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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 white matter (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 μM 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.

1. Introduction

Preeclampsia (PE) is a hypertensive complication of pregnancy involving widespread maternal vascular dysfunction, including endothelial activation, hypercoagulation, and increased vascular stiffness (Lamarca, 2012; Sankaralingam et al., 2009; Davidge et al., 2014; Hausvater et al., 2012). In the brain, PE affects the cerebral microcirculation that can manifest with neurological symptoms including persistent headache, cortical blindness and de novo seizure (eclampsia) (Lindheimer et al., 2014; Zeeman et al., 2009). PE is a leading cause of maternal morbidity and mortality worldwide, with 40% of maternal deaths involving complications of the cerebrovasculature (MacKay et al., 2001). Not only is PE dangerous during the acute pregnancy phase (index pregnancy), but is also associated with long-term adverse neurological function (Aukes et al., 2007; Postma et al., 2016). 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 (Postma et al., 2016; Postma et al., 2013). 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 (Aukes et al., 2012; Wiegman et al., 2014). WM lesions, defined radiologically as subcortical hyperintensities of WM on T2-weighted magnetic resonance imaging (MRI), are strongly associated with cognitive decline (Lin et al., 2017; Iadecola, 2013).

The underlying etiology of WM lesions remains unclear, but is thought to be vascular in nature (Fernando et al., 2006). In addition, under pathological conditions including hypertension and hyperlipidemia, both of which are present in PE, cerebrovascular dysfunction may perpetuate WM injury (Iadecola, 2013; Girouard and Iadecola, 2006; Belo et al., 2002). For example, hypertension-induced endothelial dysfunction and vascular inward remodeling can compromise the

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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ability of blood flow to match neuronal metabolic demand and lead to chronic hypoperfusion (Girouard and Iadecola, 2006). Although WM is less metabolically active than gray matter, it is particularly susceptible to vascular insufficiency (Iadecola, 2013; Joutel, 2014). 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 (Lima et al., 2011). Increased very low-density lipoproteins (vLDL) have been shown in women with PE that favors oxidation and formation of oxidized low-density lipoprotein (oxLDL) (Belo et al., 2002; Lima et al., 2011; Hubel et al., 1998; Buhimschi et al., 1998). 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 (Hubel et al., 1998; Chen et al., 2002; Ogura et al., 2009; Qiu et al., 2006). 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 (Schreurs et al., 2013). Further, this model of experimental PE (ePE) has increased blood-brain barrier permeability that was shown to be LOX-1-dependent (Schreurs et al., 2013; Schreurs and Cipolla, 2013; Johnson and Cipolla, 2017). 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 (Jensen and Holstein-Rathlou, 2013). 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.

2. Materials and methods

2.1. 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.

2.2. 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 (Sankaralingam et al., 2009; Belo et al., 2002; Hubel et al., 1998; Schreurs et al., 2013; Schreurs and Cipolla, 2013; Schreurs and Cipolla, 2014). 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 (Johnson and Cipolla, 2017). This model has been previously shown to induce dyslipidemia and cause other PE-like symptoms including maternal endothelial dysfunction and increased blood pressure (Schreurs et al., 2013; Schreurs and Cipolla, 2013). 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 (Schreurs et al., 2013).

2.3. 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 (TNFα) and oxLDL were measured in serum from Nonpreg, Preg, and ePE rats (n = 5–8/group) using commercially available ELISA kits for TNFα (R&D Systems, Minneapolis, MN, USA) and oxLDL (Biomatik, Wilmington, DE, USA). Samples were measured undiluted and in duplicate.

2.4. 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 (Fig. 1) (Johnson and Cipolla, 2017; Cipolla et al., 2011).

PAs were equilibrated at 20 mm Hg for 1 h, after which intravascular pressure was increased to 120 mm Hg 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 mm Hg for the remainder of the experiment. Vessels that did not develop myogenic tone along the 700 μm 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 μM) and the IK channel inhibitor TRAM-34 (1.0 μM) were added to the bath and lumen diameters recorded after 25 min. 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 mm Hg.

2.5. Local application of KCl and adenosine to measure conducted vasodilations

Using a micromanipulator (Narishige International USA, East Meadow, NY, USA), a glass micropipette (20 μm diameter tip) that was