc (Indianapolis, IN).
Animals were housed in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle. All of the experimental procedures executed in this study were in accordance with National Institutes of Health guidelines for use and care of animals, and the institutional animal care and use committee at the University of Mississippi Medical Center approved all of the protocols. Placental ischemia was induced by RUPP and was created on gestation day 14, as described previously.8 Nonpregnant and normal pregnant rats were used as controls.

Mean Arterial Pressure
Rats were catheterized on day 18 of gestation under isoflurane anesthesia, as described previously, and pressure was measured on day 19, as described previously.8 The blood pressure recorded at day 19 in pregnant animals from this study is consistent with our blood pressure values recorded on day 19 of gestation by radiotelemetry in normal pregnant rats.11

Vascular Function
Rats were euthanized immediately after measuring pressure. The brain was quickly removed, weighed, and placed in physiological saline solution (pH adjusted to 7.4 with NaOH; containing [in millimoles per liter]: 130 NaCl, 4 KC1, 1.2 MgSO4, 4 NaHCO3, 1.8 CaCl2, 10 HEPES, 1.18 KH2PO4, 6 glucose, and 0.03 EDTA) on ice. The M1 segment of the MCA was dissected from the circle of Willis and kept in physiological saline solution on ice until the experiment.

Vessel Preparation
MCAs were transferred to a vessel chamber (model CH/1/SH, Living Systems, Burlington, VT), and the proximal end of the artery was cannulated with a tapered glass pipette, secured in place with a single strand of silk ligature, and gently flushed to remove any residual blood from the lumen. The distal end of the vessel was cannulated, and the artery was gently stretched longitudinally to approximate its in situ length and pressurized to 75 mm Hg at 37°C. The vessel chamber was mounted on a Nikon Eclipse TE2000-S microscope equipped with a CoolSnap CCD camera (Roper Scientific, Trenton, NJ). The distal stopcock was closed to conduct the experiment under 0 flow conditions and eliminate potential effects of flow-induced shear stress. The lumen pressure was controlled with a peristaltic pump regulated by a servo-controller (Living Systems) or by raising and lowering the height of an adjustable water column. Vessel diameter and wall thickness were measured in the center of the vessel using the Nikon Imaging Software, Elements.

Protocol 1
Changes in MCA diameter were tested after an initial decrease in lumen pressure to 25 mm Hg followed by 25 mm Hg increments until the lumen pressure reached 150 mm Hg. Each pressure step was held for 5 minutes. This protocol was performed under active (in the presence of extracellular calcium) and passive (absence of extracellular calcium) conditions.

Protocol 2
Concentration response curves (10−9 to 10−4 m) to acetylcholine, ADP, and sodium nitroprusside were performed in vessels at 75 mm Hg after preconstriction with the a1-adrenergic agonist phenylephrine (10−5 m). It is common to preconstrict isolated middle cerebral vessels to increase tone and to have a wider dynamic range with which to quantify the vasodilator response.12–16 Importantly, the tone generated by MCA in the present work is consistent with previously published work of others.17,18 All of the vasoactive agonists were added to the 5-mL chamber bathing the vessels. Phenylephrine was used for preconstriction, because this was used in previously published work from our laboratories.19 Vasodilatation was calculated as the percentage of dilation.

Calculations
The following calculations were made as described previously by others.20 Vessel tone was calculated as ([ØPpass−ØAactive]/ØPpass)*100 and is expressed as percentage of tone. ØPpass and ØAactive are the internal diameters in microns under passive and active conditions at each pressure, respectively. The wall:lumen ratio is calculated as the ðInner/[ØInner], where ð is the wall thickness and ØInner is the ID in microns. Circumferential wall stress is calculated as T/[ø], where T is the wall tension. Tension is calculated as T=pressure*Ø, where pressure is in dynes per centimeter squared (1 mm Hg=1333.2 dynes/cm2) and r is the radius (ØInner/2). The measurements used to calculate wall:lumen ratio and circumferential wall stress were made under passive conditions at a luminal pressure of 75 mm Hg and under the assumption that the vessel is a cylindrical tube.

Brain Water Content
Brain water content, as a marker of edema, was calculated as the wet weight of the brain immediately on removal minus the dry weight (weighed 3 days after drying in a laboratory oven), with this value divided by the wet weight. The data are presented as a percentage of the total wet weight. A similar method has been used previously by others.21

Western Analysis
Superficial blood vessels were dissected from the inferior side of the brain, and total protein homogenates were analyzed for the β subunit of the epithelial sodium channel (βENaC) by standard Western blotting methods. Blots were incubated with a sheep anti-βENaC (1:1000) and a mouse anti-β actin (1:5000, Gentest) overnight at 4°C as published previously by us.22

Statistics
For studies examining vessel function, we used a 1-way ANOVA with repeated measures with a Newman-Keuls multiple comparison post hoc test. GraphPad Prism software was used to generate linear regressions for myogenic responses in each rat. The slopes were compared between each group using a 1-way ANOVA with a Newman-Keuls post hoc test. This was also used to assess statistical differences in pressure and brain water content. Data are presented as the mean±SEM and were considered statistically different at P values <0.05.

Results
This study tested whether placental ischemia (induced by RUPP in pregnant rats) leads to impaired cerebral vascular myogenic responses. As expected, normal pregnant rats have a lower mean arterial pressure (103±2 mm Hg; n=19; P<0.05 versus control and RUPP rats) when compared with nonpregnant control animals (116±1 mm Hg; n=14). Consistent with our previous studies, RUPP in pregnant rats significantly increases mean arterial blood pressure (135±3 mm Hg; n=20; P<0.05 versus control and pregnant rats).

Figure 1 shows the change in MCA diameter under active and passive conditions in response to pressure for nonpregnant control rats, normal pregnant rats, and pregnant rats with RUPP. In control animals, the difference between active and passive responses to pressure is significantly different beginning at 75 mm Hg. The difference between active and passive is blunted in normal pregnant rats. In pregnant rats with RUPP, the change in diameter caused by pressure increments is not different under active and passive conditions. Starting basal diameters were not statistically different between the experimental groups under active conditions (Table).

Vessel tone was calculated using active and passive internal diameters and was plotted as a function of increasing pressure (Figure 2). This relationship can be used as an indicator of vessel responsiveness to changes in pressure. The slope of this relationship in control rats was 0.08±0.01 (r²=0.9693), and this relationship was not statistically different in normal pregnant rats
(0.05±0.01; r²=0.9487). In pregnant rats with RUPP, the relationship between tone and intraluminal pressure was absent (0.01±0.003; r²=0.4389), suggesting an impairment of the cerebral vascular response to pressure.

There was no difference in the vessel response to ADP or acetylcholine between the experimental groups at any concentration (Figure 3B and 3C). Similarly, the response to the endothelial independent dilator sodium nitroprusside was not different between groups (Figure 3A).

Brain water content was significantly increased in pregnant rats with RUPP when compared with control rats and normal pregnant rats (Figure 4). The wall:lumen ratio, as a marker of vascular remodeling, was not different among experimental groups, nor was calculated wall stress (Table).

Previous studies from our laboratory (H A Drummond) show that βENaC is an important mechanotransducer in the cerebral vasculature of rats and mice that mechanistically contributes to the myogenic response. Figure 5 shows that the protein expression of βENaC in isolated cerebral vessels is reduced in animals with RUPP when compared with pregnant rats.

**Discussion**

The major new finding of the present study is that the induction of placental ischemia in pregnant rats leads to impaired cerebral vascular responses to incremental pressure changes that are associated with increased brain water content and decreased cerebral vascular expression of βENaC. This is an important discovery, because the mechanisms leading to encephalopathies during hypertensive pregnancies remain unclear, and experimental animal models that closely mimic preeclampsia have been limited. Therefore, this study describes a new experimental model and a potentially important experimental pathway (βENaC) on which one can focus to explore mechanisms leading to cerebral changes during preeclampsia.

**RUPP Model of Placental Ischemia**

Preeclampsia is characterized by high blood pressure and proteinuria beginning in the late second or early third trimester of pregnancy that remits on delivery of the fetus. The cause of hypertension during preeclampsia remains unclear; however, many consider impaired vascularization of the placenta leading to ischemia as a key initiating event. RUPP in the pregnant rat induces many characteristics of preeclampsia by mimicking the placental ischemia. Similar methods to restrict uterine blood flow have been used to model preeclampsia in nonhuman primates, dogs, and rabbits. Examples of similarities between the RUPP model in rats and preeclampsia in humans, in addition to the placental ischemia, include the development of impaired renal hemodynamics, impaired endothelial function in the peripheral vasculature, immune system activation, and altered levels of angiogenic factors, such as soluble fms-like tyrosine kinase 1. All of these are consistent with preeclampsia in women. Therefore, the major advantage of the RUPP model is that it mimics a large number of characteristics of human preeclampsia, whereas other models typically exhibit only the increased blood pressure commonly observed during preeclampsia.

**MCA Structure and Autoregulatory Function**

The brain is a highly perfused organ system and, as a result, tight regulation of flow is required to prevent blood pressure changes from damaging neuronal tissue. To accomplish this, autoregulatory mechanisms in the cerebral vasculature help to maintain constant cerebral blood flow over a range of arterial pressures from ~60 and 160 mm Hg. The myogenic response is an intrinsic property of vascular smooth muscle to constrict or dilate in response to increases or decreases in pressure, respectively. It is an important autoregulatory mechanism that, when impaired, can lead to pressure-mediated tissue injury and increase downstream hydrostatic

<table>
<thead>
<tr>
<th>Vessel Property</th>
<th>CTRL</th>
<th>Pregnant</th>
<th>RUPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Øactive, μM</td>
<td>203±5 (n=14)</td>
<td>183±6 (n=16)</td>
<td>192±7 (n=13)</td>
</tr>
<tr>
<td>Øpassive, μM</td>
<td>230±5 (n=14)</td>
<td>207±6 (n=16)</td>
<td>215±9 (n=13)</td>
</tr>
<tr>
<td>W:L</td>
<td>0.087±0.005 (n=7)</td>
<td>0.083±0.005 (n=6)</td>
<td>0.076±0.004 (n=6)</td>
</tr>
<tr>
<td>Wall stress, dyne/cm×10⁵</td>
<td>3.9±0.3 (n=7)</td>
<td>4.0±0.2 (n=6)</td>
<td>4.4±0.4 (n=6)</td>
</tr>
</tbody>
</table>

Ø indicates ID at 75 mm Hg; W:L, wall:lumen ratio; CTRL, control; RUPP, reduced uterine perfusion pressure; MCA, middle cerebral artery. W:L ratio and wall stress were calculated under passive conditions at 75 mm Hg.
pressure to promote edema. Previous studies in isolated posterior cerebral arteries from late pregnant rats (last 7 days of gestation) show that this myogenic response is impaired. Experimental evidence is also suggestive of a change in autoregulatory function in vivo during hypertensive pregnancies. For example, at a given blood pressure, late pregnant rats with hypertension caused by N^G-nitro-L-arginine methyl ester have greater cerebral blood flow than nonpregnant rats with N^G-nitro-L-arginine methyl ester–induced hypertension. This suggests that the cerebral blood flow regulation is altered when hypertension is superimposed on pregnancy. The mechanisms for impaired myogenic responses in the RUPP model, or other models of hypertension during pregnancy, remain undefined. Although the possibility exists that hypertension resulting from placental ischemia could contribute to the impaired myogenic responses, we believe this to be unlikely given that hypertension in nonpregnant animals typically results in vascular wall changes that shift the autoregulatory curve to higher pressures.

Chronic hypertension is commonly associated with medial hypertrophy that encroaches on the lumen of vessels. Inward vascular remodeling can also lead to a smaller ID and OD, with no change in cross-sectional area during hypertension. Both hypertrophy and remodeling of the cerebral vessels can help to maintain constant cerebral blood flow and protect the brain from pressure-induced damage. Impor-tantly, evidence from studies in rats shows that pregnancy prevents and reverses both medial hypertrophy and inward remodeling, making the brain vulnerable to sudden changes in blood pressure. This is in contrast to data from nonpregnant rats showing that increased pressure, even over short time courses, is associated with a smaller ID of cerebral vessels. Our data showing that the wall:lumen ratio, wall stress, and IDs under active and passive conditions (Table) are similar in pregnant rats with or without RUPP are consistent with these previous findings.

**Consequence of Impaired Myogenic Responses**

Preeclampsia may precede the more serious eclampsia charac-terized by seizures, and both are considered among disorders that constitute posterior reversible encephalopathy syndromes. Posterior reversible encephalopathy syndrome can also occur at blood pressures that are not considered to be hypertensive. A prevailing theory is that posterior reversible encephalopathy syndromes are caused in part by impaired cerebral blood flow autoregulation; however, the mechanisms for this remain elusive. Previous work from Drummond and colleagues shows an important role for ENaC as a mechanosensor that contributes to the myogenic response in different vascular beds, including cerebral vessels. In the present study, we show that the expression of ENaC from cerebral vessels in RUPP rats is significantly reduced when compared with pregnant controls. Therefore, although the mechanisms for impaired vessel tone in pregnancy-induced hypertension remain unclear, this provo-

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**Figure 2.** The relationship between middle cerebral artery (MCA) tone and pressure is blunted in pregnant rats with reduced uterine perfusion pressure (RUPP) vs nonpregnant controls (CTRLs) and normal pregnant (Pregnant) rats. One-way ANOVA, *P*<0.05 vs CTRL and Pregnant (CTRL n=14; Pregnant n=16; RUPP n=13).

**Figure 3.** Isolated middle cerebral artery (MCA) responses to the endothelial-independent dilator sodium nitroprusside (A, SNP) and endothelial-dependent dilators acetylcholine (B, ACh), or ADP (C) in nonpregnant control (CTRL), normal pregnant (Pregnant), and reduced uterine perfusion pressure (RUPP) pregnant rats. Vessels were preconstricted with phenylephrine (10 μM) (CTRL n=7; Pregnant n=9; RUPP n=6 for SNP and ACh; Pregnant n=4, RUPP n=4 for ADP).

**Figure 4.** Brain water content (percentage of total weight) is significantly greater in pregnant rats with reduced uterine perfusion pressure (RUPP). One-way ANOVA, *P*<0.05 vs CTRL and Pregnant (CTRL n=11; Pregnant n=24; RUPP n=22).
imbalance between proangiogenic (ie, vascular endothelial growth factor) and angiostatic (soluble fms-like tyrosine kinase 1 or soluble endoglin) factors has been reported to mechanistically contribute to the development of preeclampsia.\textsuperscript{42,43} We demonstrated recently that these factors are important in the vascular phenotypes and hypertension associated with the RUPP model.\textsuperscript{44,45} The direct effect of proangiogenic and antiangiogenic factors on cerebral vascular function in the RUPP model will be an important line of future investigation.

Cerebral Endothelial Function

In addition to myogenic responses, we tested whether endothelial-dependent dilation was impaired in the MCAs from pregnant rats with RUPP. The data show that the concentration response to the endothelial-dependent agonist ADP or acetylcholine and endothelial-independent agonist sodium nitroprusside is not altered. These data differ from previously published work in the RUPP model showing impaired endothelial function.\textsuperscript{9} The reason for these disparate responses can be attributed to potential vascular bed-specific differences within this model (aorta versus cerebral). Indeed, dramatic differences in endothelial responses to acetylcholine have been reported in the carotid artery and aorta from the same strain of mouse.\textsuperscript{46} Another factor that likely contributes to the disparate responses is the experimental preparation. Early studies showing impaired endothelial function were conducted using aortic tissue cut into strips and mounted in organ chamber baths. The present study uses intact, pressurized MCAs. The level of preconstriction achieved with 10 μmol/L of phenylephrine was not different between groups.

Perspectives

Preeclampsia affects \textasciitilde5% to 8% of all pregnancies and is associated with an increased risk for a variety of encephalopathies. The mechanisms that lead to cerebral impairment continue to be investigated but are thought to be related to changes in vascular function and permeability. This study demonstrates that placental ischemia in pregnant rats caused by RUPP is associated with alterations in cerebral vascular function and that reduced \( \beta \)-ENaC expression in cerebral vessels from RUPP rats is associated with the impaired function. Because many characteristics of preeclampsia are exhibited, this model will be a useful tool to further investigate the underlying mechanisms that promote cerebral vascular changes during pregnancy and preeclampsia.

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