Published in final edited form as:

Microvasc Res. 2018 September ; 119: 64–72. doi:10.1016/j.mvr.2018.04.007.

Impaired Function of Cerebral Parenchymal Arterioles in Experimental Preeclampsia

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Abstract

Preeclampsia (PE), a dangerous hypertensive complication of pregnancy, is associated with widespread maternal vascular dysfunction. However, the effect of PE on the cerebral vasculature that can lead to stroke and cognitive decline is not well understood. We hypothesized that function of cortical parenchymal arterioles (PAs) would be impaired during PE. Using a high cholesterol diet to induce experimental PE in rats (ePE), we studied the function and structure of isolated and pressurized PAs supplying frontoparietal WM tracts and cortex and compared to normal pregnant (Preg) and nonpregnant (Nonpreg) Sprague Dawley rats (n=8/group). Myogenic reactivity and tone were similar between groups; however, constriction to intermediate-conductance calcium-activated potassium (IK) channel inhibition was diminished and dilation to inward-rectifying K⁺ (K_{IR}) channel activation was impaired in PAs from ePE rats, suggesting altered ion channel function. Conducted vasodilation was significantly delayed in response to 12 mM KCl, but not 10 μ M adenosine, in PAs from ePE rats versus Preg and Nonpreg rats (940 ± 300 ms vs. 70 ± 50 ms and 370 ± 90 ms; $p < 0.05$). Overall, dysfunction of PAs supplying frontoparietal WM and gray matter was present in ePE. If persistent these changes could potentiate neuronal injury that over time could contribute to WM lesions and early-onset cognitive decline.

Keywords

Preeclampsia; Pregnancy; Cerebral Arterioles; Cerebrovascular Dysfunction; Conducted Vasodilation

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Introduction

Preeclampsia (PE) is a hypertensive complication of pregnancy involving widespread maternal vascular dysfunction, including endothelial activation, hypercoagulation, and increased vascular stiffness.^{1–4} In the brain, PE affects the cerebral microcirculation that can manifest with neurological symptoms including persistent headache, cortical blindness and de novo seizure (eclampsia).^{5, 6} PE is a leading cause of maternal morbidity and mortality worldwide, with 40 % of maternal deaths involving complications of the cerebrovasculature.⁷ Not only is PE dangerous during the acute pregnancy phase (index pregnancy), but is also associated with long-term adverse neurological function.^{8, 9} For example, formerly PE women scored worse on a Cognitive Failures Questionnaire and self-report cognitive impairment more frequently than women with prior normotensive pregnancies.^{9, 10} In addition, compared to women who had normal pregnancies, formerly PE women had increased incidence and severity of cerebral white matter (WM) lesions predominantly affecting the frontal lobes.^{11, 12} WM lesions, defined radiologically as subcortical hyperintensities of WM on T2-weighted magnetic resonance imaging (MRI), are strongly associated with cognitive decline.^{13, 14}

The underlying etiology of WM lesions remains unclear, but is thought to be vascular in nature.¹⁵ In addition, under pathological conditions including hypertension and hyperlipidemia, both of which are present in PE, cerebrovascular dysfunction may perpetuate WM injury.^{14, 16, 17} For example, hypertension-induced endothelial dysfunction and vascular inward remodeling can compromise the ability of blood flow to match neuronal metabolic demand and lead to chronic hypoperfusion.¹⁶ Although WM is less metabolically active than gray matter, it is particularly susceptible to vascular insufficiency.^{14, 18} However, despite evidence of increased WM lesion burden and impaired cognition later in life, little is known about how PE affects cerebrovascular function of arterioles perfusing frontoparietal WM and cortex during the index pregnancy that may predispose to WM lesions and cognitive decline.

Hyperlipidemia is a consequence of normal pregnancy that is exaggerated in PE and thought to underlie maternal vascular dysfunction.¹⁹ Increased very low-density lipoproteins (vLDL) have been shown in women with PE that favors oxidation and formation of oxidized low-density lipoprotein (oxLDL).^{17, 19–21} oxLDL interacts with its receptor lectin-like oxLDL receptor 1 (LOX-1) that induces endothelial damage and dysfunction in several pathological states including hypertension, atherosclerosis and PE.^{20, 22–24} Importantly, pregnant rats maintained on a high cholesterol diet for days 7 – 20 (of a 22 day) of gestation develop preeclamptic-like symptoms including elevated blood pressure, fetal growth restriction, and maternal endothelial dysfunction.²⁵ Further, this model of experimental PE (ePE) has increased blood-brain barrier permeability that was shown to be LOX-1-dependent.^{25–27} However, the effect of ePE on cerebral parenchymal arterioles perfusing WM and deep cortical structures that could impact WM and gray matter integrity during the index pregnancy has yet to be determined.

In the current study, we investigated the function and structure of cerebral parenchymal arterioles (PAs) that supplied the frontoparietal WM tracts and the basal ganglia in rats with

ePE compared to normal pregnant (Preg) and nonpregnant (Nonpreg) rats. We investigated myogenic reactivity of PAs to intravascular pressures and conducted vasodilatory responses to mediators involved in coupling neuronal activity to cerebral blood flow including extracellular K⁺ and adenosine.²⁸ Finally, we investigated ePE-induced structural remodeling of arterioles, including changes in lumen diameter, vascular wall thickness, and vessel stiffness. We hypothesized that the function of arterioles from ePE rats would be impaired, compromising the ability of arterioles to conduct vasodilatory responses upstream. We further hypothesized that ePE would cause structural remodeling of PAs to be smaller and stiffer than arterioles from Preg and Nonpreg rats.

Materials and Methods

Animals

All experiments were conducted using virgin, Nonpreg or timed-Preg Sprague Dawley rats between 12 and 14 weeks of age (Charles River, Canada). Preg rats were used late in gestation (day 20 of a 22 day gestation). Rats were housed singly with environmental enrichment in the University of Vermont Animal Care Facility, an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) accredited facility. Rats were maintained on a 12-h light/dark cycle and allowed access to food and water ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The investigator was not blinded to animal group during experiments, as this was not possible due to the obvious visual differences in body size during pregnancy. However, the order of experiments was randomized by animal group using an online randomization tool (random.org). All euthanasia was under isoflurane anesthesia according to NIH guidelines.

Rat Model of Experimental PE (ePE)

PE is a state of dyslipidemia that is thought to contribute to maternal endothelial dysfunction and the pathogenesis of PE through elevated oxLDL and LOX-1 activation.^{2, 17, 20, 25, 26, 29} We used an established model of ePE that involved maintaining pregnant rats on a high cholesterol diet (Prolab 3000 rat chow with 2 % cholesterol and 0.5 % sodium cholate; Scotts Distributing Inc., Hudson, NH, USA) days 7 – 20 of gestation.²⁷ This model has been previously shown to induce dyslipidemia and cause other PE-like symptoms including maternal endothelial dysfunction and increased blood pressure.^{25, 26} Although uteroplacental vascular function was not measured, this model is associated with fetal growth restriction suggesting it encompasses impaired uteroplacental blood flow and/or placental dysfunction.²⁵

Measurement of Circulating Factors via Enzyme-Linked Immunosorbent Assays (ELISAs)

To assess systemic markers of inflammation and oxidative stress in the ePE model, circulating levels of tumor necrosis factor alpha (TNF α) and oxLDL were measured in serum from Nonpreg, Preg, and ePE rats (n=5 – 8/group) using commercially available ELISA kits for TNF α (R&D Systems, Minneapolis, MN, USA) and oxLDL (Biomatik, Wilmington, DE, USA). Samples were measured undiluted and in duplicate.

In-vitro Isolated Arteriole Experiments

Rats that were either Nonpreg, Preg, or with ePE (n=8/group) were decapitated under deep isoflurane anesthesia (3 % oxygen) and brains immediately removed and placed in cold, oxygenated artificial cerebrospinal fluid (aCSF). Immediately after brains were dissected out of the skull, sections of cerebral cortex that were 2 mm thick containing the middle cerebral artery (MCA) were dissected from the brain. PAs branching from the MCA that were > 1.0 mm in length and entering the cerebral cortex through the lateral olfactory tract were dissected, mounted and pressurized in an arteriograph chamber as previously described (Figure 1).^{27, 30}

PAs were equilibrated at 20 mmHg for one hour, after which intravascular pressure was increased to 120 mmHg in a stepwise manner to determine if vessels developed spontaneous myogenic tone and to measure myogenic reactivity. Lumen diameter and wall thickness were recorded at each intravascular pressure. Pressure was then returned to 40 mmHg for the remainder of the experiment. Vessels that did not develop myogenic tone along the 700 μ m length of vessels segment that indicated damage to the vascular wall were excluded. To investigate the response of PAs to some mediators of dilation, reactivity to various pharmacological agents was measured: NS309, a small- and intermediate-conductance calcium-activated potassium (SK/IK) channel agonist (10^{-5} M); extracellular KCl (3 – 40 mM); adenosine (10^{-8} – 10^{-4} M). Cumulative doses of the SK channel inhibitor apamin (0.3 μ M) and the IK channel inhibitor TRAM-34 (1.0 μ M) were added to the bath and lumen diameters recorded after 25 minutes. At the end of each experiment, aCSF was replaced with aCSF containing zero calcium, 0.5 mM EGTA, papaverine (10^{-4} M) and diltiazem (10^{-5} M) to fully relax the vascular smooth muscle, and passive structural measurements made within the pressure range of 5 – 120 mmHg.

Local Application of KCl and Adenosine to Measure Conducted Vasodilations

Using a micromanipulator (Narishige International USA, East Meadow, NY, USA), a glass micropipette (20 μ m diameter tip) that was connected to a micro-injection system (Picospritzer III, Parker Hannafin Corp., Pine Brook, NJ, USA) was inserted into the bath and positioned 50 μ m from the distal-most portion of the vessel (Figure 1). Glass micropipettes were backfilled with aCSF containing 12 mM KCl (to activate inward rectifier potassium, K_{IR} , channels) or 10 μ M adenosine that was ejected through the micropipette with the ejection pressure set to 3 psi using 100 % nitrogen gas for a pulse duration of 10 sec. The bath flow of the arteriograph chamber was 4 mL/min in the proximal-to-distal direction to eliminate diffusion of drug proximally along the length of the vessel that was confirmed by locally applying trypan blue dye in preliminary studies. Lumen diameters were simultaneously and continuously recorded 700 μ m upstream using an IonOptix system (Milton, MA, USA). Conducted vasodilatory measurements to 12 mM KCl were repeated after cumulative treatment of apamin and TRAM-34 to determine the role of SK/IK channels in K_{IR} -dependent conducted vasodilation.

To investigate pregnancy- and ePE-induced changes in conducted vasodilation in response to local application of 12 mM KCl and 10 μ M adenosine, several parameters were measured in response to drug application: diameter after treatment compared to baseline (starting)

diameter, percent tone at maximum diameter, the duration for vessels to dilate to maximum diameter in response to local application of vasoactive substance ($t_{\max\phi}$) (Figure S1), the duration for vessels to constrict back to lumen diameters that were 67% of the total diameter change (τ ; Figure S1), the percent of vasodilation that was conducted upstream, and the delay in conduction was determined by measuring the time between local dilation and remote dilation 700 μm upstream. Two different mediators were investigated to determine conducted responses involving K_{IR} channel activation (by 12 mM KCl application),^{31, 32} and receptor-mediated, endothelium-independent vasorelaxation via adenosine A_{2A} receptor activation.³³

Drugs and Solutions

Adenosine, NS309, KCl, and papaverine were purchased from Sigma Aldrich (St. Louis, MO, USA). Apamin and TRAM-34 were purchased from Tocris (Minneapolis, MN, USA). Diltiazem was purchased from MP Biomedicals (Santa Ana, CA, USA). Adenosine stock solution was made every other day, and stock solutions of papaverine and diltiazem were made weekly and stored at 4 °C until use. NS309 and TRAM-34 were diluted in DMSO, and apamin in double-distilled H_2O , and stock solutions aliquoted and stored at -20 °C until use. Isolated PA experiments were performed using aCSF containing (mM): NaCl 122.0, NaHCO_3 26.0, NaH_2PO_4 1.25, KCl 3.0, MgCl_2 1.0, CaCl_2 2.0, and glucose 4.0. aCSF with higher concentrations of KCl (8 – 40 mM) were made with reduced amounts of NaCl to maintain constant osmolality. Buffer solutions were made each week and stored without glucose at 4 °C. Glucose was added immediately prior to each experiment. aCSF was aerated with 5 % CO_2 , 10 % O_2 and 85 % N_2 to maintain pH at 7.40 ± 0.05 and the temperature within the arteriograph chamber bath was maintained at 37.0 ± 0.2 °C throughout the experiments.

Data Calculations

At each intravascular pressure, percent tone was calculated by the equation: % Tone = $[1 - (\phi_{\text{active}}/\phi_{\text{passive}})] \times 100$ %; where ϕ_{active} is lumen diameter under physiological conditions and ϕ_{passive} is lumen diameter under fully relaxed conditions. Dilation to NS309 was calculated from the equation: % Dilation = $[(\phi_{\text{dose}} - \phi_{\text{baseline}})/(\phi_{\text{passive}} - \phi_{\text{baseline}})] \times 100$ %; where ϕ_{dose} is the diameter of the vessel after treatment with a specific concentration of drug and ϕ_{baseline} is the starting diameter before any drug treatment. Percent change in diameter from baseline with different extracellular KCl concentrations was calculated using the equation: % Change = $[(\phi_{\text{dose}} - \phi_{\text{baseline}})/\phi_{\text{baseline}}] \times 100$ %. Percent constriction to apamin and TRAM-34 was calculated using the equation: % Constriction = $[(\phi_{\text{baseline}} - \phi_{\text{dose}})/\phi_{\text{baseline}}] \times 100$ %. To compare parameters of conducted vasodilations, tau was calculated by the equation: $\tau = t_{67\%} - t_0$, where $t_{67\%}$ is the time at which the diameter constricted to 67% of the total dilation, and t_0 is the time at which maximum diameter was reached (Figure S1). Conducted vasodilation was calculated as the % of vasodilation conducted 700 μm upstream by the equation: % Conducted Vasodilation = $(\phi_{\text{remote}}/\phi_{\text{local}}) \times 100$; where ϕ_{remote} is the change in lumen diameter at the remote site 700 μm proximal to the application site and ϕ_{local} is the change in lumen diameter at the site of application. Outer diameter (ϕ_{outer}) was calculated at each pressure by the equation: $\phi_{\text{outer}} = \phi_{\text{inner}} + 2WT$; where ϕ_{inner} is the lumen diameter of the vessel fully relaxed and WT is the measured wall thickness. Cross-sectional

area (CSA) was calculated by the equation: $CSA = \pi(\phi_{outer}/2)^2 - \pi(\phi_{inner}/2)^2$ at intravascular pressures 5 – 120 mmHg. For wall tension, wall stress and wall strain, all diameter and WT measurements were converted to cm. Wall tension was calculated across the pressure range 5 – 120 mmHg by converting pressure (mmHg) into dynes/cm² x ($\phi_{inner}/2$). Wall stress was calculated at each pressure by the equation: Wall Stress = wall tension/WT. Wall strain was calculated by the equation: Wall Strain = $(\phi_{passive} - \phi_{mmHg})/\phi_{mmHg}$; where ϕ_{mmHg} is the passive diameter at each pressure.

Statistical Analyses

The number of animals used was determined by a statistical power calculation using a two-sided 95 % confidence interval for a single mean and $1 - \beta$ of 0.80 based on our previous studies using similar methodology.^{27, 30} Results are presented as mean $\pm$ SEM. Differences between three groups were determined by one-way ANOVA. For conducted vasodilation comparisons, diameters were compared to baseline and between groups using a two-way ANOVA. The role of SK/IK channels in the parameters of conducted vasodilation were determined by comparing arteriolar responses in the absence and presence of SK/IK channel inhibition by a repeated measures one-way ANOVA. Differences were considered significant at $p < 0.05$. All ANOVAs had *post-hoc* Bonferroni's test to correct for multiple comparisons and were performed using Graph Pad Prism 6 (Graph Pad Software, Inc., San Diego, CA, USA).

Results

Elevated Serum Markers of Inflammation and Oxidative Stress in Rats with ePE

To determine if ePE rats were in a state of inflammation and oxidative stress that could contribute to cerebrovascular dysfunction, circulating levels of TNF α and oxLDL were measured via ELISA. Serum levels of TNF α from Preg and Nonpreg rats were undetectable whereas serum TNF α level in rats with ePE was 8.01 ± 1.73 pg/mL. Circulating levels of oxLDL were significantly higher in serum from rats with ePE compared to Nonpreg and Preg rats (Figure 2). Thus, rats with ePE were in a state of inflammation and oxidative stress.

Reactivity of Isolated and Pressurized PAs

To investigate if ePE affected the cerebrovasculature supplying WM tracts and frontoparietal cortical structures, we studied the function of isolated PAs from ePE, Preg and Nonpreg rats. Figure 3A shows changes in lumen diameters of arterioles over the pressure range of 20 – 120 mmHg. As intravascular pressure increased, lumen diameters remained relatively stable with similar levels of tone (Figure 3B). Upon return of intravascular pressure to 40 mmHg, percent tone in PAs from ePE rats was 44 ± 3 %, 40 ± 2 % for Preg rats, and 43 ± 2 % for Nonpreg rats ($p > 0.05$).

SK and IK channels are present in the endothelium of PAs, and activation of SK/IK channels causes hyperpolarization and subsequent vasodilation;^{34, 35} however, whether these channels are functionally present in PAs from Preg or ePE rats is not known. A single high dose of NS309 caused dilation of arterioles in all groups, with maximum dilation being similar between groups (90 ± 3 % in ePE, 92 ± 3 % in Preg, and 95 ± 2 % in Nonpreg; $p > 0.05$). To

determine if SK and/or IK channels were basally active and inhibited myogenic tone, we administered pharmacological inhibitors cumulatively and measured changes in lumen diameters. Figure 4A shows representative tracings of lumen diameters of arterioles from Nonpreg, Preg and ePE rats in response to the bath application of apamin (0.3 μ M), and TRAM-34 (1.0 μ M). Apamin resulted in little-to-no change in lumen diameter of PAs from any groups (Figure 4B); however, TRAM-34 caused ~ 20 % constriction of arterioles from Preg and Nonpreg rats that was significantly less in those from ePE rats (Figure 4C).

To determine the effect of ePE on the arteriolar response to mediators of vasodilation, responses to globally (bath) applied extracellular K^+ and adenosine were studied. Increasing extracellular KCl within the range that activated K_{IR} channels (8 – 15 mM) elicited dilation of PAs from all groups (Figures 5A and 5B). At 8 mM KCl, diameters of PAs from Preg rats were significantly larger than those from ePE and Nonpreg rats (Figure 5A), demonstrating enhanced vasodilation of PAs from Preg rats that was absent in those from ePE rats. When extracellular KCl was increased to 15 mM, arterioles from ePE rats had significantly smaller lumen diameters than those from Preg rats (Figure 5A), demonstrating vasoconstriction in response to 15 mM KCl in PAs from ePE rats. As extracellular KCl was further increased, vasoconstriction occurred in arterioles from all groups, with similar constriction in response to 40 mM KCl (Figures 5A and 5B). Adenosine caused vasodilation in arterioles from all groups in a concentration-dependent manner, with the maximum concentration (100 μ M) eliciting similar dilations between groups (32 ± 7 % in ePE, 29 ± 3 % in Preg, and 28 ± 4 % in Nonpreg; $p > 0.05$).

Conducted Vasodilation of PAs

Figure 6A shows representative tracings of changes in lumen diameter of a PA from a Nonpreg rat at the local and remote application sites during administration of 12 mM KCl. Robust dilation occurred along the length of the arteriole, indicated by similar dilation occurring locally and 700 μ m proximally. Arterioles had similar lumen diameters at baseline in all groups, and dilated similarly 700 μ m proximal from the site of KCl application (Figure 6B). Percent tone of arterioles at maximum dilation to 12 mM KCl was 29 ± 3 % in ePE, 25 ± 4 % in Preg, and 25 ± 3 % in Nonpreg rats ($p > 0.05$). Tau was similar between groups (8.8 ± 3.2 sec in ePE vs. 7.1 ± 2.3 sec in Preg vs. 4.4 ± 0.7 sec in Nonpreg; $p > 0.05$). PAs from Preg rats had significantly greater $t_{max}\phi$ under control conditions compared to arterioles from PE and Nonpreg rats (Figure S2). The percent of vasodilation conducted 700 μ m proximally was ~ 90 % for PAs in all groups (Figure 6C); however, conducted vasodilation of PAs from ePE rats was significantly delayed compared to arterioles from Preg rats (Figure 6D). SK/IK channel inhibition did not affect the delay in conducted vasodilation in arterioles from Nonpreg and ePE rats; however, SK/IK channel inhibition increased the delay in arterioles from Preg rats compared to control conditions, that was not statistically significant ($p = 0.078$ by repeated-measures ANOVA; Figure 6D). SK/IK channel inhibition had no effect on any of the measured outcomes. There were no differences in the presence of apamin and TRAM-34 on the percent of conducted vasodilation (Figure 6C), $t_{max}\phi$ (Figure S2), the conducted vasodilatory response (Figure S3), percent tone at maximum dilation (data not shown), or tau (data not shown).

Local application of 10 μ M adenosine caused modest dilation upstream of PAs in all groups (Figure 7A and 7B). The $t_{\max\phi}$ (Figure S4) and tau (2.6 ± 1.2 sec in ePE vs. 6.5 ± 2.7 sec in Preg vs. 6.5 ± 1.8 sec in Nonpreg; $p > 0.05$) were similar between groups. The percent of vasodilation conducted upstream was $\sim 50 - 60$ % (Figure 7C), and the delay in conducted vasodilation was $\sim 3 - 5$ sec (Figure 7D). Thus, the conduction of adenosine-evoked vasodilation was less robust and slower than that of 12 mM KCl and was similar between groups.

Structural and Biomechanical Properties of PAs

Passive lumen diameters (Figure S5A) and outer diameters (Figure S5B) of PAs from Preg, ePE and Nonpreg rats were similar. The vascular wall of PAs from Preg rats was thinner (Figure S5C) and cross-sectional areas smaller (Figure S5D) than PAs from ePE and Nonpreg rats across the pressure range studied. In addition, wall stress-strain curves were calculated to investigate changes in vessel stiffness in pregnancy and ePE and were similar between groups (Figure S5E).

Discussion

Formerly PE women have greater WM lesion burden and cognitive impairment than women who had normotensive pregnancies that may be due to adverse effects of PE on the function and structure of the cerebrovasculature during the index pregnancy.^{10–12} One theory is that women who develop PE are predisposed to cardiovascular disease prior to pregnancy and that the physiological stress of pregnancy unmasks vascular dysfunction. However, another theory is that PE causes long-lasting vascular damage that persists into the post-partum period.³⁶ The current study focused on understanding the effect of ePE on the function and structure of the cerebrovasculature during the acute pregnancy phase as a starting point to understanding PE-induced cerebral vasculopathies that may contribute to WM injury and cognitive decline later in life. The main findings of the current study were that: 1) rats with ePE had elevated circulating proinflammatory cytokines and were in a state of oxidative stress, as indicated by elevated serum oxLDL and TNF α , 2) PAs supplying frontoparietal WM tracts and cortical structures had impaired conducted vasodilation along their length in ePE, and 3) structural remodeling of PAs that was associated with normal pregnancy was prevented in rats with ePE; however, the myogenic response that contributes to blood flow autoregulation remained intact. Overall, these findings suggest that some cerebrovascular dysfunction was present in ePE that may increase the susceptibility to WM injury.

Local control of cerebral blood flow appears to occur at the level of pre-capillary arterioles and upstream penetrating arterioles, making the function of PAs critical in maintaining neuronal and glial health.³⁷ Further, the ability of PAs to conduct local vasodilation upstream to decrease segmental vascular resistance and increase local perfusion in response to neuronal activity is an important aspect of cerebrovascular function.^{16, 38} Impaired conducted vasodilation of PAs in ePE suggests there may be a mismatch of neuronal metabolism and local blood flow in frontoparietal WM and cortical structures that, if persistent after the index pregnancy, may potentiate development of WM disease and cognitive impairment.

K_{IR} channels are effective mediators of conducted vasodilation.³¹ In PAs, extracellular KCl concentrations between 8 – 15 mM activate K_{IR} channels on both vascular smooth muscle cells (VSMCs) and endothelial cells (ECs), resulting in rapid hyperpolarization and vasodilation that is prevented in the presence of the K_{IR} channel blocker barium chloride.^{31, 32, 39} In the current study, local activation of K_{IR} channels by 12 mM KCl caused a robust vasodilation that was conducted upstream, was similar between groups and not dependent upon SK/IK channel function. However, in arterioles from ePE rats, this conducted vasodilation was significantly delayed, taking nearly one second (~ 950 ms) for dilation to occur 700 μ m proximally, a > 10-fold decrease in the speed of conductance of PAs from normal pregnant rats (~ 70 ms). Interestingly, adenosine-mediated conducted vasodilation that is thought to be endothelial-independent was similar between groups.³³ The mechanism by which the speed of K_{IR} -dependent conductance was compromised in ePE remains to be determined, but could be due to changes in K_{IR} channel function and/or expression, or differential expression of gap junctions.³¹

The K_{IR} -dependent vasodilation of PAs appears critical to neurovascular coupling.^{40, 41} In a rat model of transient global cerebral ischemia neurovascular coupling was impaired due to the loss of functional K_{IR} channels in PAs.⁴¹ Further, in a study investigating the mechanism of stress-induced impaired neurovascular coupling in the amygdala of rats, Longden et al. (2014) showed decreased K_{IR} channel activity and expression in isolated PAs in-vitro that contributed to impaired neurovascular coupling in-vivo.⁴⁰ In the current study, the global dilatory response to 8 mM KCl was more robust in normal pregnancy compared to the nonpregnant state that was absent in ePE. These findings suggest pregnancy may increase K_{IR} expression and/or activity in VSMCs and/or ECs of PAs that contributes to the greater dilatory response to 8 mM KCl in PAs from Preg rats. The effect of pregnancy and ePE on cell-specific K_{IR} channel expression requires further investigation in order to determine the endothelial or smooth muscle contributions to the differences in global dilation and speed of conduction in response to K_{IR} channel activation. Regardless, the lack of this adaptation of PAs from ePE rats could decrease the local response of arterioles to elevated extracellular K^+ that occurs during neuronal activity.¹⁶ While the effect of K_{IR} channel-dependent dilation on neurovascular coupling in ePE was not determined in the current study, in combination with slowed conducted vasodilation could result in a mismatch of local blood flow and metabolic demand.⁴² The findings in the current study revealed changes in cerebrovascular function in ePE during the index pregnancy that could result in impaired neurovascular coupling and, if persistent over time, potentiate changes in gray matter, WM, and cognitive function that occur in formerly PE women.^{11, 43, 44}

Impaired neurovascular coupling has been demonstrated in women that formerly had PE.⁴⁴ Studies utilizing a visual stimulation task and transcranial Doppler to assess neurovascular coupling in women with PE reported impaired coupling of blood flow and neuronal activity six years after the index pregnancy that was not present mid-gestation prior to PE onset.^{44, 45} These findings suggest that cerebrovascular function is intact prior to development of PE, and that cerebrovascular dysfunction is a consequence of PE that may progress over time after the index pregnancy.⁴⁴ Although the findings of the current study support that PE exerts deleterious effects on cerebrovasculature function during the index pregnancy, a limitation is that WM integrity and cognitive function were not measured. However, WM

lesions and cognitive decline, while strongly associated, develop over time, making it unlikely to be present during the index pregnancy, especially in the short gestation of ePE. Further, cognitive function was reported to be similar in women during PE and normotensive pregnancies, further supporting that the effects of PE on WM and cognition are progressive and take years to manifest symptomatically.^{10, 12, 46} In addition, while we did not find an effect of ePE on myogenic tone or reactivity in PAs, it is not known how this might change in the post-partum state and over time.

The adaptation of PAs to normal pregnancy includes peroxisome proliferator-activated receptor gamma (PPAR γ)-dependent outward hypertrophic remodeling, resulting in a larger lumen diameter and thinner walls compared to the nonpregnant state.^{30, 47} Pregnancy-induced structural remodeling may be protective by contributing to the extension of the cerebral blood flow autoregulatory curve that occurs in normal pregnancy.^{48, 49} Pregnancy-induced thinner walls and increased wall stress could predispose PAs to increased permeability or rupture under pathological conditions such as acute hypertension.³⁰ However, in the current study we show that myogenic tone was sustained in arterioles at elevated intravascular pressure, suggesting PAs are capable of maintaining cerebral vascular resistance in response to elevated pressure despite the structural remodeling in pregnancy. Interestingly, pregnancy-induced remodeling of PAs was prevented in rats with ePE. Vascular wall thickness and lumen diameters were similar to the nonpregnant state that may be due to impaired PPAR γ signaling in the brain required for pregnancy-induced remodeling in ePE. The lack of this potentially protective adaptation of PAs in ePE could predispose the brain to injury, particularly as it relates to cerebral autoregulation. However, the relationship between the lack of structural remodeling of PAs in ePE and blood flow autoregulation, and the role of PPAR γ signaling remains to be determined. Regardless, PE appears to affect both the structure and function of the cerebral vasculature that could contribute to cerebral pathologies associated with PE.

SK and IK channels are present in ECs in cortical PAs and appear to be basally active to inhibit tone in male rats; however, little is known about their expression and activity in PAs from female rats.³⁵ The findings of the current study that pharmacological activation of SK/IK channels caused dilation indicates these channels are expressed in PAs of female, pregnant and ePE rats. Interestingly, IK, but not SK, channels contributed to the inhibition of myogenic tone that was significantly reduced in ePE. However, the apparent IK channel dysfunction did not overtly result in increased myogenic tone in arterioles in ePE, potentially due to EC-derived vasoactive factors that provide additional mechanisms that contribute to tone.⁵⁰ Interestingly, the findings of the current study that conducted vasodilation to 12 mM KCl was unaffected by SK/IK channel inhibition suggests that K_{IR}-dependent conducted vasodilation of cerebral arterioles does not depend upon SK/IK channel involvement. Regardless, IK channel dysfunction suggests endothelial dysfunction was present in arterioles in ePE.

Conclusions

The strong associations between cerebral small vessel disease, WM hyperintensities and cognitive impairment in the aged and elderly populations are well-established.^{14, 51, 52} That

women who had pregnancies complicated by PE have increased incidence and severity of WM lesions and reported cognitive impairment at ages as young as 30 – 40 years old suggest that PE may cause long-lasting cerebrovascular dysfunction that contributes to WM lesion burden and early-onset cognitive decline.^{10, 11} The findings in the current study support that ePE adversely affects the penetrating arterioles supplying frontoparietal WM tracts, cortices and basal ganglia critical to cognitive function. In addition, we have previously shown that arterioles in the cognition-centric hippocampus are smaller, stiffer and also have diminished dilatory responses to elevated K⁺ in ePE.²⁷ Together, these studies suggest PE has a wide-spread detrimental effect on the function and structure of arterioles supplying gray and WM that may initiate degenerative processes, although implications of such vascular dysfunction on persistent and progressive changes in WM integrity and cognitive function remain to be investigated. Taken together with the clinical findings of structural and functional changes in gray matter, WM, and cognitive function 5 – 8 years after PE,^{10–12, 43} we speculate that PE may be a unique form of cerebral small vessel disease. Understanding the effects of PE on the cerebrovasculature may allow for investigation into novel vascular therapeutic targets to prevent the detrimental effects of PE on neuro-vascular function and health, and improve the quality of life of these young mothers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

This work was supported by the NIH National Institute of Neurological Disorders and Stroke R01 NS045940; the Preeclampsia Foundation; The Totman Medical Research Trust; and the Cardiovascular Research Institute of Vermont. The authors have no competing interests.

Abbreviations

PE	Preeclampsia
ePE	Experimental preeclampsia
Preg	Pregnant
Nonpreg	Nonpregnant
WM	White matter
PA	Parenchymal arteriole
K_{IR}	Inward rectifier potassium channel
IK	Intermediate-conductance calcium-activated potassium channel
SK	Small-conductance calcium-activated potassium channel

oxLDL	Oxidized low-density lipoprotein
LOX-1	Lectin-like oxidized low-density lipoprotein receptor 1
TNFα	tumor necrosis factor alpha
PPARγ	peroxisome proliferator-activated receptor gamma
VSMCs	Vascular smooth muscle cells
ECs	Endothelial cells

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Highlights

- Investigated cerebral arterioles that supply frontoparietal white and gray matter
- Isolated arterioles had diminished dilation to 8 mM K⁺ in experimental preeclampsia
- Impaired conducted vasodilation of isolated arterioles from rats with preeclampsia
- Conducted vasodilation did not involve endothelial Ca²⁺-activated K⁺ channels

Figure 1. Dissection of Parenchymal Arterioles (PAs)

(A) Ventral surface of a rat brain with visible middle cerebral arteries (MCAs) at their point of origination off of the Circle of Willis (black arrows). Dotted line indicates the coronal section of cortex containing the MCA and downstream PAs dissected from the brain. White arrows indicate the lateral olfactory tract (LOT). (B) Anterior surface of the coronal section of brain containing PAs. Black arrowheads indicate the PA penetrating through the LOT (white arrow) towards the corpus callosum (CC; dotted line). (C) Illustration of the coronal section of rat brain at the level of the MCA showing brain regions (left) and the vasculature supply those regions (right). The PA studied entered the cortex through the LOT and ran inferior or superior to the CC (black oval). (D) PA secured on a glass cannula and pressurized to 40 mmHg with myogenic tone and lumen diameter of $\sim 60 \mu\text{m}$. Glass micropipette inserted in bath to administer local application of 12 mM KCl to distal end of PA. Blue regions of interest (ROI) continuously and simultaneously record lumen diameters along the 700 μm length of the arteriole. Local application was applied to ROI 1 and diameters measured and compared to ROI 4. Scale bar is 50 μm . Cing.- cingulate, Somat.- somatosensory, Ins.- insular, Pyrif.- pyriform cortices; Acc.- nucleus accumbens; Sp.- septal nucleus; V- lateral ventricles.

Figure 2. Circulating levels of oxidized low-density lipoprotein (oxLDL)
oxLDL levels were higher in serum from rats with preeclampsia (ePE) compared to pregnant (Preg) and nonpregnant (Nonpreg) rats. * $p < 0.05$ vs. Nonpreg by one-way ANOVA with post-hoc Bonferroni test.

Figure 3. Myogenic reactivity of PAs

(A) Pressure-diameter curves of PAs from nonpregnant (Nonpreg), pregnant (Preg) and preeclamptic (ePE) rats and (B) percent tone across the pressure range 20 – 120 mmHg.

Figure 4. The role of SK and IK channels in inhibiting basal tone

(A) Representative lumen diameter tracings of PAs from nonpregnant (Nonpreg; top panel), pregnant (Preg; middle panel), and preeclamptic (ePE; bottom panel) rats in response to cumulative treatment with apamin and TRAM-34. Percent constriction from baseline of PAs in response to SK channel inhibition (B) and IK channel inhibition (C). * $p < 0.05$ by one-way ANOVA with post-hoc Bonferroni test.

Figure 5. Reactivity of PAs to elevated extracellular KCl

Graphs showing lumen diameter responses (A) and percent change in diameter (B) of PAs from nonpregnant (Nonpreg), pregnant (Preg) and preeclamptic (ePE) rats to increased extracellular KCl. * $p < 0.05$ vs. PE and Nonpreg, # $p < 0.05$ vs. Preg by one-way ANOVA with post-hoc Bonferroni test.

Figure 6. Conducted vasodilation in response to local application of 12 mM KCl

(A) Representative lumen diameter traces of a PA from a nonpregnant (Nonpreg) rat at the site of 12 mM KCl application and 700 μ m proximal. Arrow indicates the time at which 12 mM KCl was locally applied. (B) Diameter responses of PAs from Nonpreg, pregnant (Preg) and preeclamptic (ePE) rats. (C) Percent of vasodilation that was conducted upstream in the absence and presence of SK/IK channel inhibition. (D) The delay in conducted vasodilation of PAs without and with SK/IK channel inhibition. ** $p < 0.01$ vs. baseline by two-way ANOVA with post-hoc Bonferroni test; # $p < 0.05$ vs. Preg by one-way ANOVA with post-hoc Bonferroni test.

Figure 7. Conducted vasodilation in response to local application of adenosine

(A) Representative lumen diameter traces of a PA from a nonpregnant (Nonpreg) rat at the local site of 10 μM adenosine application and 700 μm proximal. Arrow indicates the time at which adenosine was locally applied. (B) Diameter responses of PAs from Nonpreg, pregnant (Preg) and preeclamptic (ePE) rats. (C) Percent of vasodilation that was conducted upstream. (D) The delay in conducted vasodilation of PAs.