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Cerebral Blood Flow Autoregulation, Blood-Brain Barrier Permeability, and the Effects of Magnesium Sulfate Treatment During Pregnancy and Hypertension

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CEREBRAL BLOOD FLOW AUTOREGULATION, BLOOD-BRAIN BARRIER PERMEABILITY, AND THE EFFECTS OF MAGNESIUM SULFATE TREATMENT DURING PREGNANCY AND HYPERTENSION

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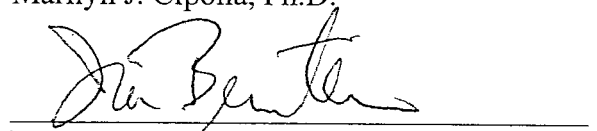

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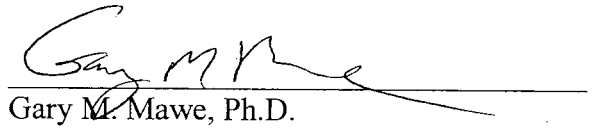

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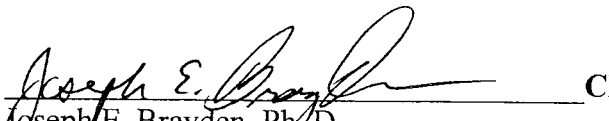

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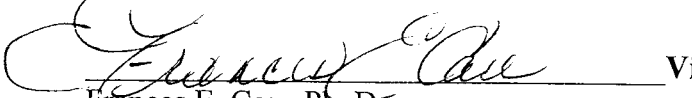

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ABSTRACT

Eclampsia is a hypertensive disorder of pregnancy and a leading cause of maternal death. The primary explanation for eclampsia is that it represents a form of hypertensive encephalopathy (HTE) with neurological symptoms including headaches, nausea, vomiting, visual disturbances, and seizures. The etiology of HTE involves an acute increase in arterial blood pressure that exceeds the autoregulatory capacity of the brain leading to forced dilatation of cerebral vessels, decreased cerebrovascular resistance, hyperperfusion, blood-brain barrier (BBB) disruption, and vasogenic cerebral edema formation. Due to the central role of the cerebral circulation in mediating these symptoms, a better understanding of how pregnancy affects the cerebral circulation is important to the treatment and prevention of eclampsia.

A central goal of this dissertation was to determine pregnancy's effect on cerebral blood flow (CBF) autoregulation, edema formation, and BBB permeability during acute hypertension. Women with eclampsia often seize at lower blood pressures than HTE patients. We hypothesized that pregnancy may predispose the brain to eclampsia by lowering the pressure of autoregulatory breakthrough and enhancing cerebral edema formation. Using an *in vivo* model of HTE, we found that the pressure of autoregulatory breakthrough was not different between nonpregnant (NP) and late-pregnant (LP) rats; however, cerebral edema formation was significantly increased only in LP animals. Nitric oxide synthase inhibition significantly increased the upper limit of autoregulation in both NP and LP animals and attenuated cerebral edema formation in LP animals. BBB permeability during acute hypertension was not different between these groups.

Magnesium sulfate ($MgSO_4$) is widely used to treat eclampsia despite an unclear mechanism of action. A second goal of this dissertation was to determine the cerebrovascular effects of $MgSO_4$ during pregnancy. Specifically, we investigated the effect of $MgSO_4$ on *in vitro* resistance artery vasodilation and *in vivo* BBB permeability during acute hypertension. We hypothesized that dilation to $MgSO_4$ would be greater in mesenteric than cerebral vessels. $MgSO_4$ elicited concentration-dependent vasodilation in all arteries, as determined by measuring lumen diameter of isolated and pressurized arteries, however, mesenteric arteries were considerably more sensitive than cerebral arteries. In addition, there was no effect of pregnancy on $MgSO_4$ sensitivity in mesenteric arteries, whereas pregnancy decreased sensitivity to $MgSO_4$ in cerebral arteries. We further hypothesized that $MgSO_4$ would decrease BBB disruption during acute hypertension, thereby protecting the brain in eclampsia. Using an *in vivo* model of HTE, we showed that $MgSO_4$ treatment decreased BBB permeability during acute hypertension in LP rats, with the greatest effect observed in the posterior cerebrum.

In conclusion, this dissertation determined CBF autoregulation and cerebral edema formation during pregnancy, and also the effect of $MgSO_4$ on cerebral resistance artery vasodilation and BBB permeability during acute hypertension in LP rats. Although pregnancy did not influence autoregulatory breakthrough, cerebral edema formation was enhanced in LP animals and this may potentiate neurological symptoms in eclampsia. In addition, $MgSO_4$-induced cerebral vasodilation is likely not a primary mechanism of eclampsia treatment, rather $MgSO_4$ may limit edema formation by attenuating BBB permeability during hypertension.

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CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

1.1. Eclampsia

1.1.1. Introduction

Since the times of Ancient Greece, it has been observed that "In pregnancy, drowsiness with headache, accompanied by heaviness and convulsions, is generally bad" (Chadwick and Mann 1950). Today this condition is known as eclampsia, a pregnancy-specific syndrome consisting of preeclamptic symptoms (elevated blood pressure and proteinuria) combined with the new onset of seizures (Cunningham et al. 2001; National High Blood Pressure Education Working Group on High Blood Pressure in Pregnancy 2000). Eclampsia has a range of neurological signs and symptoms including nausea, vomiting, headaches, visual disturbances, and seizures and coma in the most severe cases (Chames et al. 2002; Douglas and Redman 1994; Katz, Farmer, and Kuller 2000; Sibai 2005).

Eclampsia is a serious complication of pregnancy. The incidence of eclampsia was estimated to be 3.0 per 1,000 live births for 2003 in the United States (Martin et al. 2005), and 1 in 2,000 pregnancies in the United Kingdom in 1992 (Douglas and Redman 1994). The risk of pregnancy complications increases with eclampsia, and these complications can include abruptio placentae, preterm birth, fetal growth retardation, HELLP (Hemolysis, Elevated Liver enzymes, and Low Platelets) syndrome, disseminated intravascular coagulopathy, pulmonary edema, acute renal failure, and neurological deficits (Douglas and Redman 1994; Katz, Farmer, and Kuller 2000; Lopez-Llera 1992; Mattar and Sibai 2000; Sibai 1990, 2005). Eclampsia is a

leading cause of maternal death throughout the world, with a higher mortality rate in developing countries compared to Western countries (Berg et al. 2003; Duley 1992; Khan et al. 2006). Worldwide, it is estimated that at least 50,000 women die each year from eclampsia (Duley 1992). Importantly, approximately 40% of eclamptic deaths are due to cerebral complications (Berg et al. 2003; Donaldson 1989; MacKay, Berg, and Atrash 2001).

The pathogenesis of the neurological symptoms of eclampsia has been debated between two opposing theories. Eclamptic seizures have been proposed to be caused by either vasospasm with decreased cerebral blood flow (CBF), or alternatively by hyperperfusion in the cerebrovasculature. To better comprehend the origins of eclampsia and its related neurological complications, the effects of pregnancy, and hypertension in pregnancy, on the cerebral circulation must be understood. The overall goal of this dissertation project was to determine how pregnancy affects CBF autoregulation and blood-brain barrier (BBB) permeability during acute hypertension, and how the common treatment of magnesium sulfate may influence these cerebrovascular parameters.

1.1.2. Related Disorders: Posterior Reversible Encephalopathy Syndrome and Hypertensive Encephalopathy

In 1996, Hinchey and colleagues published a report of cases with similar clinical signs and neuroimaging findings that resulted from various causes including eclampsia and hypertensive encephalopathy (HTE) (Hinchey et al. 1996). Common presenting

symptoms include headaches, nausea, vomiting, altered mental status, visual disturbances, and generalized seizures (Hinchey et al. 1996; Schwartz et al. 1992; Servillo et al. 2003; Stott, Hurrell, and Anderson 2005). These patients shared a common neuroimaging finding of white matter edema in posterior cerebral regions, particularly bilaterally in parietal and occipital areas. In the authors' opinion, the acute and reversible nature of the white matter abnormalities seen in this cohort was best explained by hypertension and an altered BBB, and they termed this syndrome Reversible Posterior Leukoencephalopathy Syndrome (RPLS) (Hinchey et al. 1996). Alternative names have been proposed for this syndrome, such as Posterior Reversible Encephalopathy Syndrome (PRES) (Casey et al. 2000; Lamy et al. 2004) and Posterior Leukoencephalopathy Syndrome (Ay et al. 1998), to emphasize the possibility for gray matter involvement and irreversible damage to occur (Casey et al. 2000; Schwartz 1996).

Advances in neuroimaging techniques have further characterized PRES, and thus HTE and eclampsia. Magnetic resonance imaging (MRI) scans and fluid attenuated inversion recovery (FLAIR) images from PRES patients show cerebral edema in the posterior cerebral hemispheres (Stott, Hurrell, and Anderson 2005). FLAIR sequences allow areas of cerebral edema to appear more prominently and can improve cortical lesion detection (Casey et al. 2000). In one study, the use of FLAIR sequences identified cortical involvement in 94% of PRES patients examined in addition to white matter lesions (Casey et al. 2000).

Imaging technology can also provide pathophysiological information. Traditional MRI cannot distinguish between edema caused by hypoxia (cytotoxic) and edema caused by BBB disruption (vasogenic) (Ebisu et al. 1993). However, diffusion-weighted imaging (DWI) and apparent diffusion coefficient (ADC) images are sensitive to microscopic random motion of water molecules and can potentially differentiate between cytotoxic and vasogenic edema (Ebisu et al. 1993; Mukherjee and McKinstry 2001; Provenzale et al. 2001; Schwartz et al. 1998; Sevick et al. 1992). Increased ADC values, consistent with vasogenic edema, have been found in PRES and HTE patients (Ahn et al. 2004; Gocmen, Ozgen, and Oguz 2007; Provenzale et al. 2001; Schaefer et al. 1997; Schwartz et al. 1998). These imaging modalities provide clinical support for the hyperperfusive theory of PRES, and thus eclampsia, in which CBF autoregulation is overcome by elevated blood pressure causing cerebral vasodilation, BBB disruption, and vasogenic cerebral edema (Mukherjee and McKinstry 2001; Provenzale et al. 2001).

A major cause of PRES is HTE, an acute brain syndrome that occurs when the upper limit of cerebral autoregulation is exceeded by a sudden elevation in blood pressure, also known as autoregulatory breakthrough (Phillips and Whisnant 1992; Vaughan and Delanty 2000). (Particular features of the cerebrovasculature, including CBF autoregulation, will be described in detail in following sections.) In 1928, Oppenheimer and Fishberg introduced the term "hypertensive encephalopathy" to describe the cerebral changes associated with arterial hypertension, and associated the severity of HTE clinical manifestations with increased water in the brain (Oppenheimer

and Fishberg 1928). Clinically, HTE is characterized by headaches, altered mental status, nausea, vomiting, visual disturbances (including blindness), and seizures in the setting of elevated blood pressure (Hauser, Lacey, and Knight 1988; Johansson 1997; Phillips and Whisnant 1992). If left untreated, HTE can progress to more serious complications such as cerebral hemorrhage, coma, and death (Dinsdale 1978). Fortunately, the neurological signs of HTE are largely reversible if blood pressure is promptly lowered (Johansson 1997).

The pathophysiology of HTE is relevant because eclampsia is thought to be a form of HTE (Easton 1998; Phillips and Whisnant 1992). The more recent controversy over the etiology of eclampsia echoes a similar difference of opinion over the pathogenesis of HTE decades ago. The debate involved two diametrically opposed etiologies, cerebrovascular vasospasm and ischemia versus excessive vasodilation of the cerebral circulation and hyperperfusion of the brain (Byrom 1969; Lassen and Agnoli 1972). Historically, it was thought that an acute and severe increase in arterial blood pressure (ABP) caused excessive vasoconstriction in the cerebrovasculature with resulting areas of infarction (Johansson 1997; Paulson et al. 1989). Experimentally, Byrom observed alternating areas of constriction and dilation in the cerebral arteries of rats fitted with cranial windows following experimental renal hypertension, and these areas of focal contraction were shown to completely normalize when hypertension was abolished (Byrom 1954). This pattern of alternating regions of constriction and dilation has been referred to as a "sausage-string" (Auer 1978; Goldby and Beilin 1972; MacKenzie, Strandgaard et al. 1976), and has been observed in different vascular beds,

both cerebral and systemic, in a variety of animal models of hypertension (Giese 1973; Rodda and Denny-Brown 1966; Werber and Heistad 1984). Byrom also reported that 87% of rats showed trypan-blue staining (indicative of BBB disruption) in the cerebral cortex in response to hypertension, and edema was increased in the stained areas. Byrom stated that "spasm of the cerebral arteries and focal edema are closely correlated with acute cerebral symptoms" (Byrom 1954), and hypothesized that hypertension-provoked vasospasm caused cerebral ischemia with subsequent BBB disruption and edema formation. This hypothesis was later supported by other studies (Dinsdale, Robertson, and Haas 1974; Meyer, Waltz, and Gotoh 1960; Rodda and Denny-Brown 1966).

Byrom interpreted the narrower cerebral artery segments he observed as the pathological condition, and the areas of dilation between them the normal state. However, these observations can also be considered in reverse. In later studies, direct observations of pial arteries during acute hypertension revealed that the narrow segments of the sausage-string pattern represented maximal autoregulation. The dilated segments were abnormal and had force dilated due to rising intraluminal pressure, and with time the constricted segments of pial arteries would also dilate (Auer 1978; MacKenzie, Strandgaard et al. 1976). This supported a new hypothesis that forced vasodilation of the cerebrovasculature leads to BBB disruption, edema formation, and the symptoms of HTE (Lassen and Agnoli 1972). This theory is supported by work showing increased vascular permeability in dilated, but not constricted, arterial segments during induced hypertension to both colloidal carbon (Giese 1964; Goldby

and Beilin 1972, 1972) and Evan's blue (EB) tracers (Auer 1978). It was also observed that vessel segments with vascular permeability have "widely patent lumens" (Giacomelli, Wiener, and Spiro 1970). Studies have also suggested that BBB disruption preceded or occurred independently of cerebral ischemia in animals with symptoms of encephalopathy (Sadoshima and Heistad 1982; Tamaki et al. 1984). Additionally, CBF was shown to be increased in regions with BBB disruption (Tamaki et al. 1984), not decreased as would be predicted by the vasospasm theory of HTE. Many studies have shown that severe and acute hypertension leads to a passive dilatation of cerebral vessels and BBB disruption (Heistad and Marcus 1979; Johansson et al. 1970; Kontos et al. 1981; MacKenzie, Strandgaard et al. 1976; Nag, Robertson, and Dinsdale 1979). In fact, Byrom himself later reversed his interpretation and supported forced vasodilation as the key event in the pathogenesis of HTE (Byrom 1969).

Increasingly, human studies also support a vasodilatory mechanism for HTE. Direct measurements of CBF in a patient with HTE symptoms demonstrated autoregulatory breakthrough (Skinhøj and Strandgaard 1973), and studies have also shown no evidence of cerebral vasospasm in patients at high blood pressures (Strandgaard et al. 1973). Computed tomography (CT) scans of a HTE patient show widespread low density areas in the cerebrum with a particularly defined area of low density in the occipital region indicating intracerebral edema (Jespersen, Rasmussen, and Hennild 1989). T_2-weighted MRI scans of HTE patients show focal high-intensity lesions involving the cortex and white matter, especially the occipital lobes, with lesion

resolution on follow-up imaging (Hauser, Lacey, and Knight 1988; Schwartz et al. 1992). These high-intensity lesions on MRI were interpreted as protein and fluid extravasation across the BBB caused by breakthrough of cerebral autoregulation. Single-photon emission computed tomography (SPECT) studies during hypertensive crisis have shown increased vascular perfusion adjacent to areas with abnormal signals on MRI scans (Schwartz et al. 1992), suggesting increased CBF in the abnormal areas following a hypertensive insult.

Due to the emergence of more effective anti-hypertensive medications, HTE is no longer as comprehensively studied. It has been said that the brain is the "organ that has benefited most unequivocally from modern antihypertensive treatment"(Paulson, Strandgaard, and Edvinsson 1990). However, studies on the pathogenesis of cerebral HTE symptoms offer important clues about the etiology of neurological symptoms in eclampsia.

1.1.3. Hemodynamics during Pregnancy and Eclampsia

Eclampsia is considered to be a form of both PRES and HTE in which acute elevations in blood pressure cause autoregulatory breakthrough and a subsequent decrease in cerebrovascular resistance (CVR) (Easton 1998; Phillips and Whisnant 1992). These hemodynamic changes increase hydrostatic pressure and lead to increased BBB permeability and edema formation, which may cause neurological complications due to pathologically increased intracranial pressure (ICP) and brain compression (Hatashita, Hoff, and Ishii 1986; Kimelberg 1995; Kongstad and Grande

2001). Seizures often occur at lower blood pressures in eclamptic women compared to HTE patients (Douglas and Redman 1994; Leveno and Cunningham 1999), suggesting that there are important differences in the effect of acute hypertension on the cerebrovasculature during pregnancy. The overall goal of this project was to gain a better understanding of the effects of pregnancy on the cerebral circulation that may predispose the brain to eclampsia.

Among the many maternal physiological adaptations to pregnancy, there are significant changes in the cardiovascular system. Early in gestation, peripheral vascular resistance begins to decrease (Clapp and Capeless 1997; Robson et al. 1989). During a normal singleton gestation, maternal plasma volume increases approximately 40% (Pritchard 1965; Pritchard, Cunningham, and Pritchard 1984), and cardiac output increases 30-50% (Clapp and Capeless 1997; Monga 2004; Robson et al. 1989). The increased cardiac output is distributed among many maternal organs. Most notably uterine blood flow increases ten-fold (Gant and Worley 1989), and renal blood flow increases approximately 50% (Dunlop 1981; Lindheimer and Katz 1970). In contrast to other organs, relatively little is known about changes in the cerebrovasculature and CBF during pregnancy.

Transcranial Doppler (TCD) ultrasonography has been widely used to study cerebral hemodynamics during pregnancy. TCD is a non-invasive method that can measure changes in blood flow velocity, but cannot directly determine vessel diameter (Kassab et al. 2007). Thus, changes in CBF and vessel caliber are inferred (Clyde et al. 1996), and concerns about the validity of extrapolating CBF from TCD studies have

been expressed (Kontos 1989). In healthy pregnant women, decreased blood flow velocity has been measured in the middle cerebral artery (MCA) with advancing gestation by several groups (Belfort et al. 2001; Serra-Serra et al. 1997; Sherman et al. 2002; Williams and Wilson 1994), though the interpretation of these common results varied. These data were taken as evidence of physiological cerebral vasodilation, thus decreased CVR, in late gestation by some authors, presumably limiting data interpretation to the insonated vessel versus downstream arterioles (Serra-Serra et al. 1997). Others have reasoned that if ABP remained constant, decreased arteriolar resistance would cause an increase in both arterial blood volume and velocity (Belfort et al. 2001). (Following this reasoning, the decreased velocities observed in normal pregnancy would seem to suggest increased downstream CVR (Kassab et al. 2007).) However, the final conclusion from this study was that because pregnancy caused a decrease in systolic and mean velocities, without changes in diastolic velocity, these data indicated an increase in arteriolar distensibility and a decrease in CVR with pregnancy (Belfort et al. 2001). In the internal carotid artery, mean blood flow velocity was also found to decrease during the third trimester, and this was interpreted as decreased CBF based on the assumption that the internal carotid, a large extracranial artery, would not change diameter during pregnancy (Ikeda and Mori 1990). It is recognized that TCD cannot determine if increased mean velocities are related to vasospasm or hyperemia (Romner et al. 1996; Clyde et al. 1996). Velocity-encoded phase-contrast MRI can measure vessel diameter, and potentially determine absolute CBF (Morriss et al. 1997). Using this technique, absolute CBF was shown to be

decreased ~20% in normal pregnancy due to a decrease in blood flow velocity (similar to TCD studies) not a change in vessel diameter (Zeeman, Hatab, and Twickler 2003). However, this study used postpartum values for comparison, which may not be the most appropriate data evaluation.

Other measures of cerebral hemodynamics can be determined from TCD studies. Cerebral perfusion pressure (CPP) can be calculated from TCD and ABP measurements (Belfort et al. 2000), and has been shown to increase up to 50% during gestation (Belfort et al. 2001; Sherman et al. 2002; Williams and Wilson 1998). The resistance index (calculated as $[velocity_{systolic} - velocity_{diastolic}] / velocity_{systolic}$) is thought to represent the resistance of vessels distal to the location of TCD investigation; an increased resistance index implies increased resistance in downstream vessels and a decreased resistance index indicates lowered resistance downstream (Belfort, Saade, Grunewald, Dildy, Varner et al. 1999). During normal pregnancy, the MCA resistance index decreases significantly in the third trimester (Belfort et al. 2001; Williams and Wilson 1994), supporting cerebral vasodilation during pregnancy. In a study where the CPP and resistance index were determined simultaneously, it was concluded that the increased CPP observed together with decreased CVR, as suggested by a lower resistance index, likely increased CBF during pregnancy (Belfort et al. 2001).

Preeclamptic and eclamptic women have been studied with TCD in an attempt to better understand the cerebral pathophysiology of these disorders. Increased MCA mean velocities have been found in preeclamptic and eclamptic women versus those

with uncomplicated pregnancies (Demarin, Rundek, and Hodek 1997; Naidu et al. 1997; Ohno et al. 1997; Vliegen et al. 1993; Zunker et al. 1995; Zunker et al. 2000). Velocities are more greatly increased in severe preeclampsia, cases with neurological symptoms or higher blood pressures, and eclampsia (Belfort, Grunewald et al. 1999; Demarin, Rundek, and Hodek 1997; Ohno et al. 1997; Qureshi et al. 1996; Zunker et al. 1995; Zunker et al. 2000). Indications of hyperperfusion have been observed in a preeclamptic patient with increased MCA velocities (Vliegen et al. 1993). Elevated CPP is more common in patients with severe preeclampsia than those with mild preeclampsia (Belfort, Grunewald et al. 1999; Belfort et al. 2002), and severe preeclamptics also have a lower resistance index (P=0.06) (Belfort, Grunewald et al. 1999), suggesting higher CBF and impaired CBF autoregulation. These findings agree with other reports suggesting impaired autoregulation in preeclamptic women in both the MCA (Belfort, Saade, Grunewald, Dildy, Varner et al. 1999) and renal circulations (Kublickas et al. 1996). Impaired autoregulation, suggested by increased CPP or mean arterial pressure (MAP) with a decreased resistance index, is present in preeclamptic women with headache, a common precursor to seizure, but not in preeclamptic women without headache (Belfort, Saade, Grunewald, Dildy, Varner et al. 1999). These data may suggest that a headache (a neurologic symptom) coincides with the loss of MCA autoregulation (Belfort, Saade, Grunewald, Dildy, Varner et al. 1999). Headache in preeclamptic women is also strongly associated with abnormally high CPP, suggesting that severe headache may be linked to hyperperfusion (Belfort, Saade, Grunewald, Dildy, Abedejos et al. 1999). Using MRI, women with severe preeclampsia were found

to have significantly greater CBF with no changes in vessel diameter compared to normotensive pregnant women (Zeeman, Hatab, and Twickler 2004), suggesting that increases in CBF likely precede the onset of eclamptic convulsions.

There are other findings in eclampsia that suggest disordered autoregulation and support a hyperperfusive pathogenesis. During normal pregnancy, cerebral autoregulation is similar to non-pregnant patients, as assessed by transient hyperemic response and TCD (Sherman et al. 2002). However, preeclamptic women exhibit decreased cerebrovascular reactivity to carbon dioxide (CO_2) inhalation and isometric hand-grip tests (Riskin-Mashiah et al. 2001). In addition, a diffuse loss of autoregulation in preeclamptic and eclamptic patients is suggested by dynamic cerebral autoregulation studies (Oehm et al. 2003; Oehm et al. 2006). Similar to studies of HTE, both CT and MRI brain scans in eclamptic patients suggest edema formation (Crawford et al. 1987; Dahmus, Barton, and Sibai 1992; Fredriksson et al. 1989; Manfredi et al. 1997; Raroque, Orrison, and Rosenberg 1990; Vandenplas et al. 1990). A classic description of eclamptic imaging findings is high signal intensity bilaterally on T_2-weighted MRI with marked involvement of the parietal and occipital lobes (Manfredi et al. 1997). As with HTE and PRES, in eclamptic patients DWI has shown the presence of vasogenic edema (Apollon et al. 2000; Kanki et al. 1999; Schaefer et al. 1997), ruling out cerebral infarction or transient ischemia as the primary insult in eclampsia. The reversibility of cerebral lesions and abnormalities on follow-up imaging also supports edema caused by hyperperfusion and BBB disruption because lesions caused by ischemia or hemorrhage do not resolve quickly, if at all (Crawford et

al. 1987; Fredriksson et al. 1989; Raroque, Orrison, and Rosenberg 1990; Schwaighofer, Hesselink, and Healy 1989). Additionally, clinical reports demonstrate increased CBF, determined by TCD and MRI, both before and after the onset of eclamptic seizures (Belfort 2005; Oehm et al. 2003; Ohno et al. 1999; Zeeman, Hatab, and Twickler 2004; Zunker et al. 2000), further supporting eclampsia as a hyperperfusive disorder.

Eclampsia has many similarities to HTE and is probably best classified as a subset of this syndrome or as a subcategory with HTE within PRES. However, a notable difference between HTE and eclampsia is the blood pressure at which seizures occur. Clinical evidence demonstrates the onset of seizures at relatively low MAP when compared to HTE patients (Donaldson 1989; Ohno et al. 1999), suggesting that the autoregulatory curve may shift to lower pressures during pregnancy. When blood pressure was measured within one hour of seizure onset, the average diastolic blood pressure was 97 mmHg (Douglas and Redman 1994). This same study found that only 19% of eclamptic women cared for in the United Kingdom in 1992 had a diastolic blood pressure that was considered high (≥120 mmHg) before seizure onset (Douglas and Redman 1994). *In vitro* studies in our lab have shown that cerebral vessels from late-pregnant (LP) animals force dilatated at significantly lower pressures than vessels from nonpregnant (NP) animals (Cipolla, Vitullo, and McKinnon 2004). However, our *in vivo* studies have shown that there is no difference in cerebral autoregulation between NP and LP rats as measured by laser Doppler (Euser and Cipolla 2007). While autoregulation may not differ with normal gestation, it is possible that

generalized endothelial dysfunction associated with eclampsia may also contribute to the onset of seizures.

Because most women who develop eclampsia are normotensive prior to pregnancy, we had previously hypothesized that pregnancy alone predisposes the maternal cerebral circulation to eclampsia by lowering the pressure at which autoregulatory breakthrough occurs and by enhancing vascular permeability and cerebral edema formation. Therefore, a primary goal of this dissertation was to further investigate CBF autoregulation, cerebral edema formation, and BBB permeability *in vivo* using a model of HTE in the pregnant rat. Although vascular changes during pregnancy have been well-studied in other organs, changes in cerebrovascular autoregulation and BBB function remained largely unknown prior to this dissertation. A better understanding of the effects of pregnancy, and hypertension during pregnancy, on the cerebral circulation is important to the treatment and prevention of eclampsia.

1.2. Cerebral Blood Flow and Autoregulation

1.2.1. Cerebral Circulation and Cerebral Blood Flow

The blood supply of the brain is delivered by two paired arteries, the right and left internal carotid arteries and the right and left vertebral arteries, and the latter combine to form the basilar artery (Sokoloff 1997). At the base of the brain these arteries form a complete ring of anastomoses called the circle of Willis (Figure 1), and the major arteries of the cerebral circulation originate here (Augustine 2001). The cerebral cortex is supplied by three pairs of arteries, the anterior cerebral arteries,

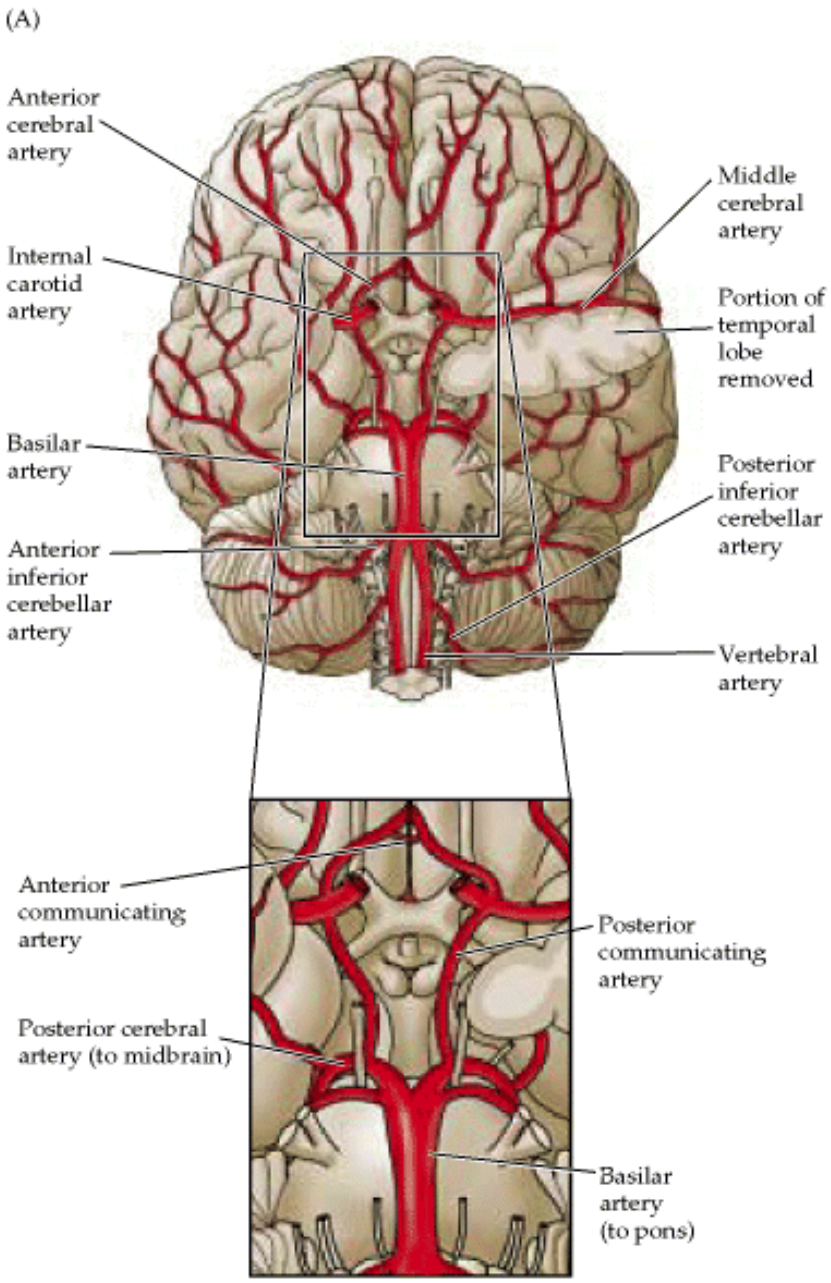


Figure 1: Ventral view of brain showing the cerebral circulation and circle of Willis, taken from Augustine 2001 and used with the permission of Sinauer Associates, Inc.

middle cerebral arteries, and the posterior cerebral arteries (PCA, which also supply the midbrain) (Augustine 2001; Brust 1991). The cerebral circulation must provide the brain with adequate oxygen, glucose, and other nutrients, as well as remove metabolic byproducts. The brain has a high metabolic rate and typically receives 15% of total cardiac output despite accounting for only 2% of total body weight (Brust 1991; Sokoloff 1997). Total CBF is estimated to be 50 mL per 100 g brain tissue per minute provided that CPP is between 50 and 150 mmHg (Agnoli et al. 1968; Hurn and Traystman 1997; Kety and Schmidt 1948).

It is known that **CBF** is dependent on **CPP** (the difference between ABP and ICP) and **CVR**, defined by the relationship **CBF = CPP/CVR** (Paulson et al. 1989; Skinhøj 1977). Under normal conditions, arterial blood pressure (ABP) at the level of the head adequately represents CPP (Lassen 1959), and CPP varies in parallel with ABP (Paulson et al. 1989). Thus, in order to maintain a constant CBF, CVR must vary inversely with changes in ABP. Poiseuille's Law determines CVR, such that $\mathbf{R} = \mathbf{(8\eta \mathit{l})/(\pi r^4)}$, where **R** represents resistance to blood flow, **η** represents blood viscosity, ***l*** is the length of the vessel, and **r** is radius of the vessel (Hurn and Traystman 1997). In general, the length of any vessel is virtually constant, and the viscosity of blood may vary slightly with changes in the hematocrit. Therefore, vessel diameter greatly influences CVR, and small adjustments in diameter can cause major changes in CVR. Consequently, because CBF is inversely related to CVR, small changes in luminal diameter can also lead to considerable changes in flow (Ku and Zhu 1993).

1.2.2. Autoregulation

The ability of any vascular bed to maintain relatively constant blood flow despite changes in blood pressure is termed autoregulation, and this mechanism is tightly controlled in the brain (Lassen 1959; Paulson, Strandgaard, and Edvinsson 1990). Originally, it was thought that CBF was passively dependent on arterial and venous pressures (Bayliss, Hill, and Gulland 1895). This theory was partly based on the Monro-Kellie doctrine which stated that the cranium was a rigid structure containing a "nearly incompressible" brain with a total constant volume. The Monro-Kellie doctrine predicted that any increase in the volume of cranial contents, i.e. blood, would elevate intracranial pressure, and reasoned that no significant changes in intracranial blood volume or vascular diameter were likely to occur (Bayliss, Hill, and Gulland 1895; Lassen 1959; Weed 1929). However, subsequent studies challenged this belief and suggested that the cerebral circulation did have intrinsic control of CBF. Regulation of CBF was first suggested by observations that changes in CPP elicited changes in arterial diameter in feline pial arteries, and these alterations in arterial diameter were independent of the method used to decrease or elevate blood pressure (Fog 1937, 1939). Fog concluded that the active regulation of cerebrovascular tone with variations in blood pressure was a form of autoregulation, possibly due to an effect of intravascular pressure on the pial arterial wall (Fog 1939). The concept of pressure-dependent activity in resistance vessels through a direct effect on vascular smooth muscle has been termed the Bayliss effect (Folkow 1989), and was first observed in the canine peripheral circulation (Bayliss 1902). A review of multiple studies in patients

with various conditions suggested that CBF is similarly independent of ABP within a wide range of pressures in humans (Lassen 1959).

Autoregulation in the brain can be defined in terms of diameter changes and vascular resistance, such that CBF autoregulation is the occurrence of vasodilatation as ABP decreases and vasoconstriction as ABP increases (Heistad and Kontos 1983). Cerebral vessels operate in a state of myogenic tone that provides a set point from which arteries can either constrict or dilate to modulate blood flow (Osol, Osol, and Halpern 1989). Animal studies have demonstrated that large cerebral arteries contribute substantially to vascular resistance and CBF autoregulation control (Harper, Bohlen, and Rubin 1984; Heistad, Marcus, and Abboud 1978; Kontos et al. 1978; Stromberg and Fox 1972). In the canine and feline cerebral circulation, ~40-50% of the total vascular resistance takes place between the aorta and the cerebral pial arterioles (30-40 μm) (Heistad, Marcus, and Abboud 1978; Heistad and Kontos 1983; Shapiro et al. 1971). This segmental vascular resistance accounts for an arterial pressure difference from 122 mmHg at the aorta to 63 mmHg in a 400 μm pial artery as determined in cats (Shapiro et al. 1971). The resistance of large cerebral arteries protects smaller arteries and capillaries from changes in aortic pressure by reducing microvascular blood pressure (Heistad and Kontos 1983).

Several mechanisms have been proposed to explain CBF autoregulation, including myogenic, metabolic, and neurogenic mechanisms. The myogenic theory suggests that vascular smooth muscle can respond to changes in transmural pressure (Folkow 1964; Heistad and Kontos 1983). The myogenic mechanism is pressure-

dependent, and the stimulus for this response may be changes in either wall tension or stretch of vascular smooth muscle cells (Heistad and Kontos 1983). Studies have shown that the cerebral autoregulatory response can be initiated within seconds of changes in transmural pressure (Busija, Heistad, and Marcus 1980; Kontos et al. 1978; Symon, Held, and Dorsch 1973), further supporting the myogenic mechanism.

Autoregulation has also been proposed to be a metabolic mechanism and flow-dependent. In this theory, the accumulation of metabolic products influences the activity of vascular smooth muscle to cause vasodilation, thus increasing CBF in more metabolically active areas (Heistad and Kontos 1983). A close correlation exists between increases in regional CBF and areas of local activity in the cerebral cortex (Ingvar 1976) and areas of activity seen on electroencephalogram (EEG) (Ingvar, Sjölund, and Ardö 1976; Paulson and Sharbrough 1974). The identity of the vasoactive metabolic byproducts that may mediate this mechanism is unclear. Adenosine is generated in response to tissue hypoxia and is a candidate metabolite (Heistad and Kontos 1983; Winn et al. 1980). It is also known that CO_2 has a powerful effect on CBF and CVR (Traystman 1997), and the local vasodilator effect of CO_2 is thought to be mediated by changes in extracellular fluid pH (Kontos, Raper, and Patterson 1977). An increase in arterial pCO_2 by 1 mmHg can cause a ~4% increase in CBF (Skinhøj 1977).

Neurogenic control of CBF autoregulation may occur through either intrinsic or extrinsic innervation. Under normal conditions within the autoregulatory range, resting CBF seems to be minimally affected by sympathetic stimulation (Harper et al. 1972;

Heistad and Marcus 1978) or denervation (Werber and Heistad 1984), though sympathetic stimulation can attenuate transient CBF increases within the autoregulatory range (Busija, Heistad, and Marcus 1980). However, sympathetic stimulation has been shown to shift the upper limit of autoregulation to higher pressures and decrease BBB permeability (Bill and Linder 1976; Gross et al. 1979; Heistad and Marcus 1979; MacKenzie et al. 1979). Conversely, acute sympathetic denervation was shown to shift autoregulation to lower blood pressures (Sadoshima et al. 1985). The shift in CBF autoregulation with sympathetic stimulation may be a physiological mechanism in place to protect the brain against increased blood pressure associated with sympathetic activation (Paulson, Strandgaard, and Edvinsson 1990).

1.2.3. Limits of Autoregulation

Autoregulation of CBF is a balance between passive distension of vessels due to changes in transmural pressure and changes in vessel diameter due to vascular smooth muscle reactivity (Heistad and Kontos 1983). The balance of these forces is represented by a pressure-flow curve, a diagram of autoregulation. A hypothetical autoregulatory curve is shown in Figure 2. At very low pressures (designated by the letter A), increases in ABP result in increased blood flow because cerebral vessels are maximally and passively dilated and CVR is low. The autoregulatory plateau is the range in which most physiological blood pressures occur (letter B), and where CBF is most tightly regulated. In this range, increases in flow are proportionally less than increases in pressure due to increased CVR evidenced by vasoconstriction. At higher

pressures (indicated by letter C), the autoregulatory range is exceeded; CBF increases linearly with pressure and CVR decreases due to force dilatation of the cerebral arteries (Chillon and Baumbach 1997; Heistad and Kontos 1983; Paulson, Strandgaard, and Edvinsson 1990). When autoregulation is abolished, either above or below the autoregulatory range, CBF changes linearly with ABP (Paulson, Strandgaard, and Edvinsson 1990). Experimentally, autoregulation of CBF has been demonstrated in several mammalian species, including rats (Hernandez, Brennan, and Bowman 1978; Koo and Cheng 1974), cats (Busija, Heistad, and Marcus 1980; MacKenzie, Strandgaard et al. 1976), dogs (Busija, Heistad, and Marcus 1980; Ekström-Jodal et al. 1977; Rapela and Green 1964), and baboons (Strandgaard et al. 1974; Symon, Held, and Dorsch 1973). In humans, CBF autoregulation has been demonstrated by several

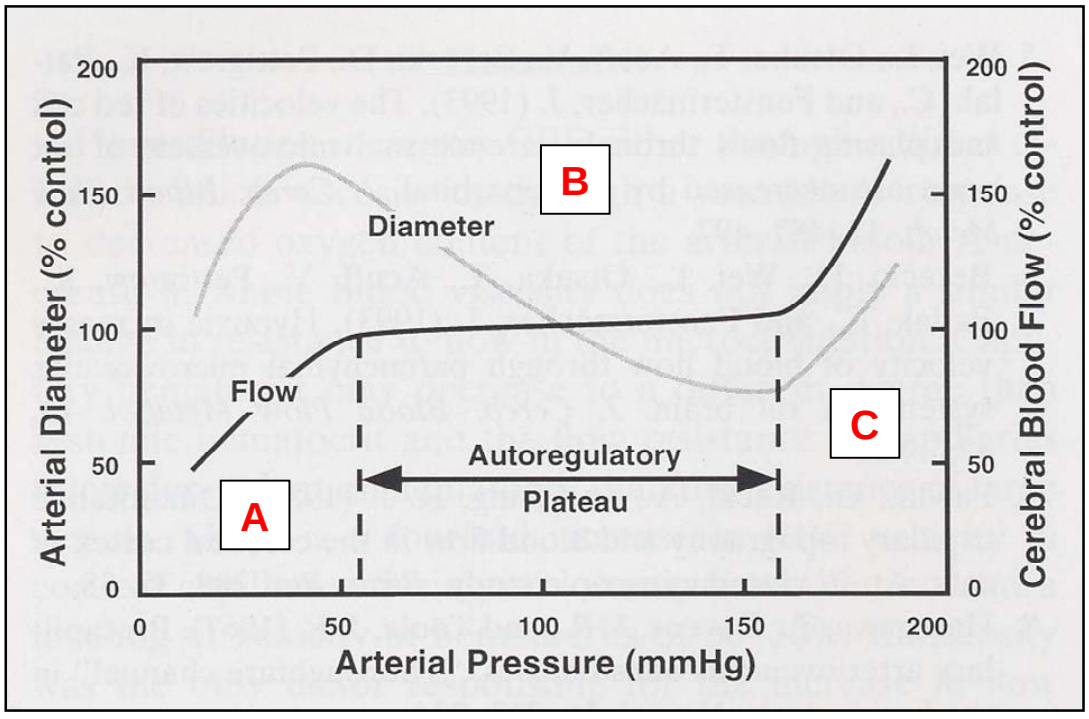


Figure 2: Hypothetical autoregulatory curve, adapted from Chillion and Baumbach 1997 and used with the permission of Elsevier Limited for Academic Press

groups reporting only minimal changes in CBF with ~30-40 mmHg increases in MAP, induced by either intravenous angiontensin or metaraminol (Agnoli et al. 1968; McHenry et al. 1974; Olesen 1973). Generally, in humans the limits of CBF autoregulation are ~60 mmHg at the lower end and ~150 mmHg at the upper end (Paulson, Strandgaard, and Edvinsson 1990; Strandgaard et al. 1973).

If blood pressure (and thus CPP) falls below a certain point, termed the lower limit of CBF autoregulation, CBF decreases. Because of the high metabolic demand for oxygen in the brain, limited CBF may lead to neurological complications, such as dizziness, confusion, loss of consciousness, and ultimately ischemic brain damage (Finnerty, Witkin, and Fazekas 1954; Paulson et al. 1989; Paulson, Strandgaard, and Edvinsson 1990). The lower limit of CBF autoregulation has been observed at 50-70 mmHg in normotensive individuals (Lassen 1959; Strandgaard et al. 1973; Olesen 1973). In rats, the lower limit of CBF autoregulation has been observed at ~30 mmHg (Koo and Cheng 1974), 50-69 mmHg (Barry et al. 1982) and 80 mmHg (Hernandez, Brennan, and Bowman 1978). The discrepancy in these observations is most likely due to variations in technique and experimental definition of the loss of autoregulation.

Evidence suggests that at high pressures cerebral resistance vessels are unable to further constrict to counteract elevated CPP and subsequently force dilatation occurs and CBF increases linearly with pressure (MacKenzie, Strandgaard et al. 1976; Paulson, Strandgaard, and Edvinsson 1990). The existence of an upper limit to CBF autoregulation has been shown in several mammalian species, including rats (Euser and Cipolla 2007; Hernandez, Brennan, and Bowman 1978), cats (MacKenzie, Strandgaard

et al. 1976), dogs (Ekström-Jodal et al. 1977), baboons (Strandgaard et al. 1974; Strandgaard et al. 1975), and in man (Skinhøj and Strandgaard 1973; Strandgaard et al. 1973). Classically, pial arteriolar constriction is observed as ABP is increased, thereby maintaining a constant CBF (MacKenzie, Strandgaard et al. 1976). With continued intraluminal pressure increases, segmental dilation (the "sausage-string" pattern) is observed followed by uniform dilation as segments of the resistance arteries are forcefully dilatated (MacKenzie, Strandgaard et al. 1976; Paulson, Strandgaard, and Edvinsson 1990). As the vessels become progressively and forcefully dilatated, CVR drops and CBF increases (Ekström-Jodal et al. 1977; Hernandez, Brennan, and Bowman 1978; Strandgaard et al. 1974). This hyperperfusion of the brain can potentially cause BBB damage and edema formation (Sokrab et al. 1988; Westergaard, van Deurs, and Bronsted 1977). This progression of events is the etiology of HTE (Lassen and Agnoli 1972; Paulson, Strandgaard, and Edvinsson 1990; Skinhøj and Strandgaard 1973).

Lassen and Agnoli first used the term "breakthrough of autoregulation" to characterize the excessive cerebral arteriolar distension caused by severe hypertension (Lassen and Agnoli 1972). The upper limit of autoregulation has been reported at ~160 to 180 mmHg in rats (Euser and Cipolla 2007; Hernandez, Brennan, and Bowman 1978), ~160-170 mmHg in cats (MacKenzie, Strandgaard et al. 1976), 180-200 mmHg in dogs (Ekström-Jodal et al. 1977), and ~140 to 150 mmHg in primates and man (Skinhøj and Strandgaard 1973; Strandgaard et al. 1973; Strandgaard et al. 1974).

1.2.4. Modulators of Autoregulation – Hypertension and Nitric Oxide

Chronic Hypertension - It has been noted that "virtually every tissue readily adjusts its structural design to changes in functional load, and blood vessels are no exception" (Folkow et al. 1973), and this is true during chronic hypertension. Although hypertensive patients without neurological deficits have similar CBF as normotensive individuals, as determined by the nitrous oxide method, CVR is significantly increased 88% (Kety et al. 1948), suggesting an adaptation of the cerebral circulation during chronic hypertension. It appears that the adaptation of the cerebral circulation to sustained hypertension affects CBF autoregulation. In patients with severe hypertension, the lower limit of CBF autoregulation was 120 mmHg on average, much higher than 70 mmHg as observed in normotensive controls (Strandgaard et al. 1973). Interestingly, the lower limit of CBF autoregulation is more severely elevated in patients with greater levels of hypertension (Strandgaard et al. 1973). Similarly, hypertensive patients show a reduced tolerance of acute hypotension (Lassen 1959), and signs of cerebral ischemia manifest at significantly higher blood pressures in patients with malignant hypertension versus normotensive controls (Finnerty, Witkin, and Fazekas 1954). The signs of cerebral ischemia are closely related to a mean CBF of 31.5 mL per 100 g brain tissue per minute irrespective of the subject's blood pressure, supporting the concept that the autoregulatory curve is shifted to higher blood pressures with chronic hypertension (Finnerty, Witkin, and Fazekas 1954). The lower limit of CBF autoregulation is also shifted to the right in baboons (Jones et al. 1976) and rats (Barry et al. 1982; Fujishima and Omae 1976; Harper and Bohlen 1984). Both

renal and spontaneously hypertensive rats exhibit a shift of the lower limit of CBF autoregulation and greater incidence of hypotensive ischemic brain damage (Barry et al. 1982). These data suggest that the adaptation of CBF autoregulation to hypertension is dependent on structural changes of the vasculature as opposed to other factors which would likely be different between two different animal models of chronic hypertension.

Chronic hypertension also shifts the upper limit of CBF autoregulation. In spontaneously hypertensive rats, the upper limit of autoregulation is increased by ~50 mmHg (Harper and Bohlen 1984), and this shift is closely related to the rise in basal blood pressure (Sadoshima et al. 1985). In baboons, the upper limit of CBF autoregulation is similarly increased by chronic renal hypertension (Strandgaard et al. 1975). These authors suggested that a shift of the upper limit of CBF autoregulation would partially protect the patient against the effects of hypertension and could explain why some patients exhibit severe hypertension without signs of HTE (Strandgaard et al. 1975). Results from studies of hypertensive patients also suggest that the upper limit of CBF autoregulation is shifted rightward with chronic hypertension because normal CBF is observed at MAP greater than 150 mmHg (Kety et al. 1948; Strandgaard et al. 1973). Experimental animal models of chronic hypertension also demonstrate normal CBF despite elevated ABP (Jones et al. 1976; Sadoshima, Busija, and Heistad 1983; Werber and Heistad 1984).

The rightward shift in the CBF autoregulatory curve to higher pressures in chronic hypertension is likely due to structural remodeling and hypertrophy of the cerebral arteries (Faraci, Baumbach, and Heistad 1989). Hypertrophy increases the

cross-sectional area of the vessel wall and remodeling typically refers to a decrease in outer diameter of the vessel (Schachter 2002; Schiffrin 1992). These structural changes have been shown to occur in the cerebral circulation (Nordberg and Johansson 1980; Baumbach and Heistad 1989; Hart, Heistad, and Brody 1980), and likely contribute to the increased segmental resistance of large arteries (>200 μm diameter) in chronic hypertension (Baumbach and Heistad 1983). During hypertension, elevated arterial pressure increases wall tension. This is represented by LaPlace's Law which states that for a cylinder, **T = PR**, where **T** represents circumferential wall tension, **P** is intravascular pressure, and **R** is the vessel radius (Schiffrin 1992). (It should be noted that this relationship is only true if the wall to lumen ratio is < 0.1 (Coulson et al. 2002).) Wall stress is the amount of wall tension transmitted to each point of the vessel wall, and is represented by the quotient of wall tension and wall thickness, where **wall stress = (PR)/wall thickness** (Schiffrin 1992). Thus, both wall tension and wall stress are increased by hypertension (Heistad and Baumbach 1992). Vascular hypertrophy and remodeling in response to chronic hypertension normalize wall stress through increases in wall thickness and decreased vascular diameter (Heistad and Baumbach 1992). These structural changes increase the wall to lumen ratio, and increased wall to lumen ratios have been observed in the cerebral circulations of both young and old spontaneously hypertensive rats (Nordberg and Johansson 1980; Hart, Heistad, and Brody 1980; Harper and Bohlen 1984). Vascular medial hypertrophy associated with chronic hypertension attenuates increases in microvascular pressure (Werber and

Heistad 1984; Harper and Bohlen 1984; Heistad and Baumbach 1992) and likely protects the BBB (Baumbach and Heistad 1988; Mueller and Heistad 1980).

Cerebrovascular remodeling is a physiological adaptation to hypertension that protects the brain from damage at high ABP. However, previous studies in our lab suggest that this protective adaptation does not occur during pregnancy. While hypertension-induced vascular remodeling occurred in NP control rats, this adaptation was absent in both LP rats treated with a nitric oxide synthase (NOS) inhibitor (N^G-nitro-L-arginine methyl ester, L-NAME) and Dahl salt-sensitive LP rats (Aukes et al. 2007; Cipolla, DeLance, and Vitullo 2006). Pregnancy also decreases myogenic reactivity in the posterior cerebral artery (Aukes et al. 2007) and lowers the pressure of force dilatation *in vitro* (Cipolla, Vitullo, and McKinnon 2004). As described in Chapter 3, *in vivo* studies to determine the upper limit of CBF autoregulation found no difference between control NP and LP rats; however, the consequences of autoregulatory breakthrough were greater as evidenced by increased cerebral edema formation (Euser and Cipolla 2007). In addition, altered CBF autoregulation has been reported in preeclamptic and eclamptic patients (Oehm et al. 2003; Oehm et al. 2006). Taken together, these studies suggest that pregnancy alone affects the cerebral circulation in ways that may make it more vulnerable to damage caused by acute increases in ABP.

Nitric Oxide - Nitric oxide (NO) is a well-known vasodilator, and is recognized as the chemical identity of endothelium-derived relaxing factor (Furchgott and Vanhoutte 1989; Ignarro et al. 1987; Palmer, Ferrige, and Moncada 1987). In 1998, the

Nobel Prize for Physiology and Medicine was award to three American scientists, Dr. Robert F. Furchgott, PhD, Dr. Louis J. Ignarro, PhD, and Dr. Ferid Murad, MD, PhD, for their work in identifying and characterizing NO (SoRelle 1998). NO is synthesized from L-arginine and oxygen via NOS enzymes (Chan and Vallance 2002; Palmer, Ashton, and Moncada 1988). Three isoforms of the NOS enzyme have been identified, constitutively active endothelial (eNOS) and neuronal (nNOS) forms, and an inducible (iNOS) form modulated by inflammatory mediators (Förstermann et al. 1994; Vanhoutte 2003). In cerebral vessels under normal physiologic conditions, NO is synthesized both within the endothelium via eNOS and by adventitial nerves via nNOS (Chan and Vallance 2002). All three NOS isoforms are involved in regulating vascular tone, and studies show that the continuous basal release of NO from the endothelium is an important modulator of resting vascular tone (Chan and Vallance 2002; Zatz and Baylis 1998). NOS can be inhibited pharmacologically, and L-NAME and L-NNA are L-arginine analogues that act as competitive, nonspecific NOS inhibitors (Traystman et al. 1995). Acute and chronic treatment with NOS inhibitors causes hypertension in both animals and humans (Aisaka et al. 1989; Haynes et al. 1993; Rees, Palmer, and Moncada 1989; Ribeiro et al. 1992). During pregnancy, sustained NOS inhibition causes symptoms similar to preeclampsia including hypertension, proteinuria, and fetal growth retardation in rats (Molnar et al. 1994; Yallampalli and Garfield 1993).

Production of NO is increased during pregnancy, and may play a role in the decreased peripheral vascular resistance observed during gestation (Sladek, Magness, and Conrad 1997; Williams et al. 1997; Boccardo et al. 1996). Estrogen levels are also

elevated in pregnancy, and it has been shown that estrogen treatment increases both eNOS and endothelium-derived NO within cerebral vessels (McNeill et al. 1999; Skarsgard, Van Breemen, and Laher 1997). Studies by ourselves and others suggest that NO has an active role in autoregulatory breakthrough in both female and male rats, and that inhibition of NOS significantly increases and possibly prevents autoregulatory breakthrough following acute hypertension (Euser and Cipolla 2007; Talman and Nitschke Dragon 1995). Recently, it was suggested that NO synthesized specifically by nNOS is responsible for the cerebral vasodilatation during acute hypertension (Talman and Nitschke Dragon 2007). Arterial forced dilatation prior to autoregulatory breakthrough appears to be an active process involving pressure-dependent production of NO, rather than a mechanical dilation (Euser and Cipolla 2007). Studies have also shown that inhibition of potassium channels similarly shifts the autoregulatory curve to higher pressures (Paterno, Heistad, and Faraci 2000). Together, these results suggest that autoregulatory breakthrough is an active process that may be mediated by NO and potassium channels.

1.3. The Blood-brain Barrier and Cerebral Edema Formation

1.3.1. The Blood-brain Barrier

In 1885, the first evidence of a barrier between the peripheral circulation and the central nervous system was described (Ehrlich 1885; as cited in Betz and Dietrich 1998; and Hawkins and Davis 2005). Ehrlich noted that dyes injected intravenously into rats stained all organs of the body with the exception of the brain and spinal cord

(Betz and Dietrich 1998; Hawkins and Davis 2005); he concluded that this was due to a low affinity of the dye for the brain (Ehrlich 1904; as cited in Betz and Dietrich 1998; and Hawkins and Davis 2005). This concept was later refuted by Ehrlich's student Goldmann, who demonstrated that dye injected into the cerebrospinal fluid (CSF) stained the whole of the brain but did not reach the periphery (Goldmann 1913; as cited in Betz and Dietrich 1998; and Hawkins and Davis 2005). Spatz first proposed that the site of the BBB might be the cerebrovasculature (Spatz 1934; as cited in Betz and Dietrich 1998), but it was not until the advent of electron microscopy that this could be demonstrated (Betz and Dietrich 1998). Reese and Karnovsky showed that intravenous horseradish peroxidase, an electron dense tracer, did not pass from the vessel lumen, suggesting that cerebral endothelial cell membranes comprised the BBB (Reese and Karnovsky 1967). Brightman and Reese confirmed that the BBB was at the level of the cerebral vascular endothelium, and found that tight junctions (TJ) "periodically obliterated the interspace along the apical-to-basal axis" between endothelial cells in cerebral parenchymal vessels (Brightman and Reese 1969). The BBB has been observed in all regions of the brain with the exception of the circumventricular organs (Ballabh, Braun, and Nedergaard 2004; Fenstermacher et al. 1988).

Cerebral endothelial cells possess unique characteristics that together form the BBB: a lack of capillary fenestrations (Fenstermacher et al. 1988), a low basal rate of pinocytosis (Reese and Karnovsky 1967; Sedlakova, Shivers, and Del Maestro 1999), and the presence of high-electrical resistance TJ between adjacent endothelial cells (Brightman and Reese 1969; Reese and Karnovsky 1967). Paracellular movement is

prevented by TJ, demonstrated by a high transendothelial electrical resistance indicating a high impedence to ion movement (Butt, Jones, and Abbott 1990; Crone and Olesen 1982). Transcellular transport, via pinocytosis, is very low under normal conditions. This has been demonstrated experimentally by the very limited transfer of tracers across the vascular endothelial cells of the brain under normal conditions (Brightman and Reese 1969; Brightman et al. 1970; Feder 1971; Mueller and Heistad 1980; Reese and Karnovsky 1967; Westergaard 1977).

1.3.2. Hypertension and Blood-brain Barrier Permeability

One of the perturbations that can influence BBB permeability is hypertension. At high blood pressures, above the upper limit of CBF autoregulation, arterial pressure is transmitted to small vessels and the increased pressure is exerted on the vascular walls. If this pressure is high enough, it can lead to BBB damage manifested as increased BBB permeability (Häggendal and Johansson 1971-1972). Increased permeability has been observed by many investigators using various tracers and methods of induced hypertension. Extravastion of relatively large dye-albumin complexes caused by hypertension has been described (Brightman et al. 1970; Byrom 1954; Häggendal and Johansson 1972; Johansson et al. 1970; Johansson 1974; Olsson and Hossmann 1970; Suzuki et al. 1984). Horseradish peroxidase has been used extensively with electron microscopy to show increased BBB permeability caused by hypertension on an ultrastructural level, indicated by increased pinocytosis (Giacomelli, Wiener, and Spiro 1970; Hansson, Johansson, and Blomstrand 1975; Nag, Robertson,

and Dinsdale 1977; Olsson and Hossmann 1970; Westergaard, van Deurs, and Bronsted 1977). In several of these studies, chronically hypertensive animals manifested neurological symptoms similar to HTE, including convulsions, together with observed increases in BBB permeability (Byrom 1954; Giacomelli, Wiener, and Spiro 1970).

The mechanism of increased BBB permeability induced by hypertension was not initially clear. In cats, extravasation of Evan's blue (EB) was seen when blood pressure was abruptly raised, but not in animals in which blood pressure had been increased to similar levels in a stepwise and more gradual manner (Häggendal and Johansson 1972). This suggested that increased BBB permeability was caused by a mechanical effect due to the sudden increase in intraluminal pressure, particularly when the brain was not able to properly autoregulate CBF or the pressure exceeded the autoregulatory range (Häggendal and Johansson 1972; Hardebo 1981; Johansson 1974). In many cases, CBF was found to be increased in areas of BBB disruption, supporting the idea that BBB permeability is increased following autoregulatory breakthrough (Baumbach and Heistad 1985; Häggendal and Johansson 1972; Hatashita, Hoff, and Ishii 1986; Johansson 1974; Johansson 1983; Suzuki et al. 1984). Several studies showing increased permeability during acute hypertension did not observe gross damage to the endothelial cells, and direct injury to the endothelium could not account for the transfer of tracers (Giacomelli, Wiener, and Spiro 1970; Nag, Robertson, and Dinsdale 1977; Westergaard, van Deurs, and Bronsted 1977). This is supported by indications that BBB disruption caused by acute hypertension is reversible, and further suggests that increased permeability following an uncomplicated acute rise in blood

pressure is a functional disturbance and not damage to the endothelium (Hatashita, Hoff, and Ishii 1986; Johansson and Linder 1978; Nag 1986).

There at least two ways that tracers can move from the vascular lumen into the brain parenchyma, either paracellularly through the endothelial TJ or transcellularly by way of increased pinocytosis. In both chronic and acute hypertension, horseradish peroxidase has been observed in pinocytotic vesicles and between endothelial cells through TJ (Giacomelli, Wiener, and Spiro 1970; Hansson, Johansson, and Blomstrand 1975). However, intact TJ have also been reported by numerous investigators (Hatashita, Hoff, and Ishii 1986; Nag, Robertson, and Dinsdale 1977; Westergaard, van Deurs, and Bronsted 1977). Pinocytotic vesicles are observed within two minutes after the hypertensive insult (Hansson, Johansson, and Blomstrand 1975; Nag, Robertson, and Dinsdale 1977), and an increase in the number of pinocytotic vesicles occurs in both acute and chronic hypertension (Eto, Omae, and Yamamoto 1971; Hansson, Johansson, and Blomstrand 1975; Nag, Robertson, and Dinsdale 1977, 1979). In hypertensive animals, horseradish peroxidase tracer was observed in eight times as many vesicles versus normotensive animals (Nag, Robertson, and Dinsdale 1979). Additionally, it was later shown that disruption of microtubules and microfilaments, which account for the movement of pinocytotic vesicles through the endothelial cell, was protective of the BBB during acute hypertension (Larsson et al. 1980; Nag 1995). Collectively, these observations led to the theory that endothelial cells respond to increased pressure through enhanced pinocytosis, with a resulting increase in BBB permeability (Nag, Robertson, and Dinsdale 1977; Westergaard 1977). Studies have

shown that increased pinocytosis is not caused by the tracer horseradish peroxidase (Nag, Robertson, and Dinsdale 1979), nor by the agents used to induce hypertension (Westergaard, van Deurs, and Bronsted 1977). Our lab has shown that elevated pressure alone increases pinocytosis in cerebral vessels, suggesting that vesicle formation in cerebral endothelial cells is sensitive to physical stimulation, though this mechanism has not yet been established (Cipolla et al. 2004).

High pressure can induce transendothelial pinocytotic vesicular transport of albumin and other vascular contents, and the presence of plasma proteins in the brain parenchyma is an important component of edema formation (Kuroiwa et al. 1985), further discussed below. Therefore, increased BBB permeability via an increase in transcellular transport is likely an important contributor to edema formation during acute hypertension. Notably, our lab has also shown a significant increase in pressure-induced BBB permeability, via pinocytosis, specific to LP animals (Cipolla et al. 2005). This represents a possible mechanism by which acute hypertension may increase cerebral edema formation during pregnancy and predispose pregnant women to the neurological complications of eclampsia.

Some regions of the brain appear to be more susceptible than others to increased BBB permeability during acute hypertension. Relatively high permeability occurs in the parietal, temporal, and occipital cortices (Hatashita et al. 1985; Hatashita, Hoff, and Ishii 1986; Nag, Robertson, and Dinsdale 1979; Nag 1986; Suzuki et al. 1984), as well as in the cerebrum versus the brainstem or cerebellum (Baumbach and Heistad 1985; Mayhan, Faraci, and Heistad 1986). The primary location of BBB disruption during

acute hypertension in the cerebrovasculature has been reported at various locations. Some studies suggest that the arterioles are the predominant site of BBB permeability (Hansson, Johansson, and Blomstrand 1975; Nag, Robertson, and Dinsdale 1977, 1979; Westergaard 1977; Westergaard, van Deurs, and Bronsted 1977), while other groups report that BBB disruption occurs largely in the venules (Auer 1978; Baumbach and Heistad 1985; Baumbach, Mayhan, and Heistad 1986; Mayhan and Heistad 1985). It has been suggested that these discrepancies may be caused by possible differences between pial and parenchymal vessels; intravital microscopy studies demonstrated venous disruption whereas results of histological studies indicate disruption at the level of parenchymal arterioles (Mayhan and Heistad 1985). It has also been suggested that the nature of the hypertension affects the location of BBB permeability within the vasculature, with a rapid rise and more severe, sustained increase in pressure more likely to cause arterial permeability versus venous (Mayhan and Heistad 1985). While pin-pointing the type of vessel and region of the brain most affected by acute hypertension may provide important information about the structure and function of the cerebral circulation, the net effect of increased BBB permeability is the same. Increased BBB permeability allows for a movement of water and solutes into the brain and can lead to cerebral edema formation and potentially dangerous clinical implications.

1.3.3. Various Modulators of Blood-brain Barrier Permeability

A variety of factors have been found to modulate the permeability of the BBB. Seizures, both drug-induced and electrically-stimulated, have been shown to increase BBB permeability, and this is thought to be via increased pinocytosis (Bolwig, Hertz, and Holm-Jensen 1977; Hedley-Whyte, Lorenzo, and Hsu 1977; Lorenzo et al. 1975; Lee and Olszewski 1961; Petito, Schaefer, and Plum 1977; Suzuki et al. 1984; Westergaard, Hertz, and Bolwig 1978). However, when elevations in blood pressure are prevented by cervical cordotomy prior to seizure induction, BBB disruption is minimal (Petito, Schaefer, and Plum 1977). This suggests that the seizures are probably not directly increasing BBB permeability; rather, the systemic hypertension and accompanying increases in CBF occurring with the seizures are responsible for the increased BBB permeability.

Nitric oxide (NO) is an endogenous vasodilator synthesized from the amino acid L-arginine by NOS enzymes (Chan and Vallance 2002; Palmer, Ashton, and Moncada 1988; Vanhoutte 2003). In the cerebral circulation, NO does not appear to have a role in basal BBB integrity (Mayhan 2000); however, it has been implicated in BBB disruption during acute hypertension (Mayhan 1995). In addition, NO seems to modulate BBB permeability induced by various inflammatory mediators (Mayhan 1996, 1999; Nakano, Matsukado, and Black 1996). Studies have also suggested a role for NO in mediating BBB permeability during cerebrovascular injury (Nag, Picard, and Stewart 2000, 2001; Thiel and Audus 2001), and excessive amounts of NO redox forms ($NO\bullet$, NO^+, NO^-, $ONOO^-$) can compromise the integrity of the BBB (Boje and

Lakhman 2000; Mayhan 2000). Pregnancy is known to be a state of increased NO (Boccardo et al. 1996; Sladek, Magness, and Conrad 1997; Williams et al. 1997), and it is possible that elevated levels of NO and related molecules may contribute to BBB permeability and cerebral edema formation during pregnancy as explored in this project. Our work has shown that in LP rats, cerebral edema formation caused by acute hypertension and autoregulatory breakthrough is partially attenuated by treatment with the NOS inhibitor L-NAME (Euser and Cipolla 2007).

1.3.4. Starling Forces

Water movement between the vascular compartment and tissue is largely controlled by osmotic gradients and hydrostatic pressure differences (Kimelberg 2004; Papadopoulos, Krishna, and Verkman 2002). This is represented mathematically by the Starling equation, $\mathbf{Jv = Lp\ [(P_{plasma}-P_{tissue}) - \sigma_{protein}\ (\Pi_{plasma}-\Pi_{tissue})]}$, where **Jv** is flow, **Lp** is the hydraulic conductivity of the endothelial membrane (a measure of water permeability), $\mathbf{P_{plasma}-P_{tissue}}$ represents the hydrostatic pressure difference between plasma and tissue (which is largely influenced by the systemic blood pressure), $\boldsymbol{\sigma}$ is the osmotic reflection coefficient and is inversely related to permeability, and $\mathbf{\Pi_{plasma}-\Pi_{tissue}}$ is the difference in protein oncotic pressure between plasma and tissue (Fenstermacher and Patlak 1976; Kimelberg 2004; Klatzo 1987). In the peripheral circulation, plasma entering the tissue maintains its normal salt content due to the presence of capillary fenestrations, and efflux of fluid from the vasculature due to blood hydrostatic pressure is offset by the oncotic pull of the plasma proteins retained in the

vascular compartment (Kimelberg 2004). At peripheral capillaries, $\sigma_{protein}$ is 0.93, indicating that 93% of plasma proteins are retained in the capillary (Kimelberg 2004).

In the brain, the endothelial cells are linked by TJ preventing the movement of ions and other hydrophilic substances between the endothelial cells into the tissue. Thus, due to the presence of the BBB, the Starling equation is modified to include the osmotic force of plasma salts: $\mathbf{Jv = Lp[(P_{plasma}-P_{tissue}) - \sigma_{protein}(\Pi_{plasma}-\Pi_{tissue}) - \sigma_{salt}(\Pi_{plasma}-\Pi_{tissue})]}$ (Kimelberg 2004), where σ_{salt} is the osmotic reflection coefficient for ion movement and $\Pi_{plasma}-\Pi_{tissue}$ also represents the difference between plasma and tissue salt osmotic pressure. The lack of fenestrations in the cerebral capillary endothelium causes the σ_{salt} to approach 1. The hydraulic conductivity, **Lp**, of the BBB is at least one to two orders of magnitude smaller than the peripheral capillary membranes, signifying a very strong resistance to bulk water movement across the membrane (Fenstermacher and Patlak 1976). In the cerebral circulation, ABP (P_{plasma}) must be greater than ICP (P_{tissue}) to maintain blood flow. Net transport of water is directed by osmotic forces resulting from selective solute transport in order to maintain homeostasis in the brain (Kimelberg 2004).

Under normal conditions, any movement of water into the brain would be directed by blood pressure (Go and Pratt 1975) and would be opposed immediately by the strong osmotic and oncotic pulls of the plasma salts and proteins within the vasculature (Kimelberg 2004). However, the tightly regulated flow of water into the brain can be disturbed if the BBB is damaged (Kimelberg 1995). Breakdown of the BBB causes σ_{salt} to approach zero (as in the peripheral circulation) and $\sigma_{protein}$ to

decrease (Kimelberg 2004; Rapoport 1997). At the site of BBB disruption, **Lp** and $\mathbf{P_{plasma}}$ (blood pressure) appear to determine the force with which edema spreads, and the elevation of MAP dramatically accelerates the spread of edema (Klatzo 1987). An investigation of the relationship between hydrostatic pressure and cerebral edema formation found that when both $\mathbf{P_{plasma}}$ and $\mathbf{P_{tissue}}$ were altered simultaneously (via aortic occlusion and craniectomy, respectively) protein extravasation and edema formation were greater than for either insult separately (Hatashita et al. 1985). The dominant influence of blood pressure over other Starling forces in the cerebral circulation is important to consider in any clinical situation in which the hypertension is involved. Increased hydrostatic pressure, commonly caused by hypertension, is the physiologic cause of vasogenic cerebral edema formation (Kimelberg 2004; Hatashita et al. 1985), as described below, and this contributes to the clinical symptoms of eclampsia (Kaplan 2006; Zunker et al. 1995).

1.3.5. Cerebral Edema

Brain edema is defined as an abnormal accumulation of fluid within the brain parenchyma producing a volumetric enlargement of the tissue (Klatzo 1987). Because the brain is surrounded by the rigid skull, an increase in tissue water content can rapidly produce neurological symptoms including headache, nausea, vomiting, altered consciousness, and coma through an increase in ICP (Rapoport 1997). Brain swelling can become so severe that increased ICP ($\mathbf{P_{tissue}}$ in the brain) impairs CBF or causes herniation of the brain through the foramen magnum with life-threatening implications

(Joo and Klatzo 1989; Kimelberg 1995). Similarly, the neurological symptoms of eclampsia are thought to be caused by cerebral edema formation (Kaplan 2006; Zunker et al. 1995).

Cerebral edema can form in response to a wide variety of insults and by different mechanisms. In 1967, Klatzo proposed a classification of edema based on mechanistic principles and described two primary types of edema, vasogenic and cytotoxic (also termed cellular) (Klatzo 1967). Klatzo acknowledges that both forms of edema typically co-exist to some degree, however, identifying the predominant type of edema, and thus a pathogenic mechanism, can help to understand the cerebral insult and most appropriate clinical management (Klatzo 1987). Cytotoxic edema is associated with intracellular swelling in both gray and white matter with no change in BBB permeability (Kimelberg 2004; Klatzo 1967), and the entry of water into the brain is largely due to osmotic gradients that develop due to tissue injury and disrupted cellular osmoregulation (Klatzo 1987). This form of edema commonly occurs in ischemic states, with trauma, or with acute hypo-osmolality (Fishman 1975; Kimelberg 1995; Klatzo 1987).

Vasogenic edema is caused by injury to the vessel wall that results in the movement of plasma constituents and water, as predicted by the Starling equation, into the extracellular space (Fishman 1975; Kimelberg 2004; Klatzo 1987). Implicit in the definition of vasogenic edema is a net gain of water and solutes in the brain parenchyma (Kimelberg 1995). The retention of water in the tissue, due to serum protein retention, is essential to the formation and persistence of the edema (Klatzo

1987). In animal studies, increased brain water content has been associated with the presence of extravasated proteins (Hatashita, Hoff, and Ishii 1986; Kuroiwa et al. 1985). As predicted by the Starling equation, the development of vasogenic edema is also strongly influenced by systemic blood pressure (Brightman et al. 1970). Vasogenic edema preferentially spreads and accumulates in the white matter, possibly because parallel white matter tracts are more compliant (Klatzo 1967, 1987). It has been shown that vasogenic edema begins to resolve as soon as proteins can no longer pass across the BBB (Kuroiwa et al. 1985). Generally, vasogenic edema is reversible and leaves the cellular elements of previously edematous tissue reasonably intact, however vasogenic edema can progress to cytotoxic edema if CBF becomes restricted (Klatzo 1987). In support of this, areas of cytotoxic edema have been observed clinically within areas of vasogenic edema in eclamptic patients (Loureiro et al. 2003; Zeeman et al. 2004).

It has long been recognized that eclampsia is very similar neuroradiologically to HTE, as described in section 1.1.3. In both disorders, the distinction between cytotoxic and vasogenic edema is significant because of considerable differences in prognosis and treatment. Cytotoxic edema may result in irreversible cerebral infarction whereas vasogenic edema can be readily reversibile if treated promptly, and treatment guidelines differ between the likely causes of these forms of edema (Schaefer et al. 1997). In eclamptic patients, vasogenic edema is demonstrated as increased ADCs on MRI (Engelter, Provenzale, and Petrella 2000; Kanki et al. 1999; Loureiro et al. 2003; Schaefer et al. 1997; Zeeman et al. 2004). The most common locations of

neuroradiologic abnormalities in eclampsia are the parietal and occipital lobes (Dahmus, Barton, and Sibai 1992; Loureiro et al. 2003; Naidu et al. 1997; Schwartz et al. 1992; Schwartz et al. 2000; Zeeman et al. 2004). MRI scans in eclamptic and preeclamptic patients, obtained after intravenous administration of gadopentetate dimeglumine show enhancement indicating BBB disruption (Schwartz et al. 1992; Schwartz et al. 2000), suggesting vasogenic versus cytotoxic edema formation. In studies in which a follow-up scan was obtained, the majority of cerebral abnormalities were reversible, which additionally suggests vasogenic edema as the origin of the imaging abnormalities (Loureiro et al. 2003; Manfredi et al. 1997; Schwaighofer, Hesselink, and Healy 1989; Schwartz et al. 2000). Hyperperfusion of the cerebrovasculature due to autoregulatory breakthrough is the likely cause of vasogenic edema formation in eclampsia (Kaplan 2006; Zunker et al. 1995). Thus, it is important to understand how pregnancy may affect CBF autoregulation and BBB permeability which together contribute to cerebral edema formation, and this is the overall goal of this dissertation.

1.3.6. Aquaporins

In 2003, the Nobel Prize in Chemistry was awarded to Dr. Peter Agre for the discovery of aquaporin (AQP) water channels (Knepper and Nielsen 2004). The aquaporins are a family of water channel proteins that act to facilitate water flux through cell membranes in a variety of cell types (Badaut et al. 2002). Water flow through AQPs is bi-directional and controlled by osmotic gradients (Agre et al. 2002).

Eleven AQPs have been observed in mammals, AQP0 through AQP10 (Badaut et al. 2002), and six of these have been described in the rodent brain, AQP1, AQP3, AQP4, AQP5, AQP8, and AQP9 (Elkjaer et al. 2000; Hasegawa et al. 1994; Jung et al. 1994; Nielsen et al. 1993; Nielsen, Nagelhus et al. 1997; Yamamoto et al. 2001). AQP1, formerly known as CHIP, is found in the apical membrane of choroid plexus epithelial cells and is thought play a role in CSF formation (Nielsen et al. 1993). AQP9 has been demonstrated on ependymal cells in rat brain (Elkjaer et al. 2000) and on astrocytes of the glia limitans and around the ventricles in mouse brain (Badaut et al. 2001). Transcripts of AQP3, AQP5, and AQP8 have been detected in cortical neuron and astrocyte cell cultures (Yamamoto et al. 2001), however the physiologic role of these proteins in the brain has not yet been determined.

Aquaporin-4 was first cloned from rat lung (Hasegawa et al. 1994) and brain (Jung et al. 1994). Throughout the body, AQP4 is located in a variety of tissues: kidney (Frigeri et al. 1995; Neely et al. 1999), respiratory epithelium (Nielsen, King et al. 1997), stomach (Frigeri et al. 1995; Koyama et al. 1999), and skeletal muscle (Frigeri et al. 1995; Neely et al. 1999). However, the predominant site of its expression is in the brain (Nielsen, Nagelhus et al. 1997; Venero et al. 1999). In the central nervous system, AQP4 expression has been found in several regions including cortex (Jung et al. 1994; Neely et al. 1999), hippocampus (Jung et al. 1994; Neely et al. 1999), cerebellum (Jung et al. 1994; Nagelhus et al. 1998; Neely et al. 1999), brainstem (Neely et al. 1999), spinal cord (Frigeri et al. 1995; Jung et al. 1994; Neely et al. 1999; Oshio et al. 2004), and retina (Nagelhus et al. 1998). High levels of AQP4 protein have been

found in astrocytes bordering the subarachnoid space, the ventricles, and blood vessels (Nielsen, Nagelhus et al. 1997). The expression of AQP4 was found to be highly polarized with the most abundant expression in astrocytic endfeet in direct contact with blood vessels in rat (Amiry-Moghaddam et al. 2004; Nagelhus et al. 1998; Nielsen, Nagelhus et al. 1997; Oshio et al. 2004) and human tissues (Saadoun et al. 2002). AQP4 has also been reported in cerebral endothelial cells (Amiry-Moghaddam et al. 2004) and in cerebral microvessel isolations (Kobayashi et al. 2001). Systemic capillary endothelial cells are immunoreactive for AQP1 (Nielsen et al. 1993); however, cerebral microvessels in the rat express only AQP4 mRNA (Kobayashi et al. 2001). The location of AQP4 at brain-CSF and brain-blood interfaces suggests a role in brain water homeostasis. In addition, AQP4 likely plays a role in cerebral edema, as edema is essentially the loss of water homeostasis in the brain.

AQP4 has been tied to edema formation in a variety of injury models. AQP4 null mice, which are phenotypically normal, show decreased edema formation following both acute hyponatremia and ischemic stroke (Manley et al. 2000). Additionally, when AQP4 expression was disrupted at the astrocytic endfeet, by α-syntrophin deletion, edema volume was decreased following transient cerebral ischemia (Amiry-Moghaddam et al. 2003). Brain regions with increased AQP4 expression have increased rates of edema formation, induced by acute hyponatremia (Amiry-Moghaddam et al. 2004). Expression of AQP4 is also increased after ischemia (Taniguchi et al. 2000) and traumatic brain injury (Sun et al. 2003). In general, increased AQP4 levels seem to cause greater edema formation and decreased or absent

AQP4 levels are protective against cerebral edema. However, more recently it has been suggested that the role of AQP4 in cerebral edema varies with the specific type of edema, vasogenic or cytotoxic (Manley et al. 2004). In animal models of vasogenic edema, AQP4 appears to be protective and aid the resolution of cerebral edema (Papadopoulos et al. 2004). Conversely, in instances where cytotoxic edema is more common, AQP4 expression appears to be maladaptive because it tends to increase cerebral edema formation (Amiry-Moghaddam et al. 2004). Therefore, in eclampsia, which is typically characterized as a vasogenic edema, AQP4 may be more important in resolving cerebral edema formation rather than preventing the initial formation.

Of interest to the study of eclampsia is the role of AQP4 in mediating water movement across the BBB. We have shown that cerebral edema formation is increased in LP rats but not NP rats following acute hypertension, and this is not likely due to increased solute permeability (Euser and Cipolla 2007). Our lab has also shown that AQP4 protein levels are significantly increased in pregnant and postpartum rats versus NP females (Quick and Cipolla 2005). These findings led to the hypothesis that pregnancy acts to increase water permeability (hydraulic conductivity) of the BBB by increased AQP4 expression leading to significantly enhanced edema formation.

1.4. Magnesium Sulfate

1.4.1. Clinical Usage

Magnesium sulfate ($MgSO_4$) has been used since the beginning of the 20th century to treat eclamptic seizures (Lazard 1925) and continues to be used extensively

(Working Group on High Blood Pressure in Pregnancy 1990; Sibai 1990; Witlin and Sibai 1998). Empirical evidence supports the effectiveness of $MgSO_4$ in treating eclamptic seizures (Lazard 1925; Pritchard, Cunningham, and Pritchard 1984; Sibai et al. 1981; Sibai 1990), and recently controlled clinical trials have provided evidence to support these reports (Altman et al. 2002; Chien, Khan, and Arnott 1996; Witlin and Sibai 1998). For eclamptic seizure prophylaxis in preeclamptic women, $MgSO_4$ is superior to phenytoin (an anticonvulsant drug) (Duley and Henderson-Smart 2003; Lucas, Leveno, and Cunningham 1995), nimodipine (a calcium channel blocker with specific cerebral activity) (Belfort et al. 2003), diazepam (Duley and Henderson-Smart 2003), and placebo (Altman et al. 2002). In a multinational trial, $MgSO_4$ reduced the risk of recurrent seizures in eclamptic women by 52% when compared to diazepam and by 67% when compared to phenytoin (The Eclampsia Trial Collaborative Group 1995). The publication of these trials appears to have significantly increased the use of magnesium sulfate versus other anticonvulsants in the United Kingdom and Ireland (Gülmezoglu and Duley 1998). The reported use of magnesium sulfate in preeclampsia has increased from 2% to 40%, and 60% of providers surveyed would now use magnesium as an anticonvulsant for eclamptic women versus in 1992 only 2% of eclamptic women received magnesium sulfate (Douglas and Redman 1994; Gülmezoglu and Duley 1998). Although the effectiveness of $MgSO_4$ in treating and preventing eclampsia has been established, questions still exist as to its safety and mechanism.

There are concerns regarding the possibility of hypermagnesemia toxicity in eclampsia treatment. Normal serum concentrations of Mg^{+2} are 1.5-2.5 mEq/L (1.8-3.0 mg/dL), with one third bound to plasma proteins (Donaldson 1986). Magnesium serum concentrations advocated for the treatment of eclamptic convulsions are 3.5-7 mEq/L (4.2-8.4 mg/dL) (Leveno and Cunningham 1999; Pritchard 1955). Areflexia, particularly loss of the patellar reflex, has been observed at 8-10 mEq/L, and respiratory paralysis at >13 mEq/L (Donaldson 1986; Pritchard, Cunningham, and Pritchard 1984). Progressively higher serum magnesium levels can ultimately lead to cardiac arrest (Donaldson 1986; McCubbin et al. 1981). Reports suggest that in some patients eclamptic seizures do not cease even with elevated levels of $MgSO_4$ (Pritchard, Cunningham, and Pritchard 1984; Sibai et al. 1981; Sibai et al. 1984), suggesting that $MgSO_4$ is not effective in treating all cases of eclampsia.

Though the use of $MgSO_4$ is wide-spread and effective, its mechanism of action remains unclear. Several possible mechanisms of action have been proposed, and are further discussed in following sections. $MgSO_4$ may act as a vasodilator, with actions either peripherally or in the cerebral circulation, to relieve vasoconstriction. Alternatively, $MgSO_4$ may protect the BBB and thereby decrease cerebral edema formation. Further, $MgSO_4$ may treat eclamptic seizures through a central anticonvulsant action. Aims 3 and 4 of this dissertation were designed to better understand the effects of $MgSO_4$ during normal pregnancy. The vasodilatory action on *in vitro* cerebral and systemic resistance arteries was investigated and is described in

Chapter 2, and the effect of $MgSO_4$ treatment on *in vivo* BBB permeability during acute hypertension is described in Chapter 4.

1.4.2. Magnesium-induced Vasodilation

Magnesium is a unique calcium antagonist as it can act on all types of calcium channels in vascular smooth muscle, and subsequently may lower peripheral and cerebral vascular resistance, relieve vasospasm, and decrease ABP (Altura et al. 1987). The vasodilatory effect of $MgSO_4$ has been investigated in a wide variety of vessels. For example, both *in vivo* and *in vitro* animal studies have shown that it is a vasodilator of the aorta (Aloamaka et al. 1993; Longo et al. 2001), mesenteric arteries (Altura et al. 1987; Euser and Cipolla 2005; Nishio et al. 1989; Villamor et al. 1996), skeletal muscle arteries (Altura et al. 1987), uterine arteries (Nelson and Suresh 1991), and cerebral arteries (Altura et al. 1987; Euser and Cipolla 2005; Perales et al. 1991). However, the importance of magnesium-induced vasodilation in the treatment and prevention of eclampsia is not completely understood.

The theory of cerebrovascular vasospasm as the etiology of eclampsia seemed to be reinforced by TCD studies which suggested that $MgSO_4$ treatment caused dilation in the cerebral circulation (Belfort and Moise Jr. 1992; Belfort, Saade, and Moise Jr. 1993; Naidu et al. 1996) as well as in large cerebral arteries in animal studies (Perales et al. 1991). However, a vasodilator such as $MgSO_4$ would seem to be a paradoxical treatment choice for eclamptic encephalopathy. In order to clarify the cerebral effect of $MgSO_4$, we performed *in vitro* studies to compare the effect of $MgSO_4$ treatment on

resistance arteries from cerebral and mesenteric circulations. This work is presented in detail in Chapter 2. In summary, we found that $MgSO_4$ caused a concentration-dependent vasodilatation in both cerebral and mesenteric resistance arteries; however the mesenteric arteries were significantly more sensitive to $MgSO_4$, particularly during pregnancy (Euser and Cipolla 2005). Our results of a modest vasodilatory effect in the cerebral circulation are consistent with other findings that $MgSO_4$ treatment caused no significant change in CBF, large cerebral artery diameter, or mean MCA velocity as determined by MRI (Hatab, Zeeman, and Twickler 2005) and TCD (Belfort, Saade, Yared et al. 1999; Sherman et al. 2003). The effects of $MgSO_4$ as an eclamptic seizure prophylactic may be more closely related to an effect on peripheral vascular resistance and lowering of systemic blood pressure than to a direct effect on CBF.

Reports of the effects of $MgSO_4$ treatment on ABP have been mixed. Hypotensive effects have been noted in various studies particularly with bolus injections (Belfort, Saade, and Moise Jr. 1993; Pritchard 1955; Scardo, Hogg, and Newman 1995); however the duration of decreased blood pressure was varied. In pregnant rats with L-NAME-induced hypertension, magnesium treatment resulted in significantly lower blood pressures at term and better neonatal outcomes (Standley, Batia, and Yueh 2006). It has been cautioned that magnesium should not be considered primarily an anti-hypertensive agent, and there are other drugs better suited for that purpose in eclampsia (Leveno and Cunningham 1999).

Several reports have suggested that gestation may influence vascular reactivity to $MgSO_4$ and that this sensitivity varies with vascular bed (Aloamaka et al. 1993;

Euser and Cipolla 2005; Longo et al. 2001; Nelson and Suresh 1991). Human uterine arteries from pregnant patients are three-fold more reactive to $MgSO_4$ than uterine arteries from non-pregnant patients (Nelson and Suresh 1991). In aortic smooth muscle from pregnant and non-pregnant rats, both greater and less sensitivity to magnesium-induced vasodilation have been shown based on the preconstriction agent used, suggesting that pregnancy may differentially affect receptor versus voltage-operated calcium channels (Aloamaka et al. 1993). In another study of rat aortic rings, the effect of magnesium was dependent on gestation such that vasodilation was lower at term than during late pregnancy (Longo et al. 2001). We have found that while mesenteric resistance arteries showed no change in sensitivity with gestation, posterior cerebral resistance arteries from LP and postpartum (PP) animals were significantly less sensitive to $MgSO_4$ versus those from NP animals (Euser and Cipolla 2005). This may be due to changes in the cerebrovascular vaodilatory mechanisms that have been demonstrated during pregnancy and the postpartum state (Cipolla, Vitullo, and McKinnon 2004).

$MgSO_4$ may have other effects within the vasculature that could also explain its effectiveness in eclampsia. Magnesium may act by stimulating production of prostacyclin by the endothelial cells causing vasodilation (Watson et al. 1986), or by inhibiting platelet aggregation (Ravn et al. 1996; Watson et al. 1986). In patients with pregnancy-induced hypertension, $MgSO_4$ treatment significantly decreased circulating levels of angiotensin-converting enzyme (Goldkrand and Fuentes 1986). These actions

may attenuate the endothelial dysfunction associated with eclampsia (Easton 1998; Khan et al. 2005; Roberts et al. 1989).

1.4.3. Effects on the Blood-brain Barrier

As described in detail in section 1.3.1, the cerebral endothelium is specialized and forms the BBB. Briefly, the unique features of the BBB include intercellular TJ between endothelial cells, a lack of capillary fenestrations, and a low rate of pinocytosis (transcellular transport) (Betz 1997; Hawkins and Davis 2005). Disruption of the BBB can result in vasogenic edema formation, an important component in the clinical picture of eclampsia (Kaplan 2006; Zunker et al. 1995). Recently, decreased BBB permeability with $MgSO_4$ treatment has been reported in a variety of animal models of BBB disruption including traumatic brain injury (Esen et al. 2003), septic encephalopathy (Esen et al. 2005), hypoglycemia (Kaya et al. 2001), and mannitol injection (Kaya et al. 2004). Our work has also shown decreased BBB permeability during acute hypertension in LP rats treated with $MgSO_4$ (Euser and Cipolla 2007). This work is presented in Chapter 4 of this manuscript. Various studies have also shown that $MgSO_4$ decreases cerebral edema formation after injury (Esen et al. 2003; Feldman et al. 1996; Ghabriel, Thomas, and Vink 2006; Kaya et al. 2004; Okiyama et al. 1995). However, it is not yet clear how magnesium protects the BBB and decreases edema formation.

Several mechanisms of action have been proposed to explain the neuroprotective effects of magnesium. Magnesium is a calcium antagonist that acts both intracellularly

and extracellularly (Fawcett, Haxby, and Male 1999), and may act directly on cerebral endothelial cells. It is possible that by acting as a calcium antagonist at the level of the endothelial cell actin cytoskeleton, magnesium opposes paracellular movement of solutes through the TJ. Alternatively, pinocytosis can be induced by acute hypertension and contributes to increased BBB permeability during acute hypertension. If magnesium is somehow able to decrease pinocytosis caused by acute hypertension, it may restrict the movement of water and solutes into the brain and limit edema formation thereby improving clinical outcomes in eclampsia. It has been suggested that the movement of large molecules such as EB across the BBB may implicate transcellular versus paracellular transport (Mayhan and Heistad 1985). In our study, we found that $MgSO_4$ treatment significantly decreased BBB permeability during acute hypertension to the large solute EB but not to the much smaller solute sodium fluorescein, suggesting transcellular transport (Euser and Cipolla 2007). Further studies to investigate the effects of $MgSO_4$ on the BBB may provide important information regarding the benefits of $MgSO_4$ for eclampsia treatment and prophylaxis.

Magnesium sulfate may also act to limit cerebral edema formation through an effect on aquaporin expression. Brain edema has been associated with an up-regulation of AQP4 (Papadopoulos and Verkman 2005; Taniguchi et al. 2000), and it has been suggested that $MgSO_4$ treatment attenuates cerebral edema formation by down-regulating AQP4 expression in astrocytes (Ghabriel, Thomas, and Vink 2006). This concept is particularly interesting in light of observations of increased AQP4 expression during pregnancy (Quick and Cipolla 2005), and the subsequent hypothesis

that increased aquaporin expression during pregnancy may increase the vulnerability of the cerebral circulation to damage during acute hypertension. However, in more recent studies $MgSO_4$ treatment in normotensive LP rats did not affect AQP4 protein expression versus untreated LP rats (unpublished results). In this study AQP4 expression was determined in naïve rat brains, and it is possible that $MgSO_4$ may affect AQP4 following an acute injury to the brain, such as a hypertensive insult. Magnesium treatment may also cause a redistribution of AQP4 within the brain tissue, an outcome that was not assessed by these studies.

1.4.4. Possible Anticonvulsant Activity

There is controversy regarding the use of $MgSO_4$ treatment for neurological conditions, such as eclamptic seizures. Concerns have been raised that $MgSO_4$ treatment may mask the outward signs of convulsions through its action at the neuromuscular junction without treating the cause of the seizure in the central nervous system (Donaldson 1986; Kaplan et al. 1988). Dose-related depression of neuromuscular transmission has been shown in preeclamptic women receiving traditional $MgSO_4$ therapy (Ramanathan et al. 1988). Studies have also shown that there is little to no change in EEGs obtained during $MgSO_4$ treatment, and minimal signs of central nervous system depression in both normal (Somjen, Hilmy, and Stephen 1966) and eclamptic patients (Sibai et al. 1984), and in animals (Koontz and Reid 1985). However, clinical trials have demonstrated the efficacy of $MgSO_4$ in the treatment and prevention of eclamptic seizures versus more traditional anticonvulsant

drugs (Lucas, Leveno, and Cunningham 1995; The Eclampsia Trial Collaborative Group 1995).

The possible anticonvulsant activity of magnesium may be related to its role as a N-methyl-D-aspartate (NMDA) receptor antagonist (Goldman and Finkbeiner 1988; Hallak et al. 1994; Lipton and Rosenberg 1994). For comparison, epileptic seizures are thought to be mediated at least in part by stimulation of glutamate receptors, such as the NMDA receptor (Dingledine, Hynes, and King 1986; Lipton and Rosenberg 1994). In rats, peripheral magnesium treatment results in a resistance to both electrically stimulated (Hallak et al. 1992) and NMDA-induced hippocampal seizures (Cotton et al. 1993). In addition, peripheral treatment with $MgSO_4$ causes a significant reduction in the NMDA receptor binding capacity in the brain (Hallak et al. 1994). Studies in animals have also shown that $MgSO_4$ reduces epileptic seizure activity (Borges and Gucer 1978), though these findings have been challenged due to inadequate controls (Koontz and Reid 1985).

Magnesium ions must cross the BBB in order to elicit a central anticonvulsant effect. It has been demonstrated in animal studies that $MgSO_4$ can cross the intact BBB and enter the central nervous system in correlation with the level of serum hypermagnesemia (Hallak et al. 1992). Interestingly, seizure activity increases the movement of magnesium into the brain (Hallak et al. 1992). Human studies have also shown small but significant increases in CSF concentrations of $MgSO_4$ after systemic administration (Pritchard 1955; Thurnau, Kemp, and Jarvis 1987). Conversely, other work has suggested that the BBB prevents changes in brain and CSF magnesium

concentrations (Hilmy and Somjen 1968). However, this same group later suggested that even a small amount of magnesium in the central nervous system may suppress cortical neuronal activity (Kato and Somjen 1969). The possibility remains that acute hypertension and/or acute convulsions could cause BBB disruption which could permit $MgSO_4$ to enter the brain parenchyma and act as an anticonvulsant during eclampsia. It is likely that the prophylactic effect of $MgSO_4$ in the prevention of eclamptic seizures is multi-factorial, encompassing both vascular and neurological mechanisms.

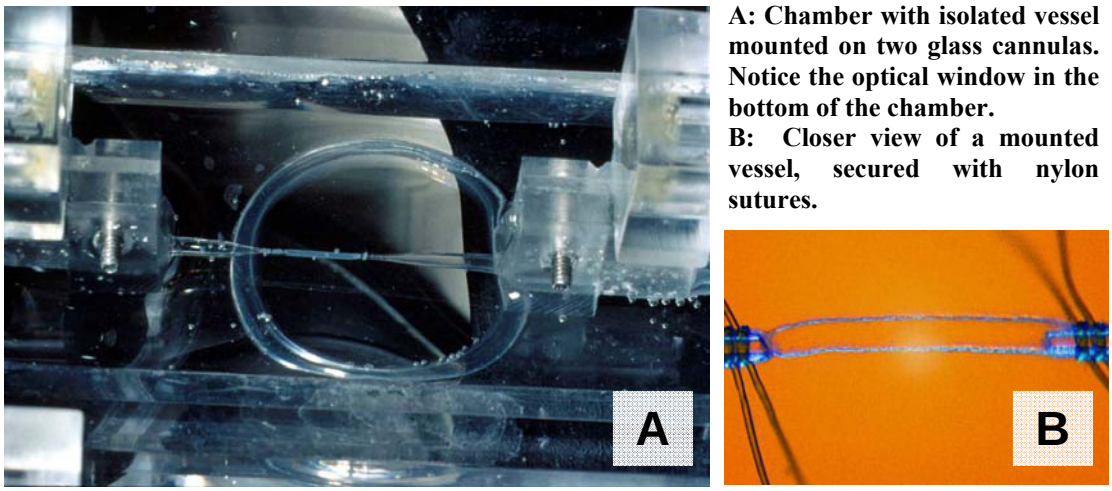
1.5. Methodology

1.5.1. Rat Model of Pregnancy

All experiments were conducted using Sprague Dawley female rats housed in the University of Vermont animal care facility. The rat is an appropriate model of pregnancy because of similar hemichorial placentation (Pijnenborg et al. 1981) and cardiovascular adaptations (Gilson, Mosher, and Conrad 1992; Barron 1987) as human pregnancy. In addition, rats have a similar cerebrovascular architecture to humans (Edvinsson and MacKenzie 2002), and rats develop HTE (Smeda and Payne 2003). For NP experiments, virgin animals were used. All LP experiments were performed in primiparous animals on day 19 to 21 of a 22 day gestation. The rats were studied during this time period in order to best focus on late gestation when eclampsia often occurs (Roberts et al. 2003). For the *in vitro* isolated vessel experiments PP animals were also studied, and they were used on the third day postpartum following their first pregnancy.

1.5.2. Arteriograph System

In vitro studies utilized the pressurized arteriograph system. This system allows the artery to remain pressurized and perfused within a solution bath and lumen diameter can be measured directly. Our lab has considerable experience using this system to study the myogenic activity and passive structural properties of small cerebral arteries (Cipolla, DeLance, and Vitullo 2006; Cipolla, Vitullo, and McKinnon 2004; Euser and Cipolla 2005). Vessels were dissected from animal tissue and mounted and secured on glass cannulas within the arteriograph chamber, as shown in Figure 3. A dual-chamber arteriograph system can be used to study two vessels simultaneously, as done in this project. The proximal cannulas are attached to an in-line pressure transducer which allows the intravascular pressure to be maintained at a constant pressure or changed at a variable rate. A state of no flow through the vessels was obtained by closing the distal cannulas. The lumen diameters were measured through optical windows in the bottom of the arteriograph chambers, also seen in Figure 3, using an inverted microscope with



A: Chamber with isolated vessel mounted on two glass cannulas. Notice the optical window in the bottom of the chamber.
B: Closer view of a mounted vessel, secured with nylon sutures.

Figure 3: Arteriograph chamber and mounted artery, images courtesy of Dr. Marilyn J. Cipolla, PhD

an attached video camera and monitor connected to a video dimension analyzer (VDA). The VDA and pressure transducer output signals were sent to a computer via a data acquisition system, providing a visual representation of lumen diameter, similar to a chart recorder.

The pressurized arteriograph system is advantageous because it allows vessel diameter to be directly measured in response to drugs and solutions or different intraluminal pressures. Pousielle's law determines CVR, such that $\mathbf{R = (8\eta \mathit{l})/(\pi r^4)}$, where **R** represents resistance to blood flow, **η** represents blood viscosity, ***l*** is the length of the vessel, and **r** is radius of the vessel (Hurn and Traystman 1997). CBF is dependent of CPP and CVR, such that **CBF = CPP/CVR** (Paulson et al. 1989; Skinhøj 1977). Because it is related inversely to CVR, CBF is proportional to vessel radius to the 4^{th} power and small changes in luminal diameter can lead to considerable changes in flow (Ku and Zhu 1993). Through observation of isolated and pressurized vessels *in vitro* under different circumstances, the global effect of such circumstances on CBF may be predicted. However, CBF autoregulation is a complex interaction of endothelial, neuronal, and metabolic influences that cannot be adequately reproduced *in vitro*, and for this reason the model of HTE was used to study CBF *in vivo*.

1.5.3. Model of Hypertensive Encephalopathy in Pregnancy

In order to investigate CBF autoregulation during pregnancy *in vivo*, we adapted a model of acute hypertension so that CBF could be measured while increasing ABP. Briefly, CBF is recorded continuously during an acute infusion of phenylephrine (PE)

to raise ABP sufficiently to cause autoregulatory breakthrough. The resulting data were then analyzed in order to determine a pressure versus flow curve and the pressure of autoregulatory breakthrough. This model could best be described as HTE during pregnancy, which we believe to be similar to eclampsia. This same model has been used by many different groups to examine cerebral hemodynamics and the effects of acute hypertension on BBB permeability and vasogenic edema formation in male animals (Hatashita, Hoff, and Ishii 1986; Hernandez, Brennan, and Bowman 1978; MacKenzie, Strandgaard et al. 1976; Mayhan, Faraci, and Heistad 1986; Talman and Nitschke Dragon 1995). In this dissertation, this model has proven useful to determine CBF autoregulation and the upper limit of autoregulation in pregnant rats as well as determine the effect of acute hypertension on BBB permeability and cerebral edema formation. This model was also used in this project to determine the effect of magnesium sulfate treatment on BBB permeability during acute hypertension. Several choices were made in adapting this model for use in our laboratory, and the advantages and possible caveats of these choices is discussed below.

A laser Doppler probe was used to continuously measure CBF transcranially. Laser Doppler flowmetry measures changes in CBF and the laser Doppler signal must be normalized to obtain a relative CBF (rCBF) to allow for interanimal comparison. This technique is limited because it is nonquantitative and multiple probes are required to determine regional blood flows (Iadecola 1997). Other techniques, such as microsphere infusion, can be used to establish absolute CBF measurements, however these studies are limited because they do not allow for continuous measurement of CBF

(Iadecola 1997). Continuous data collection is a distinct advantage of the laser Doppler technique (Iadecola 1997). For the studies within this project, the probe was positioned on the animal's skull over the MCA perfusion domain as previously described (Smeda, VanVliet, and King 1999).

For some studies, phenylephrine (PE) was used to acutely increase ABP. Phenylephrine is an α-adrenergic agonist, and is commonly used to study the effects of hypertension on the cerebral circulation *in vivo* for several reasons (Mayhan, Faraci, and Heistad 1988). Sympathomimetic agents, such as PE, contract pial vessels *in situ* and *in vitro* (Edvinsson and Krause 2002). However, α-adrenergic agonists do not readily pass the BBB (Hardebo and Owman 1980; Oldendorf 1971), and CBF is minimally affected by intravenous infusion if the BBB is intact (Tamaki and Heistad 1986; MacKenzie, McCulloch et al. 1976). Thus, PE increases peripheral vascular resistance without acting directly on cerebral vessels. In addition, when ABP elevations are prevented, intravenous infusions of PE or other α-adrenergic agonists do not have direct effects on pial arteriolar diameter (Kontos et al. 1981), cerebral vascular resistance in large or small arteries (Tamaki and Heistad 1986), or BBB permeability (Mayhan and Heistad 1985, 1986). Some studies of the cerebral circulation and acute hypertension have used angiotensin II as a pressor agent. However, we specifically chose not to use angiotensin II because refractory responses to angiotensin II have been shown during pregnancy (Gant et al. 1973). Importantly, no difference in CBF changes to the cerebrum were observed using different methods to increase ABP, aortic obstruction, aortic obstruction with angiotensin II infusion, or aortic obstruction with

norepinephrine infusion (Baumbach and Heistad 1985; Baumbach, Mayhan, and Heistad 1986).

The goal of this proposal was to study the effect of normal pregnancy on cerebrovascular parameters. For this reason, this model was appropriate because it used normal, healthy pregnant animals. However, other model systems may better represent the symptoms and endothelial dysfunction of preeclampsia and eclampsia (Khan et al. 2005; Working Group on High Blood Pressure in Pregnancy 1990; Roberts et al. 1989). The reduced uterine perfusion pressure model involves surgically reducing blood flow in the uterine and ovarian arteries and has been shown to induce hypertension, proteinuria, and intrauterine growth restriction (Alexander et al. 2001). While our results have shown that autoregulation does not differ with normal gestation (Euser and Cipolla 2007), circulating factors and/or oxidative damage as part of eclampsia could cause greater endothelial dysfunction and affect either the upper limit of autoregulation or cerebral edema formation. For this project, we believed that it was important to understand how normal pregnancy may predispose the brain to hyperperfusion and edema formation before investigating a disease state. Future experiments could utilize models of preeclampsia such as the reduced uterine perfusion pressure.

1.5.4. Cerebral Edema Quantification

Brain edema is the abnormal accumulation of fluid in brain parenchyma producing a volumetric enlargement of the tissue (Klatzo 1987). Different techniques

can be used to quantify brain water content and thus measure cerebral edema formation. For these studies, we used the ratio of wet and dry tissue weights to determine brain water content, a measure of cerebral edema (Schwab, Bauer, and Zwiener 1997). Briefly, the brain was quickly removed from the animal, weighed wet, and transferred to an oven for drying at 100° C for 24 hours, after which the brain was weighed again dry. Brain water content (in percent) was determined by the following formula: **$((weight_{wet} - weight_{dry}) \div weight_{wet}) * 100$**; where **$weight_{wet}$** is the weight of the brain immediately after removal from the skull, and the **$weight_{dry}$** is the weight of the brain after drying.

In our experience, the differences seen between groups in cerebral edema formation using a ratio of wet to dry weights have been small. However, other methods of cerebral edema quantification, such as gravimetry, are not necessarily preferable. Gravimetry uses a calibrated column of bromobenzene and kerosene to determine the specific gravity of a small piece of brain tissue, and thus the percent brain water content (Marmarou et al. 1978). Gravimetry has been used previously in our laboratory and was found to produce variable results. In addition, others have shown no significant difference in estimated brain water content determined by wet and dry weights versus gravimetry (Schwab, Bauer, and Zwiener 1997).

1.5.5. Blood-brain Barrier Permeability Model

In order to determine BBB permeability *in vivo*, the model of HTE in pregnancy was further adapted to include an *in situ* brain perfusion model. Dye tracers were

infused intravenously as an additional step prior to induction of acute hypertension. Further procedures were completed as before, however just before the experiment was ended, the animal was perfused with lactated Ringer's solution and the right side of the heart opened in order to flush dye from the cerebrovascualture. Quantification of the dye present in the brain parenchyma was determined using fluorescence spectrophotometry of the supernatant from homogenized and centrifuged tissue samples.

Dye tracers have been used to study the BBB since the earliest studies (Byrom 1954; Ehrlich 1885; Goldmann 1913), and are still used extensively as markers of BBB permeability (Auer 1978; Johansson et al. 1970; Kozler and Pokorny 2003; Mayhan and Heistad 1985; Wolman et al. 1981). Two different dye tracers were used for this dissertation, Evan's blue (EB) and sodium fluorescein (NaFl). Evan's blue binds to albumin and is commonly used as a marker of BBB permeability (Kaya et al. 2001; Wolman et al. 1981). The passage of EB across the BBB suggests that large plasma proteins are passing into the brain tissue. It has been suggested that the movement of large molecules, such as albumin and EB, across the BBB implicates transcellular versus paracellular transport (Mayhan and Heistad 1985). Conversely, NaFl was used to trace the movement of ions across the BBB, and was found to be a less sensitive and more variable marker of BBB permeability.

1.6. Project Aims and Hypotheses

The overall goal of this project was to understand how pregnancy affects CBF autoregulation and edema formation in response to acute hypertension. The permeability of the BBB during acute hypertension was also investigated as this is a principal mechanism of vasogenic edema formation. An *in vivo* model of HTE was used to determine autoregulatory curves and the pressure at which breakthrough occurs in both NP and LP female rats (Aim 1), as well as cerebral edema formation and BBB permeability (Aim 2). Because pregnancy is known to be a time of elevated NO (Sladek, Magness, and Conrad 1997; Williams et al. 1997), we also investigated the effect of NOS inhibition, induced by L-NAME treatment, on CBF autoregulation and cerebral edema formation during acute hypertension (Aims 1 and 2, respectively). This work is presented in Chapter 3.

Another goal of this project was to investigate the effects of $MgSO_4$ on the cerebral circulation during pregnancy. Magnesium is widely used to both treat and prevent eclamptic seizures (Witlin and Sibai 1998; Sibai 1990; Working Group on High Blood Pressure in Pregnancy 1990), although its mechanism of action is not clear. Because of the theorized hyperperfusive etiology of eclampsia, the known action of $MgSO_4$ as a vasodilator seems paradoxical. Thus, Aim 3 of this project explored the effect of $MgSO_4$ on cerebral and mesenteric resistance arteries, important contributors to CBF and peripheral vascular resistance, respectively. In addition, we determined if the response to $MgSO_4$ varied with gestation. We hypothesized that there may be a differential sensitivity to $MgSO_4$ in the cerebral versus the systemic vasculature, and

that the systemic vasculature would be more sensitive to $MgSO_4$. The effect of $MgSO_4$ was determined *in vitro* by directly measuring luminal diameters, and this work is presented in Chapter 2.

The results of Aim 3, together with the work of others, has shown limited effects of $MgSO_4$ on cerebral arterial diameter and CBF (Belfort, Saade, Yared et al. 1999; Euser and Cipolla 2005; Hatab, Zeeman, and Twickler 2005; Sherman et al. 2003). Therefore, we subsequently hypothesized that in eclampsia $MgSO_4$ may protect the brain by decreasing BBB disruption during acute hypertension. Protection of the BBB with $MgSO_4$ treatment has been shown in other conditions that may cause cerebral edema (Esen et al. 2003; Esen et al. 2005; Kaya et al. 2001; Kaya et al. 2004). Aim 4 determined the effect of $MgSO_4$ on *in vivo* BBB permeability during acute hypertension in LP rats using the same model of HTE used in Aims 1 and 2. This work is presented in Chapter 4. A summary of this project's aims and hypotheses follows below.

Aim 1: To determine CBF autoregulation and autoregulatory breakthrough in control NP and LP rats, and to investigate the role of NO in mediating autoregulatory breakthrough.

Hypotheses: **a)** Autoregulatory breakthrough in LP rats will occur at lower pressures versus NP rats.

b) NOS inhibition will increase the pressure of autoregulatory breakthrough.

Aim 2: To determine if pregnancy is associated with increased cerebral edema formation during autoregulatory breakthrough and the role of NO in mediating this outcome. In addition, the effect of acute hypertension on *in vivo* BBB permeability was determined in both NP and LP animals.

Hypotheses: **a)** There will be greater BBB permeability and cerebral edema formation after autoregulatory breakthrough in LP versus NP animals.

b) Cerebral edema formation will be less with NOS inhibition in both NP and LP rats.

Aim 3: To determine the effect of $MgSO_4$ on lumen diameter in cerebral and mesenteric resistance arteries *in vitro* from NP, LP, and postpartum (PP) animals.

Hypothesis: **a)** There may be a differential sensitivity to $MgSO_4$, such that systemic resistance arteries are more sensitive to the effects of $MgSO_4$ than the cerebral resistance arteries.

Aim 4: To determine the effect of $MgSO_4$ treatment on *in vivo* BBB permeability in LP rats during acute hypertension.

Hypothesis: **a)** Treatment with $MgSO_4$ will decrease BBB permeability in treated versus untreated LP rats.

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CHAPTER 2: RESISTANCE ARTERY VASODILATION TO MAGNESIUM SULFATE DURING PREGNANCY AND THE POSTPARTUM STATE

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Abstract

This study compared the vasodilatory response to magnesium sulfate ($MgSO_4$) of cerebral and mesenteric resistance arteries and determined if the response varied between different gestational groups. Third-order branches (<200 μm) of the posterior cerebral (PCA) and mesenteric arteries (MA) were dissected from non-pregnant (NP, n=6), late pregnant (LP, day 19, n=6), and postpartum (PP, d3, n=6) Sprague-Dawley rats. A concentration-response curve was performed by replacing the low $MgSO_4$ (1.2 mM) HEPES buffer solution with increasing concentrations of $MgSO_4$ (4, 6, 8, 16, 32 mM) and measuring lumen diameter at each concentration. All groups exhibited concentration-dependent dilation to $MgSO_4$, decreasing the amount of tone in the vessels. However, MA were significantly more sensitive to $MgSO_4$ than PCA. While there was no difference in response between different gestational groups in MA, the PCA from the LP and PP groups showed a significantly diminished response to $MgSO_4$. The percent dilation at 32 mM $MgSO_4$ for PCA vs. MA in NP, LP and PP animals was: 36±2 vs. 51±7% ($p<0.05$); 19±9 vs. 54±6% ($p<0.01$ vs. PCA and NP) and 12±5 vs. 52±11% ($p<0.01$ vs. PCA and NP). These results demonstrate that $MgSO_4$ is a vasodilator of small resistance arteries in the cerebral and mesenteric vascular beds. The refractory responses of the PCA in LP and PP groups demonstrate changes in the cerebrovascular vasodilatory mechanisms with gestation. The greater sensitivity of the MA to $MgSO_4$-induced vasodilation suggests that the prophylactic effect of $MgSO_4$ on eclamptic seizures may be more closely related to the lowering of systemic blood pressure than to an effect on cerebral blood flow.

Keywords: Eclampsia, Cerebral Arteries, Mesenteric Arteries, Rat

Introduction

Hypertensive disorders of pregnancy, including preeclampsia and eclampsia, affect ~8% of all pregnancies (19), however, the physician caring for a preeclamptic patient has few treatment options available to choose from. Magnesium sulfate ($MgSO_4$) has been used empirically since the beginning of the 20th century to prevent seizures, and continues to be used extensively as an eclampsia prophylactic (15, 22). Though the use of $MgSO_4$ is wide-spread and effective (2, 15, 16), its mechanism of action has historically been poorly understood. For example, studies have shown that it is a vasodilator of large, conduit arteries such as the aorta (1, 14) and mesenteric rings (21), however, its effects on the small resistance arteries that control systemic blood pressure and vascular resistance are not clear.

Resistance arteries (<200 μm in diameter) operate in a state of partial constriction, or tone, and are generally the site of vascular resistance (9). This intrinsic tone also provides a set point from which arteries can constrict or dilate to control blood flow (9, 20). Thus, the small resistance arteries have a great influence on peripheral vascular resistance and mean arterial pressure (20). In addition, because flow is dependent inversely on vessel diameter to the 4th power, small changes in luminal diameter lead to measurable changes in flow (12). It is therefore possible that as a vasodilator $MgSO_4$ prevents eclamptic seizures by lowering peripheral vascular resistance.

It was previously thought that eclampsia was due to vasospasm of cerebral vessels and the resultant ischemia was the root of the neurological complications, which include headaches, nausea, vomiting, visual disturbances, and convulsions (23). This etiology seemed to be reinforced by studies that showed $MgSO_4$ dilated the middle cerebral artery (3, 4, 18). However, more recent evidence suggests that eclampsia is similar to hypertensive encephalopathy in which an acute elevation in blood pressure overcomes the myogenic vasoconstriction, causing forced dilatation of cerebral vessels, hyperperfusion, and edema (5, 8, 17, 23). Under these conditions, it would be paradoxical that a vasodilator, such as $MgSO_4$, would be an effective prophylactic since it would cause greater hyperperfusion and promote further edema. We therefore hypothesized that there is a differential sensitivity to $MgSO_4$ between the cerebral and systemic resistance vessels such that the systemic circulation is more sensitive to $MgSO_4$ leading to a reduction in peripheral vascular resistance prior to vasodilation of cerebral vessels. To date, no studies have specifically compared the differential response of resistance arteries to $MgSO_4$ from cerebral and systemic circulations, important contributors to cerebral blood flow regulation and peripheral vascular resistance, respectively.

The goal of this study was to investigate the effects of $MgSO_4$ on small, myogenic resistance arteries that play an integral role in the modulation of peripheral vascular resistance (mesenteric) and control cerebral blood flow (posterior cerebral). The effect of $MgSO_4$ was evaluated by directly measuring the luminal diameter of isolated and pressurized vessels, a powerful indicator of flow.

Materials and Methods

Animals

Female Sprague-Dawley rats (Harlan) were used for all experiments, weighing 250-350 g. The animals were housed in the Animal Care Facility, an AAALAC accredited facility. All procedures were approved by the institutional animal care and use committee (IACUC) at the University of Vermont. Three groups of animals were compared, virgin non-pregnant (NP, n=6), late pregnant (LP, day 19, n=6), and postpartum (PP, day 3, n=6). Both the late pregnant and the postpartum animals were studied in association with their first pregnancy.

Preparation of arterial segments and pressurized arteriograph system

The animals were decapitated following anesthesia with halothane/oxygen, and the brain quickly removed and transferred to cold physiologic salt solution (HEPES buffer) at pH 7.4±0.03. A third-order branch of the posterior cerebral artery (PCA) was dissected and mounted on glass cannulas within a dual-chamber arteriograph and secured with nylon suture, as previously described (6). A branch of the PCA was chosen because the symptoms of eclampsia are focused in the occipital lobe and posterior region of the brain (10). A section of the small intestine was also quickly removed from the same animal so that experiments were paired. A third-order branch of the mesenteric artery (MA) was then dissected, and similarly mounted on glass cannulas in the second chamber. Therefore, one PCA and one MA were studied simultaneously. Both proximal cannulas were attached to an in-line pressure transducer with a controller that allowed intravascular pressure to be maintained at a constant

pressure or changed at a variable rate. The distal cannulas remained closed so that there was no flow through the arteries. Using an inverted microscope with an attached video camera and monitor connected to a video dimension analyzer (VDA), the lumen diameters were measured through optical windows in the bottom of the arteriograph chambers. A data acquisition system was used to send the VDA and pressure output to a computer, which provided visualization of the vessels' changing diameters, similar to a chart recorder.

Experimental protocol

Both vessels were equilibrated for one hour at 50 mmHg in a 1.2 mM $MgSO_4$ HEPES buffer solution. Intravascular pressure was then increased to 75 mmHg, during which time both vessels developed spontaneous tone. However, because the amount of tone was less in the MA, it was precontracted with phenylephrine (1×10^{-7} to 5×10^{-7} mM) until it attained a diameter of similar magnitude to that of the PCA. Both vessels were then exposed to increasing concentrations of $MgSO_4$ and the inner lumen diameter was recorded at each concentration (4, 6, 8, 16, and 32 mM). (The therapeutic range of $MgSO_4$ for seizure prophylaxis is 4-8 mg/dL (1, 9).) The vessels were then washed with 32 mM $MgSO_4$ HEPES solution. A single dose of papaverine (0.1 mM) was added to each bath to obtain fully relaxed diameters. While exposed to papaverine, pressure was reduced from 150 mmHg to 10 mmHg and lumen diameter measured at each pressure (150, 125, 100, 75, 50, 40, 30, 20, and 10 mmHg).

Data calculations

Percent change in diameter was calculated as the difference in diameter from the vessel diameter at the baseline $MgSO_4$ concentration of 1.2 mM following the equation: $((\Phi - \Phi_{low\ mag})/\Phi_{low\ mag})\times 100$ where Φ = diameter at the respective concentration and $\Phi_{low\ mag}$ = diameter in 1.2 mM $MgSO_4$. Percent constriction was calculated in both low magnesium (1.2 mM $MgSO_4$) and high magnesium (32 mM $MgSO_4$) with respect to the vessel's diameter in papaverine using the formula: $(1-(\Phi/\Phi_{papav})) \times 100$ where Φ = vessel diameter and Φ_{papav} = diameter in papaverine. All data are from vessels pressurized at 75 mmHg.

Drugs and solutions

HEPES, papaverine, phenylephrine, and magnesium sulfate were all purchased from Sigma. Papaverine (10^{-2} M) and phenylephrine (10^{-3} M) stock solutions were made weekly and stored at 4°C. All vessel experiments were conducted in a physiologic salt solution composed of (mM): NaCl (142.0), KCl (4.7), $MgSO_4$ (1.2), EDTA (0.50), $CaCl_2$ (2.8), HEPES (1.0), KH_2PO_4 (1.2), and glucose (5.0). A stock solution of 80 mM $MgSO_4$ was made daily in HEPES physiologic salt solution.

Statistical analysis

Data are expressed as mean ± SE. The number of animals in each group was the n-value. One MA and one PCA were taken from each animal. The differences in reactivity over gestation were determined using a one-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls test for multiple comparisons. The difference between the vessels (MA vs. PCA) within gestational groups was determined

using a paired t-test. Differences in constriction at the various concentrations were determined using a repeated measure ANOVA.

Results

Table 1 shows the percent constriction of the vessels at different stages of gestation for PCA and MA in both low (1.2 mM) and high (32 mM) concentrations of $MgSO_4$ with respect to papaverine. The percent constriction was significantly less in high $MgSO_4$ vs. low $MgSO_4$ concentrations in both vascular beds, demonstrating that $MgSO_4$ caused dilation. In addition, there was significantly less spontaneous tone in the PP vs. NP PCA in 1.2 mM $MgSO_4$.

Figures 1 and 2 show the concentration-response curves to $MgSO_4$, in both PCAs and MAs respectively, in all gestational groups. It is notable that the concentration-dependent response of the PCA was dampened in the LP and PP animals (Figure 1). It is unlikely that this relates to the diminished tone in these vessels because the calculation normalizes the response to the start diameter. The dilation of the MA was similar between all groups (Figure 2).

Across all gestational groups, the MAs were more sensitive to $MgSO_4$ than PCAs from the same animals at all concentrations. These results are shown in Figures 3, 4, and 5, each figure showing a different gestational stage. In both LP and PP animals, there was a significant difference in sensitivity between the two vascular beds (Figures 4 and 5). For the NP animals (Figure 3), though a difference between vascular beds was observed, it is not statistically significant at all concentrations.

Discussion

The results of this study demonstrate that $MgSO_4$ has a concentration-dependent vasodilatory effect on resistance arteries of the cerebral and mesenteric vascular beds. However, the sensitivity of this response was dependent on the vascular bed and the gestational stage of the animal. For example, the PCAs from LP and PP animals showed a refractory response to $MgSO_4$ when compared to the NP group. This gestational change in reactivity was not observed in the MAs. While it is not clear as to what the cause of this differential response was, it may be due to changes in the cerebrovascular vasodilatory mechanisms that have been demonstrated during pregnancy and the postpartum state (7).

Studies have shown a vasodilatory effect with $MgSO_4$ treatment on both the cerebral circulation and systemic arteries (1, 3, 4, 12, 15, 21). Belfort et. al. investigated the effect of $MgSO_4$ on the cerebral circulation in patients with pregnancy-induced hypertension (4) and showed a significant increase in mean velocity in the maternal middle cerebral artery in response to intravenous $MgSO_4$ that was interpreted as distal artery vasodilation. The current study is the first to directly examine the dilatory response of small distal cerebral arteries to $MgSO_4$ and found that it caused modest vasodilation, a response that differed with gestational stage (12-36%), shown in Figure 1. In addition, this study also demonstrated that the systemic circulation (i.e. the mesenteric vascular bed), was more sensitive to $MgSO_4$ than the cerebral circulation (12-19% vs. 50-52%), especially in LP and PP animals, as seen in Figures 4-6. This

finding suggests that as an eclamptic seizure prophylaxis the effects of $MgSO_4$ may be more closely related to an effect on peripheral vascular resistance and lowering of systemic blood pressure than to an effect on cerebral blood flow. In support of this theory, it has been shown that treatment with a 6 g intravenous loading dose of $MgSO_4$ caused a significant decrease in both maternal systolic and diastolic blood pressures (4).

Because of the difference in the amount of intrinsic tone in MA vs. PCAs, we precontracted the MA with phenylephrine to match the degree of constriction of the PCA. While there was a significant decrease in myogenic tone in the PP PCAs, we chose not to precontract those vessels for consistency within the group. It is important to note the possibility that $MgSO_4$ may dilate precontraction to phenylephrine more easily than myogenic tone, accounting for the increased sensitivity of the MA. Previous studies have shown a differential dilatory response of aortic rings from pregnant rats that depended on the method of precontraction (potassium chloride (KCl) vs. phenylephrine). However, $MgSO_4$ had a decreased vasodilatory action on vessels precontracted with phenylephrine when compared to those precontracted with KCl (1). This suggests that the difference in dilation of the PCA vs. the MA in our study could become even more significant if KCl was used for precontraction or if we compared dilation of intrinsic tone only.

$MgSO_4$ has been shown to have effects other than those on the vasculature that may relate to its effectiveness in preventing eclamptic seizures. Mg^{+2} has been shown to be a *N*-methyl-D-aspartate (NMDA) receptor antagonist (13), and it has been hypothesized that this interaction accounts for the anti-convulsant properties of $MgSO_4$.

It is not clear whether or not $MgSO_4$ can cross the blood-brain barrier. However, if the blood-brain barrier has been disrupted due to the endothelial damage caused by acute hypertension, $MgSO_4$ could enter the brain parenchyma and exert its effects. Along these lines, Mg^{+2} has been shown to be protective of the blood-brain barrier under conditions that promote disruption (11), which could perhaps slow the progression of hypertensive encephalopathy. It is likely then that the prophylactic effect of $MgSO_4$ in preventing eclamptic seizures is multi-factorial, encompassing both vascular and neurological mechanisms.

In conclusion, we found a significant difference in the vasodilatory effect of $MgSO_4$ on cerebral resistance arteries from LP and PP animals compared to NP animals, implying that there are changes in the cerebrovascular vasodilatory mechanisms that occur with pregnancy. The LP and PP PCAs were also significantly less sensitive to $MgSO_4$ than the MAs, suggesting a differential action on mesenteric vs. cerebral resistance arteries.

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Table 1: Percent constriction to magnesium sulfate at different stages of gestation

	Posterior Cerebral Artery		Mesenteric Artery	
[$MgSO_4$]	1.2 mM	32 mM	1.2 mM	32 mM
Non-pregnant	32±2	7±2**	38±3	5±1**
Late pregnant	26±6	14±5*	35±2	4±1**
Postpartum	13±5†	3±3*	36±6	7±2**

All data are expressed as mean ± SE. *=$p \leq 0.05$, **=$p \leq 0.01$ vs. percent constriction in 1.2 mM $MgSO_4$; †=$p \leq 0.05$ vs. percent constriction non-pregnant PCA in 1.2 mM $MgSO_4$

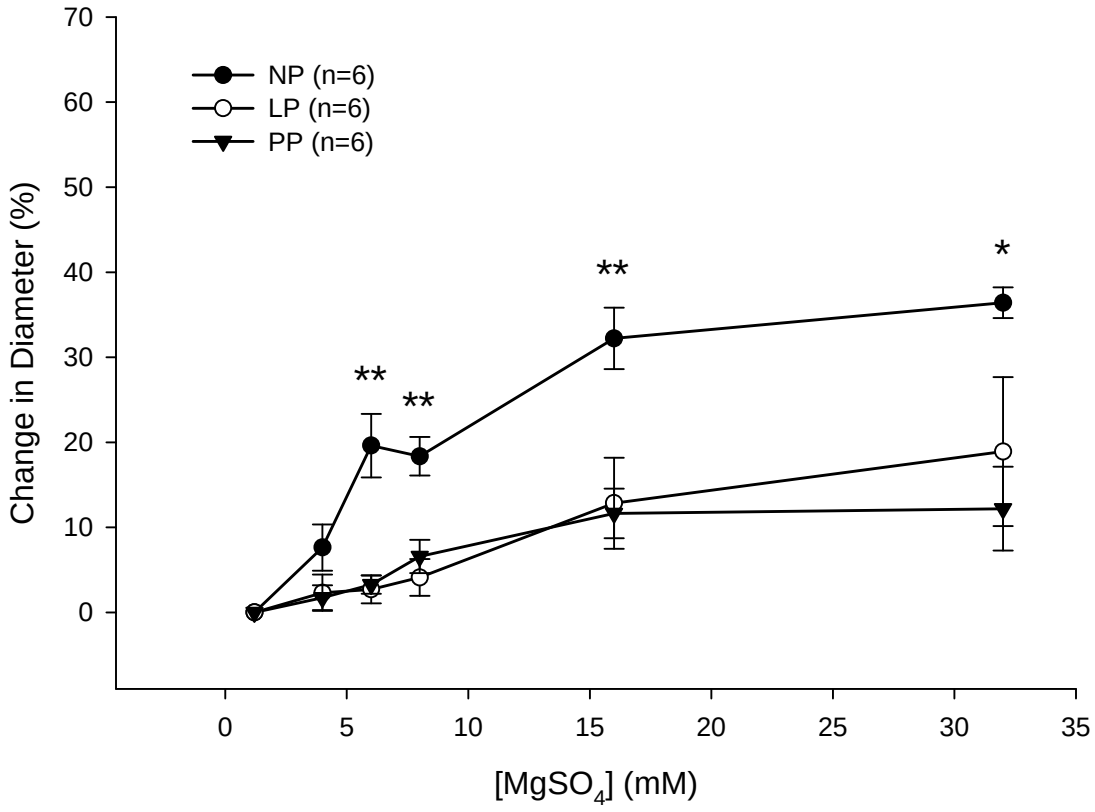

Figure 1: Percent change in diameter of the posterior cerebral artery to magnesium sulfate

Graph showing the percent change in diameter of the posterior cerebral artery (PCA) to increasing concentrations of magnesium sulfate ($MgSO_4$) at different stages of gestation, non-pregnant (NP, closed circles), late pregnant (LP, open circles), and postpartum (PP, closed triangles) animals. Notice that while there is a concentration-dependent dilation in all groups, the PCA from LP and PP animals were less sensitive to $MgSO_4$ with respect to NP animals. *=$p \leq 0.05$, **=$p \leq 0.01$ vs. LP and PP

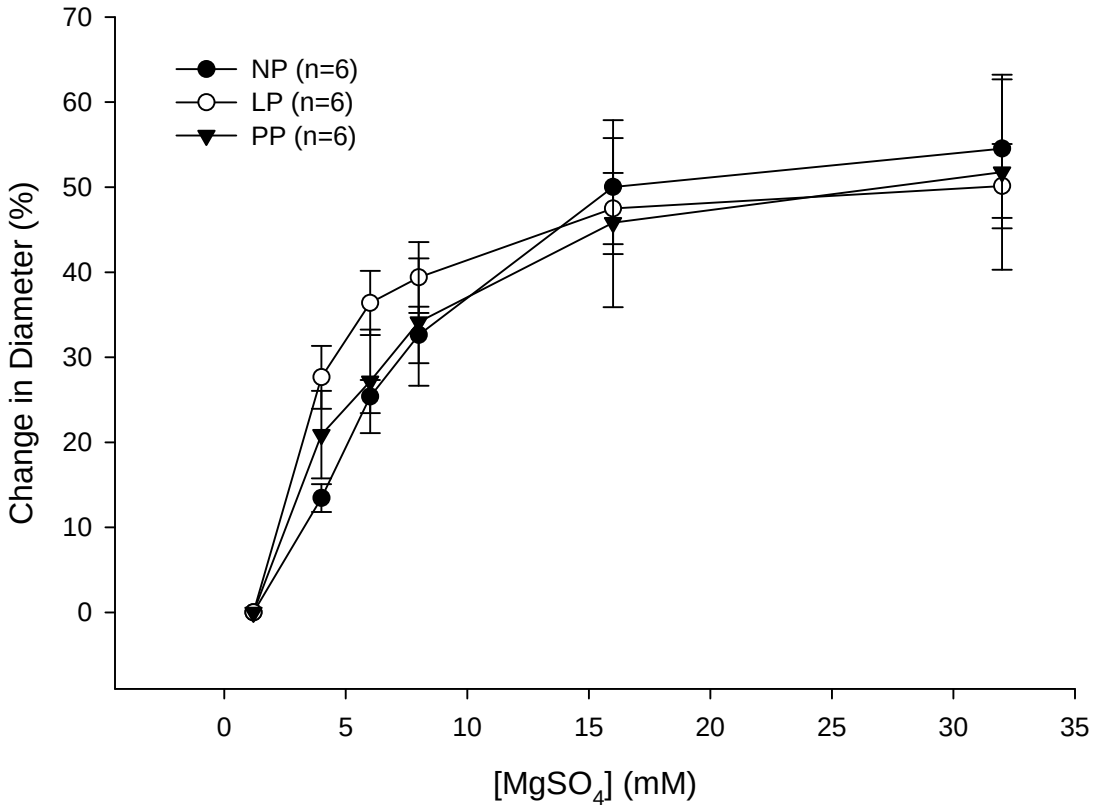

Figure 2: Percent change in diameter of the mesenteric artery to magnesium sulfate

Graph showing the percent change in diameter of the mesenteric arteries (MA) to increasing concentrations of magnesium sulfate ($MgSO_4$) at different stages of gestation, non-pregnant (NP, closed circles), late pregnant (LP, open circles), and postpartum (PP, closed triangles) animals. The dilation was concentration-dependent and did not vary between gestational groups.

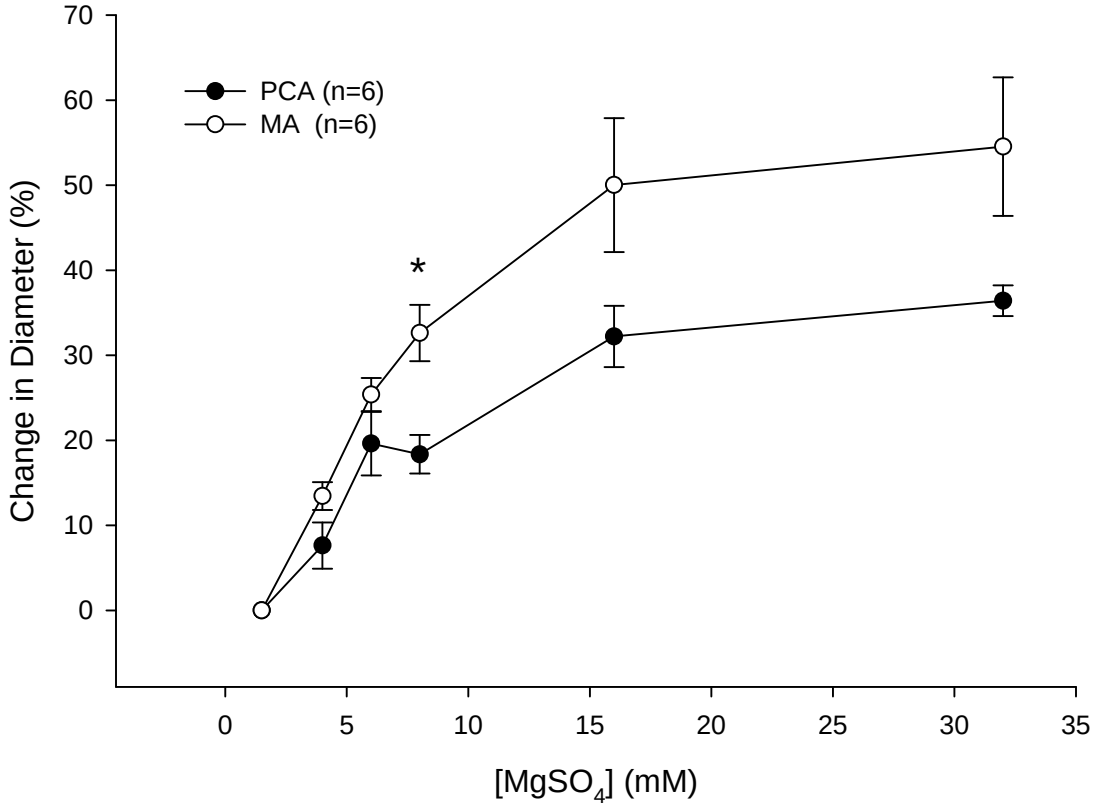

Figure 3: Percent change in diameter of the posterior cerebral artery and mesenteric artery in non-pregnant animals

Graph showing the percent change in diameter of the posterior cerebral arteries (PCA, closed circles) and mesenteric arteries (MA, open circles) in non-pregnant (NP) animals in response to increasing concentrations of magnesium sulfate ($MgSO_4$). *=$p \leq 0.05$ vs. PCA

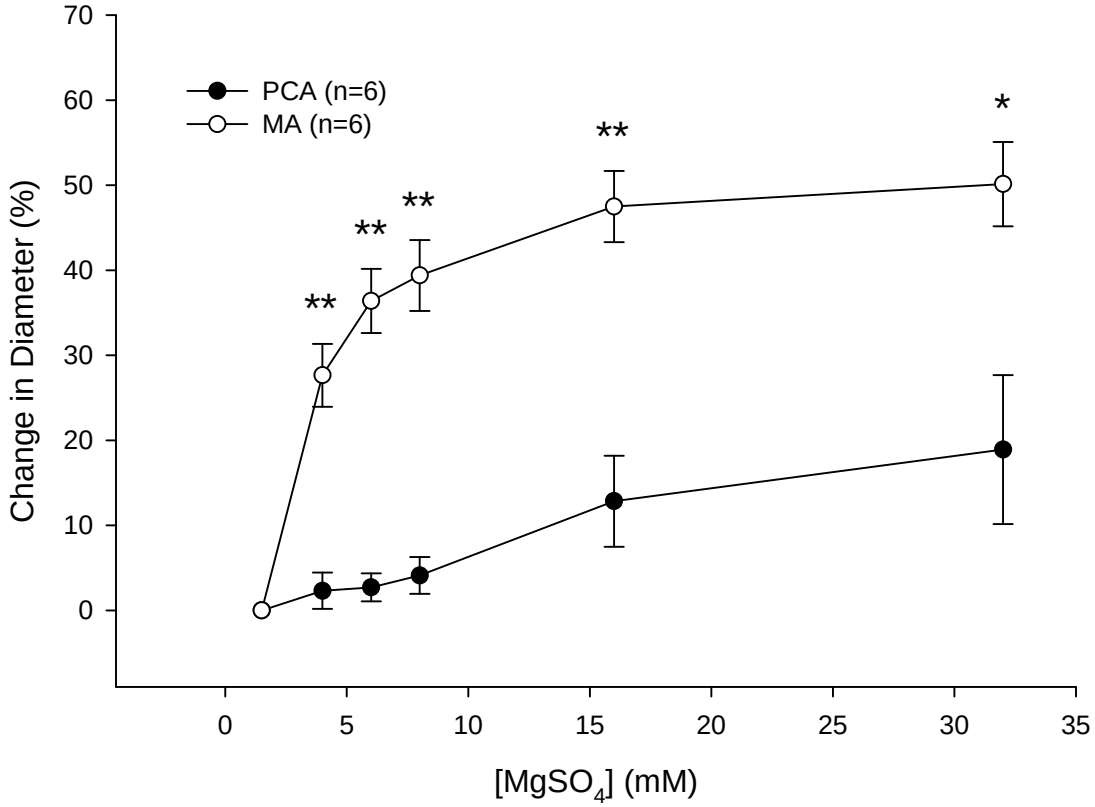

Figure 4: Percent change in diameter of the posterior cerebral artery and mesenteric artery in late pregnant animals

Graph showing the percent change in diameter of the posterior cerebral arteries (PCA, closed circles) and mesenteric arteries (MA, open circles) in late pregnant (LP) animals in response to increasing concentrations of magnesium sulfate ($MgSO_4$). Notice that the MA was significantly more sensitive to $MgSO_4$ than the PCA. *=$p \leq 0.05$, **=$p \leq 0.01$ vs. PCA

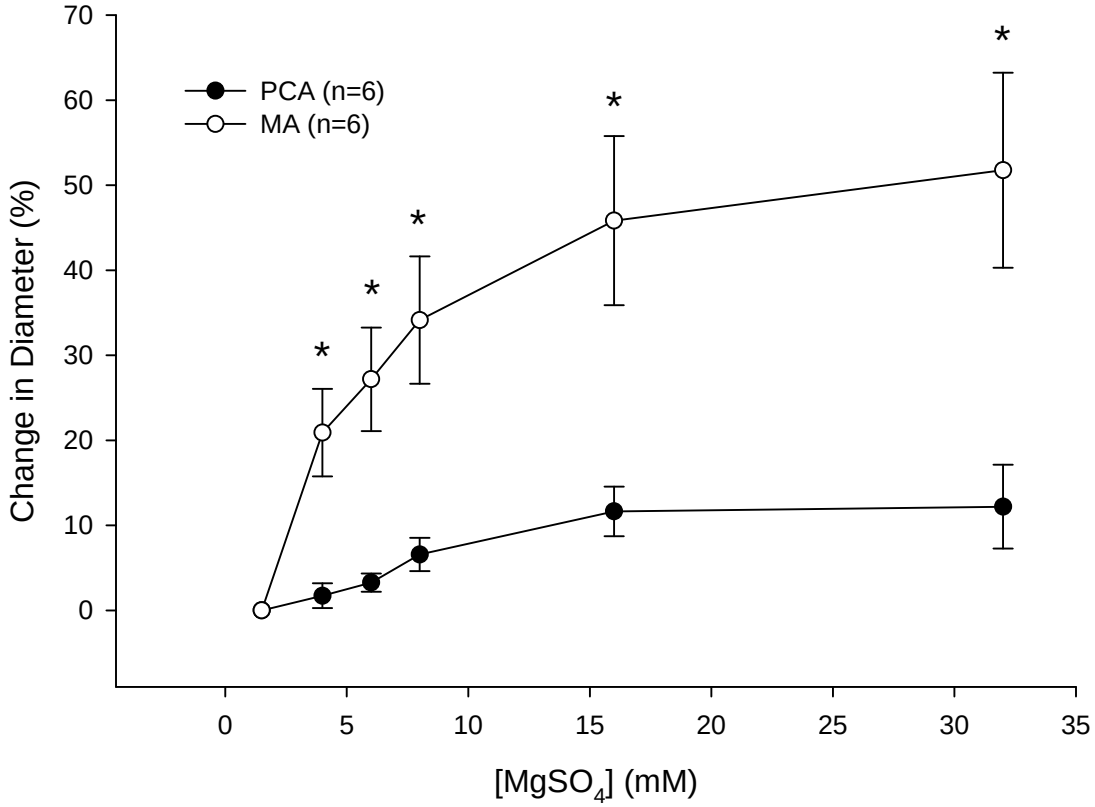

Figure 5: Percent change in diameter of the posterior cerebral artery and mesenteric artery in postpartum animals

Graph showing the percent change in diameter of the posterior cerebral arteries (PCA, closed circles) and mesenteric arteries (MA, open circles) in postpartum (PP) animals in response to increasing concentrations of magnesium sulfate ($MgSO_4$). Notice that the MA is significantly more sensitive to $MgSO_4$ than the PCA. *=$p \leq 0.05$ vs. PCA

CHAPTER 3: CEREBRAL BLOOD FLOW AUTOREGULATION AND EDEMA FORMATION DURING PREGNANCY IN ANESTHETIZED RATS

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***Hypertension* 2007; 49: 334-340**

Abstract

Eclampsia is considered a form of hypertensive encephalopathy in which an acute elevation in blood pressure causes autoregulatory breakthrough, blood-brain barrier disruption, and edema formation. We hypothesized that pregnancy predisposes the brain to eclampsia by lowering the pressure of autoregulatory breakthrough and enhancing cerebral edema formation. Because NO production is increased in pregnancy, we also investigated the role of NO in modulating autoregulation. Cerebral blood flow autoregulation was determined by phenylephrine infusion and laser Doppler flowmetry. Four groups were studied: untreated nonpregnant (n=7) and late-pregnant (d19 to 21, n=8) Sprague-Dawley rats and nonpregnant (n=8) and late-pregnant (n=8) animals treated with an NO synthase inhibitor (N^G-nitro-L-arginine methyl ester, 0.5 to 0.7 g/L). Brain water content and blood-brain barrier permeability to sodium fluorescein were determined after breakthrough. Pregnancy caused no change in autoregulation or the pressure of breakthrough. However, treatment with the NO synthase inhibitor significantly increased the pressure of autoregulatory breakthrough (nonpregnant: 183.6±3.0 mm Hg versus 212.0±2.8 mm Hg, $P<0.05$; late-pregnant: 180.8±3.2 mm Hg versus 209.3±4.7 mm Hg, $P<0.05$). After autoregulatory breakthrough, only late-pregnant animals showed a significant increase in cerebral edema formation, which was attenuated by NO synthase inhibition. There was no difference in blood-brain barrier permeability between nonpregnant and late-pregnant animals in response to acute hypertension, suggesting that pregnancy may predispose

the brain to eclampsia by increasing cerebral edema through increased hydraulic conductivity.

Key Words: autoregulation, eclampsia, L-NAME, laser Doppler flowmetry, NO synthase, pregnancy

Introduction

Eclampsia is a hypertensive disorder of pregnancy that occurs when hypertension in pregnancy presents with neurologic complications, including headache, nausea, vomiting, visual disturbances, and death.[1, 2] This disease remains a leading cause of maternal and fetal mortality worldwide.[3-5] In fact, it is estimated that 40% of eclamptic deaths are due to cerebral involvement.[1]

Eclampsia is thought to be a form of hypertensive encephalopathy.[6-8] This acute syndrome occurs from a sudden and excessive elevation of blood pressure that causes forced dilatation of the cerebrovasculature, autoregulatory breakthrough, and hyperperfusion that leads to disruption of the blood-brain barrier (BBB) and vasogenic edema formation.[6, 9, 10] There is considerable evidence to suggest that eclampsia and hypertensive encephalopathy are similar, including similar symptoms (headache, nausea, vomiting, visual disturbances, and, in the most severe cases, convulsions)[2, 6, 9] and comparable findings on imaging that indicate white matter edema and evidence of localized BBB disruption.[6, 11, 12] In addition, clinical reports demonstrate increased cerebral blood flow (CBF) both before and after the onset of eclamptic seizures,[10, 13-16] further supporting eclampsia as a hyperperfusive disorder.

Our previous studies on isolated and pressurized posterior cerebral arteries demonstrated a significant decrease in the pressure at which force dilatation occurred in late-pregnant (LP) versus nonpregnant (NP) rats.[17] These data suggest that CBF autoregulatory breakthrough may occur at lower pressures in pregnancy, perhaps predisposing women to the neurologic complications of eclampsia during episodes of hypertension. Therefore, this study sought to understand how pregnancy alone affects cerebral hemodynamics, including autoregulation, which may be an important step to preventing and treating eclampsia.

In addition to pregnancy, CBF autoregulation may be modulated by NO. It has been shown that NO synthase (NOS) inhibition during acute hypertension extended the autoregulatory range,[18] suggesting that NO may play an active role in mediating the pressure of autoregulatory breakthrough. Because NO production is significantly increased during pregnancy,[19-21] it is possible that this is a mechanism by which autoregulation is shifted during pregnancy.

The goal of the present study was to examine CBF autoregulation during pregnancy and to determine the upper limit of autoregulation in LP and NP rats. We also investigated the contribution of NOS to CBF autoregulation. Autoregulatory curves were determined in LP and NP Sprague-Dawley rats using a model of acute hypertension in vivo with and without NOS inhibition using N^G-nitro-L-arginine methyl ester (L-NAME). In addition, because eclampsia has been shown to be associated with increased cerebral edema,[6, 9, 11] we also determined cerebral edema

formation and BBB permeability after autoregulatory breakthrough, a potential mechanism of edema formation.

Methods

Animal Model

All of the experiments used a rat model of pregnancy and were conducted using Sprague-Dawley female rats (Charles River). It has been shown that gravid rats undergo many cardiovascular changes similar to those seen in human pregnancy.[22] Animals were housed in the University of Vermont Animal Care Facility, an Association for Assessment and Accreditation of Laboratory Care-accredited facility. All of the procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Virgin animals were used for NP experiments. LP experiments were conducted on day 19 to 21 of gestation. A total of 54 animals were studied. Further animal characteristics are summarized in the Table.

NOS Inhibition and Determination of Blood Pressure

For experiments investigating the effect of NOS inhibition on CBF autoregulation, the NOS inhibitor L-NAME (Sigma) was used. L-NAME was administered to animals in their drinking water for 7 days (0.5 g/L for NP animals and 0.7 g/L for LP animals). These doses have been shown to cause similar elevations of blood pressure in NP and LP animals.[23] The time course of L-NAME treatment was chosen to mimic the last trimester of pregnancy (7 days of a 22-day gestation) when

eclampsia most often occurs in gestation.[3] Based on earlier studies,[23] this period of treatment was sufficient to cause mean arterial pressure elevation as determined by the noninvasive tail cuff technique (Coda 6 System, Kent Scientific). Arterial blood pressure was monitored over the 7-day treatment period, as previously described.[23] Animals treated with L-NAME had their blood pressures monitored for 1 to 3 days before the initiation of L-NAME treatment and at least 6 of the 7 days of L-NAME treatment. For each day, the average of 3 representative measurements was taken (data not shown).

***In vivo* Acute Hypertension Model**

For determination of CBF autoregulatory curves, a model of acute hypertension was used that allowed for continuous recording of both CBF and arterial blood pressure in vivo. Anesthesia was initiated with isoflurane (≤3% in O_2, inhaled), which was then tapered off and discontinued. Anesthesia was maintained with intravenous pentobarbital (≤60 mg kg^{-1} hr^{-1}), which was decreased, as tolerated, during the surgical preparation to minimize effects on experimental parameters. Adequate anesthesia was assessed by toe pinch and changes in arterial blood pressure. Animals were mechanically ventilated with a mixture of compressed air and 100% O_2 via tracheostomy. Ventilation was adjusted to maintain arterial blood gases within physiologic ranges (pH 7.35 to 7.45, PCO_2 35 to 45 mm Hg, and PO_2 ≥100 mm Hg).

CBF was measured transcranially using laser Doppler flowmetry with a 1-mm probe (Perimed). The right side of the skull was exposed and cleared of membranes. The flow probe was affixed over a thinned area of skull posterior to the coronal suture

and lateral to the sagittal suture over the middle cerebral artery perfusion domain, as described elsewhere.[24]

A femoral arterial catheter was used to obtain blood samples for analysis (Medica), and to measure arterial blood pressures via a pressure servo transducer (Living Systems Instrumentation). A filtered solution of heparin sulfate and lactated Ringer's solution (1000 U in 6 mL) was used within the arterial catheter to prevent clotting. Two femoral venous catheters were placed in order to deliver pentobarbital and phenylephrine (PE, 0.01 g /10 mL lactated Ringer's solution, Sigma) intravenously. PE dosage was increased at regular intervals starting at 0.5 µL/min (0.5 µg/min) to ensure a consistent rise in arterial blood pressure. After CBF autoregulatory curves were obtained and evidence of breakthrough occurred, elevated blood pressure and CBF were maintained for 10 minutes. Animals were quickly decapitated, and the brain was then removed for wet and dry weight measurements. The brain was first weighed wet followed by drying in an oven at 100° C for 24 hours, at which point the brain was weighed again dry.

Four groups of animals were studied for determination of CBF autoregulation curves: NP (n=7), LP (n=8), NP animals treated with L-NAME (NP + L-NAME, 0.5 g/L for 7 days, n=8), and LP animals treated with L-NAME (LP + L-NAME, 0.7 g/L for 7 days, n=8). In addition, 2 control groups were added for brain water content analysis to control for edema because of either pregnancy and/or the response to the surgical preparation of acute hypertension. These groups are designated as NP without (w/o) hypertension (HTN) and LP w/o HTN.

Determination of CBF Autoregulatory Curves

Autoregulatory curves were determined for each animal by analysis of CBF and pressure tracings. Tracings of CBF and arterial blood pressures were collected during PE infusion using commercially available software (Figure 1; Perisoft, Perimed). During acute hypertension, the average CBF and arterial pressure were determined for the same time point over a range of pressures from baseline to autoregulatory breakthrough. These data were used to determine pressure versus flow curves for each experimental group. The point at which the curve became vertical was taken to signify autoregulatory breakthrough.

Relative CBF

Because laser Doppler units are a relative measure of changes in CBF, the laser Doppler signal was normalized to the flow at baseline (after anesthesia had been minimized and before PE administration) to determine a relative CBF (rCBF). The following equation was used: $rCBF = (CBF_x/CBF_{baseline})$, where CBF_x is the flow in laser Doppler units at various pressures and doses of phenylephrine, and $CBF_{baseline}$ is the flow in laser Doppler units at the start of the experiment. For example, rCBF of 2 signifies a 2-fold increase in CBF from baseline.

Brain Water Content

The percent of water content of the brain is a measure of cerebral edema.[25] Brain water content (in percentage) was determined by the following formula: $[(weight_{wet} - weight_{dry}) \div weight_{wet}] * 100\%$; where $weight_{wet}$ was the weight of the

brain immediately after removal from the animal, and $weight_{dry}$ was the weight of the brain after drying.

***In vivo* BBB Solute Permeability Studies**

To investigate BBB permeability in response to acute hypertension, studies were conducted which combined the in vivo acute hypertension model described above with central infusion of a dye tracer, sodium fluorescein (NaFl, 376 Da; Sigma). Animals were prepared and instrumented as described for the in vivo acute hypertension model with the addition of the placement of a catheter (22-gauge) in the right common carotid artery before the infusion of PE. A solution of 0.1% NaFl in lactated Ringer's solution filled the catheter, and 0.5 mL of this solution was infused directly into the left ventricle of the heart and allowed to circulate for 10 minutes before beginning the PE infusion (starting at 0.5 µL/min [0.5 µg/min]). Once arterial pressures had reached ≥180 mm Hg (sufficient to cause autoregulatory breakthrough), arterial blood pressure and CBF were maintained for 10 minutes, the same time course as the cerebral edema formation experiments. The cerebral circulation was then flushed by perfusion with 40 mL of lactated Ringer's solution through the carotid catheter, and at the same time the chest was opened and the right atrium snipped to allow for the removal of the dye from the vasculature. Animals were quickly decapitated, and the brain removed. Any animals in which appropriate flushing of the vasculature was not evident on gross examination were excluded from analysis. The brain was weighed and then homogenized in 10 mL 50% trichloroacetic acid (Sigma) 15 times in glass Dounce tissue grinders. Homogenized samples were centrifuged at 4000g at 4º C for 10

minutes. The supernatant was analyzed by fluorescence spectrophotometry (460 to 515 nm) to determine dye clearance into the brain tissue.

Four groups were studied for in vivo BBB solute permeability: NP Sham (n=4), NP HTN (n=4), LP Sham (n=4), and LP HTN (n=4). Sham controls were surgically prepared in an identical manner with the exception that PE was not infused; thus, no acute hypertension occurred. The time course of the sham experiments was similar to that of an experiment with acute hypertension. Data are expressed as average fluorescence counts per second (CPS) per gram brain tissue.

Statistical Analysis

All of the data are expressed as mean±SEM. Differences in arterial blood pressures at different rCBFs between NP and LP groups and between untreated control and L-NAME-treated groups were determined by an ANOVA with a posthoc Student-Newman-Keuls test for multiple comparisons. Similarly, differences in brain water content between nonsurgical controls (w/o HTN) and NP and LP groups, between nonsurgical controls (w/o HTN) and L-NAME-treated groups, and between untreated control and L-NAME-treated groups were determined by an ANOVA. Differences in BBB permeability between groups were determined by ANOVA and a posthoc Student-Newman-Keuls test for multiple comparisons. Differences were considered significant if $P < 0.05$.

Results

The Table presents data on the characteristics of each group of animals studied. L-NAME treatment during the last trimester of pregnancy did not affect average litter size, the number of animals showing fetal resorption, or the body weight of the animal. However, the body weight of pregnant animals was significantly greater than either NP group, as expected. Arterial blood pressures, determined at the time of surgery via arterial catheter, were similar between groups prior to PE infusion with the exception of the NP + L-NAME group, which had a significantly higher baseline blood pressure (Table and Figure 2).

All of the groups of animals showed CBF autoregulation over a range of pressures up to the pressure of autoregulatory breakthrough (PAB), as shown in Figure 2. The PAB was determined for each group at a rCBF of 2 and is reported in the Table. There was a dramatic increase in the PAB in L-NAME-treated animals versus NP and LP control groups, respectively (at rCBF of 2: NP animals, 183.6±3.0 mm Hg versus 212.0±2.8 mm Hg, $P<0.05$; LP animals, 180.8±3.2 mm Hg versus 209.3±4.7 mm Hg, $P<0.05$). No differences were observed between NP and LP control groups at any point on the autoregulatory curve. However, in L-NAME-treated animals, between rCBFs of 1.0 to 1.05 and 1.30 to 1.45, there was a shift to lower pressures in LP + L-NAME versus NP + L-NAME animals ($P<0.05$).

Brain water content was used as a measure of cerebral edema formation.[25] After autoregulatory breakthrough had occurred because of acute hypertension, brain water

content was determined for each animal (Figure 3). Brain water content was also determined for additional control groups that did not undergo surgery or acute hypertension (NP w/o HTN and LP w/o HTN) to control for any effects of pregnancy or the surgical preparation. There was no difference in brain water content between any of the NP groups, regardless of acute hypertension or L-NAME treatments (NP w/o HTN 77.84±0.22%, NP acute HTN 77.70±0.11%, and NP L-NAME + acute HTN 77.60±0.15%). However, there was a significant increase in brain water content after autoregulatory breakthrough in LP animals that underwent acute hypertension versus NP animals. In addition, L-NAME treatment significantly attenuated the rise in brain water content because of autoregulatory breakthrough in LP animals (LP w/o HTN 77.86±0.05%, LP acute HTN 78.56±0.10%, and LP L-NAME + acute HTN 78.28±0.08%). Lastly, increased cerebral edema was not due to pregnancy alone, because only those animals that underwent autoregulatory breakthrough had increased edema formation.

To investigate the mechanism by which brain water content was increased in LP animals in response to acute hypertension, the permeability of the BBB to a small solute under the same conditions was determined. The passage of NaFl into cerebral brain tissue was determined in response to acute hypertension as shown in Figure 4. Acute hypertension caused an increase in permeability in both NP and LP animals compared with sham controls, although this was not statistically significant. However, there was no difference in BBB permeability between NP and LP animals under either sham or acute hypertensive conditions (NP Sham 9930.0±3056.7 CPS/g versus LP

Sham 10 568.5±2564.4 CPS/g; NP HTN 17 855.0±2151.8 CPS/g versus LP HTN 16 245.8±5237.9 CPS/g).

Discussion

There were several major findings in this study that examined CBF autoregulation, cerebral edema, and BBB permeability during pregnancy. Both NP and LP animals demonstrated CBF autoregulation up to ~180 mm Hg that was similar between gestational groups. Likewise, the PAB was not different between untreated NP and LP animals. However, treatment with the NOS inhibitor L-NAME shifted the autoregulatory curve to significantly higher pressures in both NP and LP animals, suggesting that NOS has an active role in modulating the PAB. In addition, edema formation after autoregulatory breakthrough was significantly increased in LP versus NP animals, demonstrating that pregnancy alone promotes cerebral edema formation when pressure is elevated. There was no difference in BBB permeability to NaFl between LP and NP groups, suggesting that the increase in cerebral edema formation was not primarily because of increased solute permeability. Lastly, NOS inhibition attenuated edema formation after autoregulatory breakthrough in the LP + L-NAME group, further suggesting that increased NO production during pregnancy may contribute to the enhanced edema formation.

The present study is the first to examine CBF autoregulatory breakthrough and edema formation during pregnancy. Establishing the autoregulatory pressure range in female animals and the effect of pregnancy on this cerebrovascular parameter is

important because of the hyperperfusive nature of eclampsia. Clinical reports have demonstrated increased CBF in the maternal brain both preceding and after the onset of eclamptic seizures,[10, 13, 14, 16] as well as in severe preeclampsia.[15, 16] Clinical evidence also suggests that the autoregulatory curve is shifted to a lower range of pressures during pregnancy as evidenced by the onset of seizures at relatively low mean arterial pressures when compared with cases of hypertensive encephalopathy.[1, 13] In addition, our in vitro data demonstrated that the pressure of force dilatation was lower during pregnancy, also suggesting that PAB would be lower.[17] However, the results of the present study did not show a difference in CBF autoregulation or PAB between NP and LP animals. This discrepancy with our in vitro studies may be because of the fact that CBF autoregulation is a complex interaction of endothelial, neuronal, and metabolic influences that cannot be mimicked in vitro. In addition, our in vitro studies examined the posterior cerebral artery, whereas this in vivo study used laser Doppler to measure changes in flow in the middle cerebral artery perfusion domain, and it is possible that different regions of the brain have differing autoregulatory capabilities. Although autoregulation may not differ with normal gestation, it remains possible that circulating factors and/or oxidative damage as part of eclampsia could cause greater endothelial dysfunction that affects either the PAB or edema formation. This would agree with clinical reports of neurologic complications and seizures at lower pressures in settings of endothelial dysfunction.[11, 13, 26]

An important finding of the present study was the effect of NOS inhibition on CBF autoregulation. Treatment with L-NAME for 7 days shifted the autoregulatory

curve to higher pressures in both groups of animals, suggesting that NO has an active role in determining autoregulation and the PAB. In addition, arterial forced dilatation before autoregulatory breakthrough seems to be an active process that involves the pressure-dependent production of NO rather than a mechanical dilation. Studies by Talman and Dragon[18] also suggest that NO has an active role in autoregulatory breakthrough in male rats, because NOS inhibition prevented autoregulatory breakthrough. This concept is further supported by work that showed that potassium channel inhibition shifted autoregulatory curves to higher pressures.[27] Taken together, these findings suggest that autoregulatory breakthrough is an active process that is mediated by both NO and possibly potassium channels.

An alternative explanation for the shift in autoregulation because of NOS inhibition is vascular adaptation due to either hypertension-induced vascular remodeling or L-NAME-induced vasoconstriction. However, studies have shown that while L-NAME treatment for just 7 days caused medial hypertrophy and an increased wall:lumen ratio in NP animals, these same vascular adaptations were not seen in LP animals.[23] A similar lack of remodeling was seen in pregnant Dahl salt-sensitive hypertensive animals,[28] suggesting that autoregulation is shifted to higher pressures after NOS inhibition for reasons other than structural vascular adaptations. An effect of L-NAME on the contractile state and increased cerebrovascular resistance cannot be ruled out from these studies.

Cerebral edema is one of the hallmark pathologies of the eclamptic brain and is tied to the hyperperfusive nature of the disease. It has been shown that 93% of

eclamptic women studied with diffusion-weighted MRI had evidence of cerebral vasogenic edema.[11] Other classic imaging findings in eclampsia are subcortical white matter edema with evidence of localized BBB disruptions, particularly in parietal-occipital locations.[6, 7, 12] The results of the present study demonstrate that LP animals had increased cerebral edema formation after autoregulatory breakthrough that was not evident in NP animals. Because enhanced BBB permeability can cause edema formation, we determined permeability to NaFl in response to acute hypertension in NP and LP animals. Our results show that whereas there was a nonsignificant increase in permeability in response to acute hypertension, there was not a difference in permeability between NP and LP animals, suggesting that the mechanism by which pregnancy enhances edema is not due to enhanced solute permeability.

An alternative mechanism by which pregnancy may be affecting edema formation is through aquaporin expression in the brain. Aquaporin 4, located in astrocytic end feet[29, 30] and cerebral endothelium,[29] has been shown to be significantly upregulated in the brain during pregnancy.[31] This gestational effect may influence the formation and management of cerebral edema during acute hypertension. Because this study did not find a difference in BBB permeability despite the increased cerebral edema formation after acute hypertension, it is possible that in the first 10 minutes after autoregulatory breakthrough, there is a specific movement of water across the BBB independent of an increase in solute permeability. We hypothesize that pregnancy acts to increase water permeability (hydraulic conductivity) by increased aquaporin expression leading to significantly enhanced cerebral edema formation. Interestingly,

L-NAME treatment decreased cerebral edema formation in response to acute hypertension, possibly because of increased cerebrovascular resistance and reduced microvascular pressure that protects the microvessels from the increased hydrostatic pressure associated with autoregulatory breakthrough.

Perspectives

The pathogenesis of eclampsia seems to begin with an acute elevation in blood pressure leading to autoregulatory breakthrough and hyperperfusion of the brain. Subsequent vasogenic edema formation likely contributes to the clinical symptoms of eclampsia. The results from this study suggest that changes during normal pregnancy may predispose women to the occurrence of eclampsia when arterial blood pressure is acutely elevated above the reference range. In particular, the enhancement of cerebral edema formation without a change in autoregulation could potentiate the neurologic complications of eclampsia. Because we found that pregnancy did not increase BBB permeability, it seems that increased cerebral edema is because of an increase in hydraulic conductivity (possibly by an increase in aquaporin expression). In addition, it seems that NO contributes to the PAB and edema formation. These data lend further insight into the process of autoregulatory breakthrough, which is an important component in the pathogenesis of eclampsia.

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Disclosures

None.

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Table 1: Physiological characteristics of animals studied

Group	Average Litter Size	Animals with Fetal Resorption	Body Weight, g	ABP, mmHg	PAB, rCBF = 2.0, mmHg
NP (n=7)			288.6±7.2	114.4±2.6	183.6±3.0
LP (n=8)	12.4	1	323.1±8.3*	111.1±3.3	180.8±3.2
NP + L-NAME (n=8)			291.3±71	149.5±4.6*	212.0±2.8*
LP + L-NAME (n=8)	13.0	1	322.5±7.8‡	120.4±7.1‡	209.3±4.7†

Abbreviations: ABP = Arterial blood pressure, L-NAME = N^G-nitro-L-arginine methyl ester, LP = Late-pregnant, NOS = Nitric oxide synthase, NP = Nonpregnant, PAB = Pressure of autoregulatory breakthrough, rCBF = relative cerebral blood flow. *$p<0.05$ vs. NP control, †$p<0.05$ vs. LP control, ‡$p< 0.05$ vs. NP + L-NAME

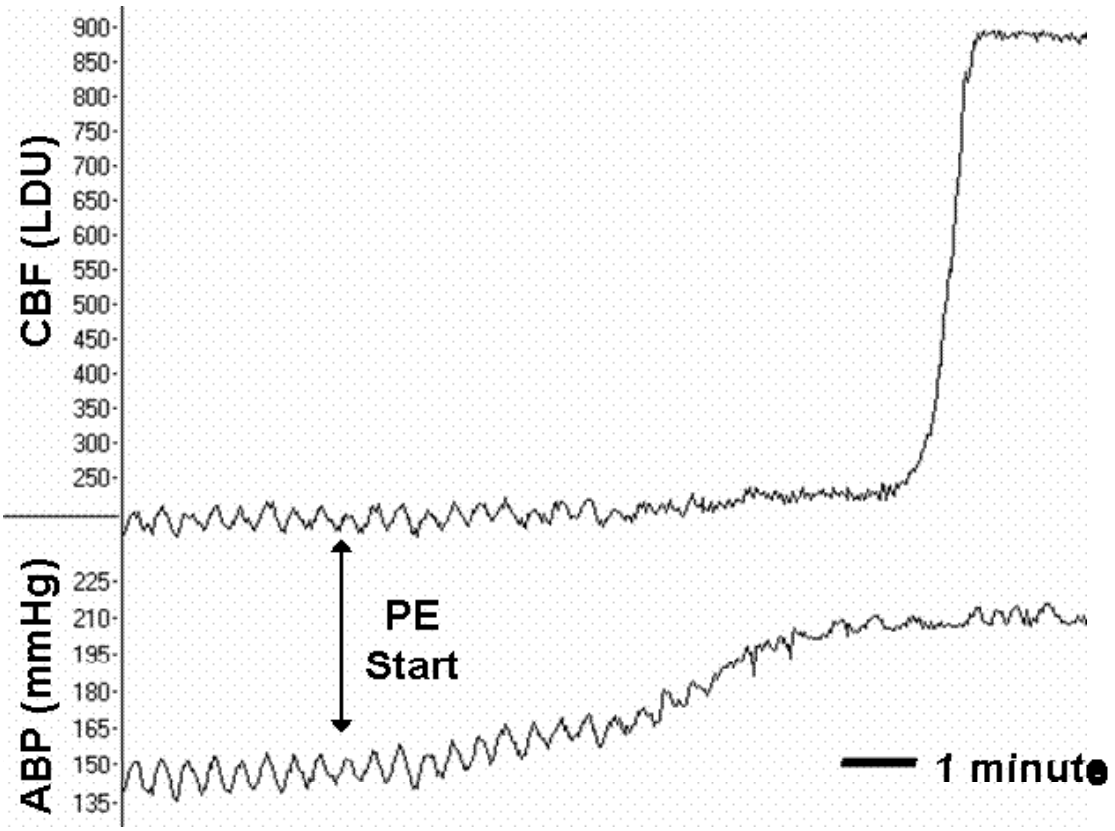

Figure 1: Tracing of cerebral blood flow and arterial blood pressure during acute hypertension

Tracing of cerebral blood flow (CBF, in laser Doppler units) and arterial blood pressure (ABP, in mm Hg) in response to increasing doses of phenylephrine (PE). In this experiment, CBF increased 4 times greater than baseline as ABP was increased from 140 to 210 mm Hg demonstrating autoregulatory breakthrough.

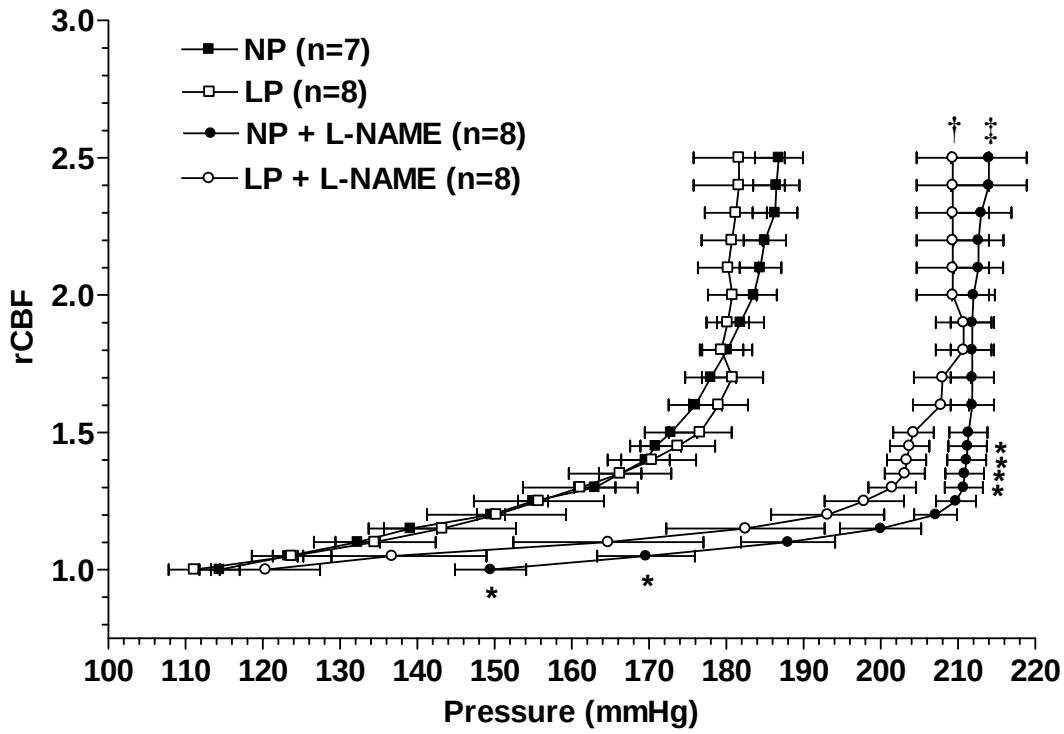

Figure 2: Autoregulatory curves for all groups studied

Graph showing autoregulatory curves for all groups of animals. All of the animals maintained cerebral blood flow autoregulation (represented as relative cerebral blood flow, rCBF) up to the pressure of autoregulatory breakthrough (PAB). There was no difference between nonpregnant (NP) and late-pregnant (LP) controls (closed and open squares, respectively). Treatment of animals with N^G-nitro-L-arginine methyl ester (L-NAME) caused a significant shift in the PAB to higher pressures for both NP and LP animals (closed and open circles, respectively). *$p<0.05$ vs. LP + L-NAME, †$p<0.05$ vs. LP control (all points rCBF >1.15), ‡$p<0.01$ vs. NP control.

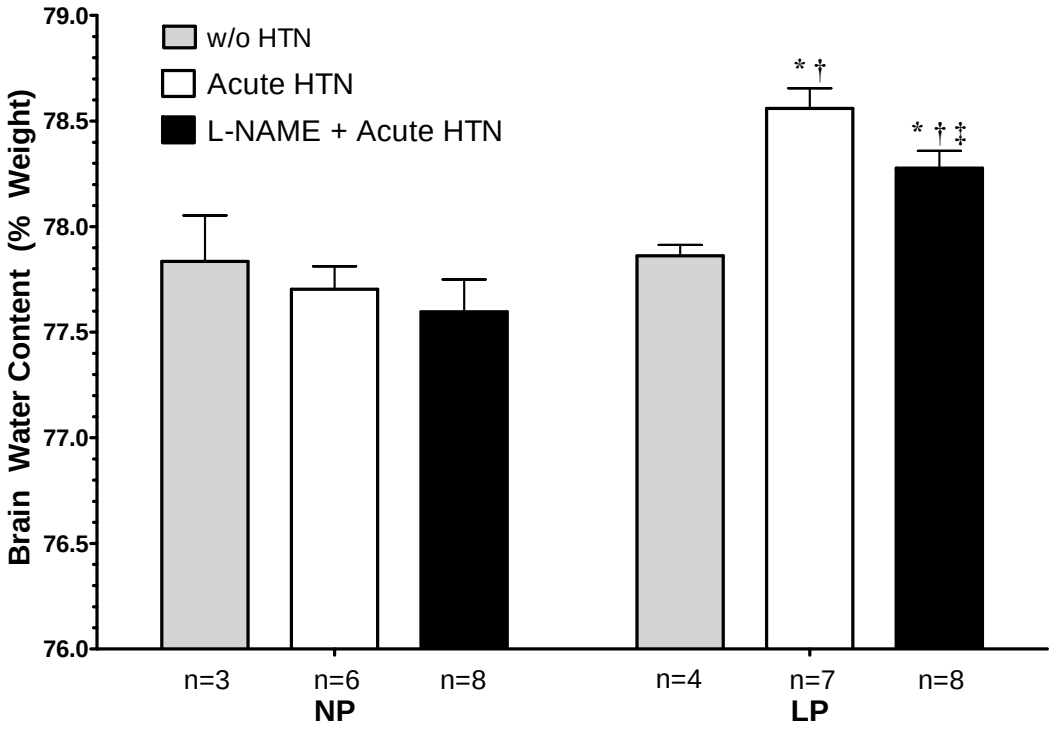

Figure 3: Brain water content for all groups studied

Graph showing percent brain water content for all groups of animals. Controls (gray bars, w/o HTN) did not undergo acute hypertension or autoregulatory breakthrough. There was no difference in brain water content between nonpregnant (NP) groups. Late-pregnant (LP) animals (white and black bars) had increased brain water content after autoregulatory breakthrough (acute HTN) compared to animals that did not undergo acute hypertension (LP w/o HTN, gray bar). Treatment with N^G-nitro-L-arginine methyl ester (L-NAME) attenuated brain water content in LP animals. *$p<0.01$ vs. respective NP control, †$p<0.01$ vs. LP w/o HTN, ‡$p<0.01$ vs. LP acute HTN group.

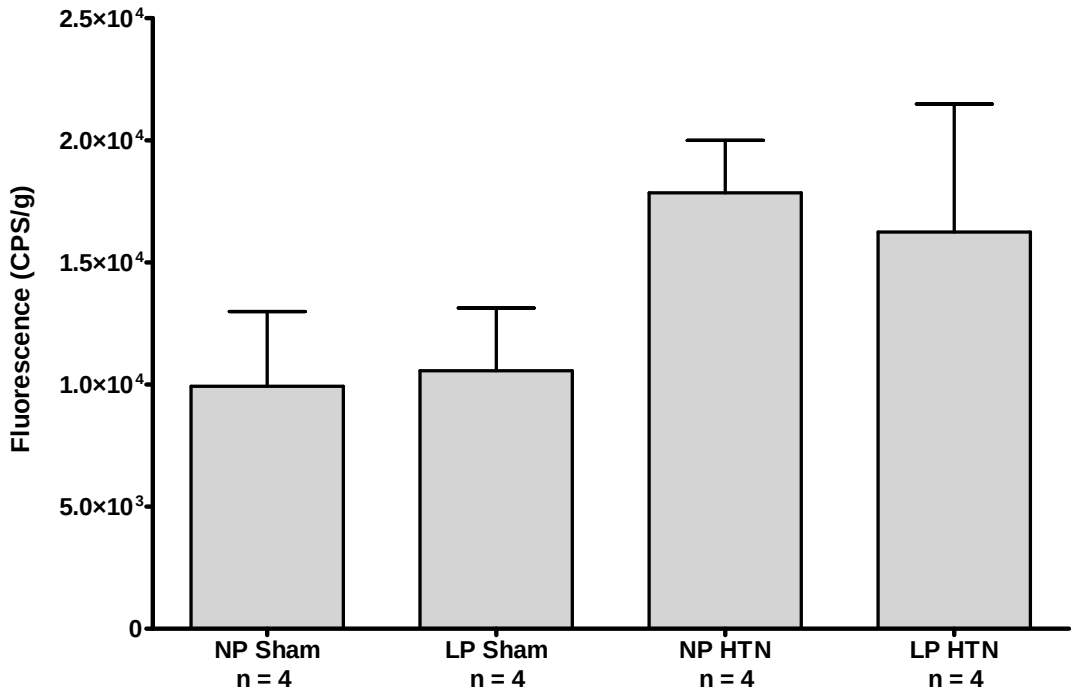

Figure 4: Average fluorescence as a measure of blood-brain barrier permeability

Graph showing average fluorescence (CPS/g) of sodium fluorescein in the brain as a measure of blood-brain barrier permeability in response to acute hypertension. Sham controls did not undergo phenylephrine infusion to cause acute hypertension. No difference in permeability was seen between nonpregnant (NP) and late-pregnant (LP) groups.

CHAPTER 4: THE EFFECT OF MAGNESIUM SULFATE ON BLOOD-BRAIN BARRIER PERMEABILITY AND BRAIN AQUAPORIN-4 EXPRESSION IN PREGNANT RATS

Abstract

Background and Purpose: Eclampsia is associated with increased blood-brain barrier (BBB) permeability and cerebral edema formation. Magnesium sulfate is used to treat eclampsia despite an unclear mechanism of action. This study's goal was to determine the effect of magnesium sulfate on *in vivo* BBB permeability during acute hypertension and on brain aquaporin-4 (AQP4) protein expression.

Methods: An *in vivo* model of hypertensive encephalopathy was used in late-pregnant (LP) rats following magnesium treatment, 270 mg/kg intraperitoneal injection every 4 hours for 24 hours. BBB permeability was determined by *in situ* brain perfusion of Evan's blue (EB) and sodium fluorescein (NaFl), and dye clearance determined by fluorescence spectrophotometry. The effect of magnesium treatment on AQP4 expression was determined by Western blot in additional LP rats.

Results: Acute hypertension increased BBB permeability in all brain regions, however, only the increase in EB was significant ($P<0.05$). Magnesium attenuated BBB permeability to EB during acute hypertension by 41% in the posterior cerebrum ($P<0.05$), and 30% in the anterior cerebrum (n.s.). Permeability to NaFl was decreased by 31% in the posterior cerebrum and 40% in the anterior cerebrum although this was not significant. AQP4 expression appeared to be increased during pregnancy in both brain regions; however, magnesium treatment had no effect on its expression.

Conclusions: Acute hypertension increased BBB permeability in LP rats and this was partially attenuated by magnesium treatment. The greatest effect on BBB permeability

to EB was in the posterior cerebrum, an area particularly susceptible to edema formation during eclampsia.

Introduction

Eclampsia is a serious hypertensive disorder of pregnancy associated with increased blood-brain barrier (BBB) permeability and subsequent vasogenic edema formation.[1-4] This condition is thought to be a form of hypertensive encephalopathy (HTE),[2, 3, 5] and both eclampsia and HTE are causes of reversible posterior leukoencephalopathy syndrome (RPLS).[6, 7] Using a model of HTE, we previously showed that breakthrough of cerebral autoregulation caused a significant increase in cerebral edema formation in late-pregnant (LP) rats which was not seen in nonpregnant (NP) controls despite similar pressures of autoregulatory breakthrough.[8] This suggests that pregnancy alone promotes edema formation under conditions of acute hypertension.

Magnesium sulfate is widely used to both prevent and treat eclamptic convulsions.[9] This treatment has been proven to be more effective than anticonvulsant drugs and placebo,[10, 11] though the mechanism of action remains unclear. Some studies suggest that magnesium may prevent eclamptic seizures through vasodilatation in the cerebral circulation.[12-14] However, magnesium treatment has also been reported to have little to no effect on cerebral hemodynamics and cerebral blood flow (CBF).[15-17] We previously showed that while magnesium sulfate has a modest vasodilatory effect on cerebral resistance arteries, the sensitivity of this response is decreased by pregnancy

and the postpartum state.[18] Furthermore, a randomized controlled trial found that when compared to nimodipine, a calcium channel blocker with specific cerebral vasodilator action, magnesium sulfate was more effective in preventing eclamptic seizures.[19] Together, these results provide evidence that the primary action of magnesium sulfate in eclampsia is likely not the relief of cerebral vasospasm.

Treatment with magnesium sulfate has been reported to decrease BBB permeability and cerebral edema formation in a variety of brain injury conditions including traumatic brain injury,[20-23] septic encephalopathy,[24] hypoglycemia,[25] and hyperosmolar mannitol injection.[26] We therefore hypothesized that the action of magnesium sulfate in eclampsia may be related to BBB protection during acute hypertension. The goal of this study was to determine the effect of magnesium sulfate treatment on *in vivo* BBB permeability following acute hypertension in LP rats. In addition, it has been proposed that magnesium sulfate may limit cerebral edema formation by decreasing the expression of aquaporin-4 (AQP4),[22] a water channel protein highly expressed in the brain, although this interaction has not been directly shown. Therefore, another goal of this study was to determine the effect of magnesium sulfate treatment on AQP4 protein expression in LP rats.

Material and Methods

Animals

All permeability experiments used a rat model of pregnancy in which primiparous Sprague-Dawley rats (Charles River, St. Constant, PQ, Canada) were

studied on day 19 to 21 of a 22 day gestation. One group of animals was treated by intraperitoneal injection of magnesium sulfate (270 mg/kg every 4 hours for 24 hours) prior to acute hypertension (HTN + Mg, n=4), and compared to untreated controls (HTN, n=4) and sham (n=4) LP animals. This dosage of magnesium sulfate has been reported to produce serum magnesium levels within the range (4.2-8.4 mg/dL, 0.35-0.70 mmol/L) recommended for eclamptic seizure prophylaxis.[27-29] Sham animals did not undergo acute hypertension, though experimental length was comparable. Separate groups of animals, LP (n=3), LP + Mg (n=3), and NP (n=3), were used for analysis of AQP4 protein expression and these animals did not undergo acute hypertension. All animals were housed in the University of Vermont Animal Care Facility, an American Association for the Accrediatation of Laboratory Animal Care accredited facility. All experimental procedures were approved by the University of Vermont Institutional Animal Care and Use Committee.

***In vivo* model of HTE and BBB permeability**

An *in vivo* model of HTE was used to determine BBB permeability during acute hypertension, as previously described.[8] Briefly, a lactated Ringer's solution containing two different-sized dye tracers was infused into the left ventricle of the heart, 0.1% sodium fluorescein (NaFl, 376 Da) and 2% Evan's blue (EB, 69 kDa; all reagents from Sigma, St. Louis, MO unless otherwise specified). This solution was allowed to circulate for 10 minutes prior to acute hypertension, induced by intravenous infusion of phenylephrine (0.01g /10 mL lactated Ringer's solution). Following 10 minutes of arterial blood pressure (ABP) of 180 mmHg or greater, sufficient to cause

autoregulatory breakthrough,[8] the animal was perfused with lactated Ringer's solution through the central catheter in order to flush the dye from the cerebral circulation. Animals were decapitated and the brain was quickly removed, sectioned, and weighed. The cerebral cortices were separated from the cerebellum and brainstem, and then further divided into anterior and posterior cerebrum by a coronal cut at the level of the optic chiasm. Any animal in which gross examination revealed inadequate flushing of the cerebrovasculature was excluded from analysis. Samples were processed as previously described,[8] and the resulting supernatant was analyzed by fluorescence spectrophotometry at 460 to 515 nm for NaFl and 620 to 680 nm for EB. Data are expressed as average fluorescence counts per second (CPS) per gram brain tissue.

Western blot analysis of AQP4 expression

Following anesthesia with isoflurane (Abbott, North Chicago, IL) and decapitation, brains were carefully removed and divided into anterior and posterior cerebrum, as described above. Brain sections were snap frozen in liquid nitrogen and stored at -80° C. For protein extraction, each section was homogenized in a glass Dounce tissue grinder with 3 mL lysis buffer consisting of 50 mM Trizma® hydrochloride, 150 mM NaCl, 10 mM EDTA, 0.25% deoxycholate, 1% nonylphenyl polyethylene glycol detergent (Calbiochem, San Diego, CA), 10% glycerol, 1% sodium dodecyl sulfate, 1 mM dithiothreitol (Bio-Rad, Richmond, CA), and 1% protease inhibitor cocktail. The homogenate was transferred and centrifuged at 3900 rcf for 10 minutes at 4° C. The supernatant was centrifuged again under the same conditions. The total amount of protein was measured using the Coomassie Plus-Bradford™ Assay

Kit (Pierce, Rockford, IL). Protein samples were incubated in Laemmli sample buffer (Bio-Rad, Richmond, CA) with 2-beta-mercaptoethanol at 95^{o}C for 10 minutes. Protein (10 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Bio-Rad, Richmond, CA). Membranes were blocked for 20 minutes at room temperature in 3% non-fat milk in phosphate buffered saline containing 0.005% Tween-20 (PBST; Calbiochem, San Diego, CA), cut vertically, and subsequently incubated overnight at 4^{o}C with two primary antibodies: an affinity purified rabbit polyclonal raised against residues 249 to 323 of rat aquaporin-4 1:1,000 (Chemicon, Temecula, CA) and a mouse monoclonal antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 1:30,000 (Biodesign, Saco, ME). Following washing steps in PBST, membranes were incubated in secondary antibodies conjugated to horseradish peroxidase for one hour at room temperature. A sheep anti-rabbit IgG (Abcam, Cambridge, MA) 1:2,000 was used for AQP4 and a goat anti-mouse IgG (Pierce, Rockford, IL) 1:3,000 was used for GAPDH. Additional washings in PBST were followed by chemiluminescence using SuperSignal West Pico Substrate and CL-XPosure Film (Pierce, Rockford, IL). Films were scanned into Adobe Photoshop CS. All experiments were done in duplicate.

Statistical Analysis

All data are expressed as mean ± SEM. Differences in animal characteristics and tissue fluorescence between treatment groups were determined by an analysis of variance with three treatment groups and a posthoc Student-Newman-Keuls test for multiple comparisons. Differences in tissue fluorescence between anterior and

posterior cerebrum within the same treatment group were determined by t-test. Likewise, differences between baseline and maximum ABP within the same treatment group were determined by t-test. Differences were considered significant if $P< 0.05$.

Results

The Table summarizes animal characteristics for *in vivo* BBB permeability experiments. There were no significant differences between treatment groups in either animal weight or litter size. Baseline ABP, measured directly by femoral arterial catheter, was not significantly different between groups. Animals receiving magnesium tended to have lower ABP, however this difference was not significant. In the groups that underwent acute hypertension, there was a significant increase in ABP accompanied by a significant increase in CBF ($P<0.05$ vs. Sham and $P<0.05$ vs. baseline ABP).

Acute hypertension significantly increased BBB permeability to EB in both the anterior and posterior cerebrum, and this was attenuated by magnesium sulfate treatment (Figure 1). In addition, acute hypertension caused a greater increase in BBB permeability to EB in the posterior cerebrum where there was a 659% increase versus sham, compared to a 365% increase versus sham in the anterior cerebrum ($P<0.05$). Magnesium sulfate treatment decreased permeability to EB in the anterior and posterior cerebrum, 31% (n.s.) and 41% ($P<0.05$) respectively.

Similar to EB, acute hypertension increased NaFl permeability in the posterior cerebrum by 184% and in the anterior cerebrum by 134%, although these increases

were not statistically significant (Figure 2). There was no difference in BBB permeability to NaFl between anterior and posterior cerebrum within the same treatment group. Magnesium sulfate treatment partially decreased NaFl permeability in the anterior and posterior cerebrum by 40% and 31% respectively (n.s.).

In both the anterior and posterior cerebrum, AQP4 expression appeared to be increased in LP versus NP animals, similar to previous results.[30] However, magnesium treatment did not have any appreciable effect on AQP4 visually. Figure 3 shows representative Western blots for both the anterior (Panel A) and posterior (Panel B) cerebrum. In all animals studied, the posterior cerebrum showed similar or increased levels of AQP4 expression, suggesting a potential regional heterogeneity in expression.

Discussion

There are several major findings of this study. First, in brains from LP animals BBB permeability to EB was increased during acute hypertension and this was partially attenuated by magnesium sulfate treatment. Second, BBB permeability varied regionally, such that the posterior cerebrum showed a greater increase in permeability during acute hypertension than the anterior cerebrum. Third, magnesium treatment did not appear to have an effect on AQP4 expression in the anterior and posterior cerebrum. These results suggest that magnesium sulfate limits BBB permeability most effectively in an area of the brain particularly affected in eclampsia. In addition, the effect of magnesium sulfate on BBB permeability does not appear to be mediated by an effect on AQP4 expression.

This study used a model of HTE during pregnancy to investigate how acute hypertension affects BBB permeability, similar to eclampsia. This model of HTE has been extensively used to investigate the effect of cerebral hemodynamics on BBB permeability and vasogenic edema formation.[8, 31-36] Previously, we showed that after ten minutes of acute hypertension, LP animals had significant cerebral edema formation that was not seen in NP animals.[8] That study also showed that acute hypertension increased BBB permeability to NaFl, but not significantly. In the present study, we used both NaFl and EB to investigate BBB permeability, and, similar to previous findings, found a non-significant increase in NaFl permeability during acute hypertension. However, permeability to EB was significantly increased during acute hypertension. Evan's blue binds to albumin and is commonly used as a marker of BBB permeability.[25] Other studies investigating the effects of magnesium sulfate on BBB permeability examined only permeability to EB,[20, 24-26] and our results agree with these reports. Importantly, magnesium sulfate treatment decreased EB permeability during acute hypertension, suggesting that it is protective of the BBB and may limit vasogenic edema under conditions that cause it.

The increase in permeability to EB during acute hypertension was the greatest in the posterior cerebrum, a region of the brain that is most susceptible to edema formation in eclampsia and RPLS.[1-4, 6, 7] A magnetic resonance imaging study showed that 93% of eclamptic women showed signs of vasogenic edema, predominantly in the posterior regions of the brain.[1] Animal studies have shown greater BBB disruption in the cerebrum versus the brainstem,[31, 34] and more specifically greater BBB permeability

has been reported in the parietal, temporal, and occipital regions of the cortex caused by acute hypertension.[33, 35, 37] Importantly, in the current study magnesium sulfate treatment decreased BBB permeability to EB most effectively in the posterior region of the brain.

The mechanism by which magnesium sulfate acts to decrease BBB permeability is not clear from this study, but it may be related to a direct effect on the cerebral endothelium. Magnesium sulfate is a calcium antagonist,[38] and may decrease paracellular transport through tight junctions by opposing calcium-induced contractions of the actin cytoskeleton in endothelial cells. Alternatively, acute hypertension has been shown to increase pinocytosis that may enhance transcellular transport in the cerebral endothelium.[37, 39, 40] Magnesium may counter this mode of transport and decrease BBB permeability during acute hypertension. In support of this concept, it has been suggested that transport of large molecules across the BBB implicates transcellular versus paracellular transport.[32] In this study, magnesium treatment had a greater effect on a large solute (EB) than on a small solute (NaFl) suggesting transcellular transport under these conditions. This result may explain why we did not observe an increase in BBB permeability to NaFl during acute hypertension in the current or previous studies.[8]

While the results of the current study support our earlier findings of increased AQP4 expression in the brain during pregnancy,[30] magnesium sulfate treatment had no effect on AQP4 expression. Magnesium treatment has been reported to cause a qualitative change in AQP4 immunoreactivity such that AQP4 distribution in

magnesium-treated animals was more similar to uninjured animals following traumatic brain injury than injured.[22] In the present study, we did not evaluate AQP4 expression after injury, but investigated the effect of pregnancy and magnesium treatment on naïve brains. The role of AQP4 in mediating cerebral edema is still not clear, however it has been demonstrated that AQP4 promotes resolution of vasogenic edema,[41] the type of edema formed following disruption of the BBB in eclampsia. From the results of this study it does not appear that magnesium sulfate has an effect on total AQP4 expression levels, and the effects of magnesium sulfate on BBB permeability do not seem to be related to AQP4 expression.

There are several limitations of the current study that are worth noting. First, the relatively small n-value in each group may have produced a type-II error in some of our analyses, such as the NaFl. This was because several animals were excluded from analysis due to technical problems, including insufficient clearance of dye during flushing. In addition, BBB permeability to NaFl was highly variable with high background fluorescence, as seen in the sham animals, which likely precluded statistically significant differences. Second, the effect of magnesium treatment on BBB permeability during acute hypertension was studied during normal pregnancy, not under conditions of oxidative stress or endothelial cell damage which may play a role in eclampsia. However, this model of HTE in normal pregnancy is valuable for evaluating regional differences in BBB permeability and the effect of magnesium treatment on these parameters. Despite these limitations, the fact that magnesium

treatment decreased BBB permeability during acute hypertension may provide insight into the beneficial effect of magnesium sulfate treatment in eclampsia.

Summary

To our knowledge, this is the first study to investigate magnesium sulfate treatment on BBB permeability during acute hypertension in pregnancy. The results demonstrate that treatment with magnesium sulfate decreased BBB permeability following acute hypertension, particularly in the posterior region of the cerebrum. Magnesium treatment may limit edema formation in eclampsia by attenuating BBB permeability in response to acute hypertension. A more complete understanding of the effect of magnesium sulfate on the BBB may provide information regarding the beneficial effect of magnesium in eclampsia treatment and prophylaxis.

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Table 1. Characteristics of late-pregnant animals studied for permeability experiments

Group	Weight (g)	Litter Size	Baseline ABP (mmHg)	Maximum ABP (mmHg)	Maximum rCBF
Sham (n=4)	336±4	12±1	118±5	126±5	1.1±0.0
HTN (n=4)	343±16	12±1	112±7	185±8*†	2.2±0.5*
HTN + Mg (n=4)	354±11	12±1	103±4	184±6*†	2.4±0.2*

Abbreviations: ABP = Arterial Blood Pressure**,** rCBF = relative cerebral blood flow,

*P<0.05 vs. sham, †P<0.05 vs. respective baseline ABP

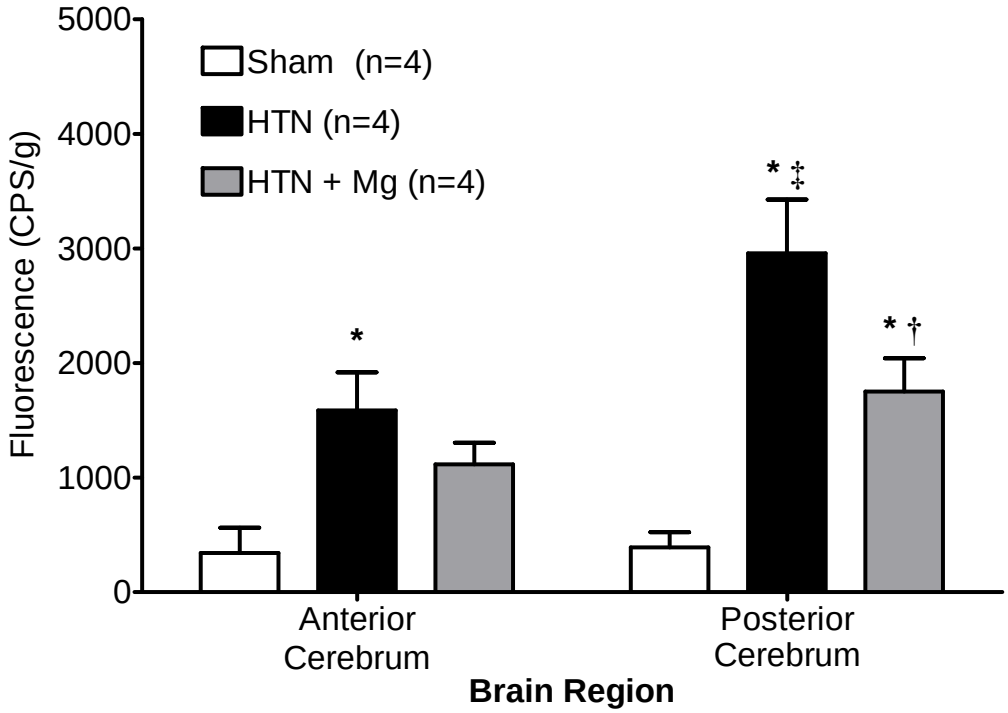

Figure 1: Graph of blood-brain barrier permeability to Evan's blue

Graph showing average fluorescence (CPS/g) of Evan's blue in the anterior and posterior cerebrum of late-pregnant animals as a measure of blood-brain barrier (BBB) permeability during acute hypertension. BBB permeability was significantly increased with acute hypertension (HTN, **P*<0.05 versus Sham), which was greater in the posterior versus anterior cerebrum (‡ *P*<0.05 versus anterior cerebrum). Magnesium treatment significantly decreased BBB permeability in the posterior cerebrum (HTN + Mg, †*P*<0.05 versus HTN).

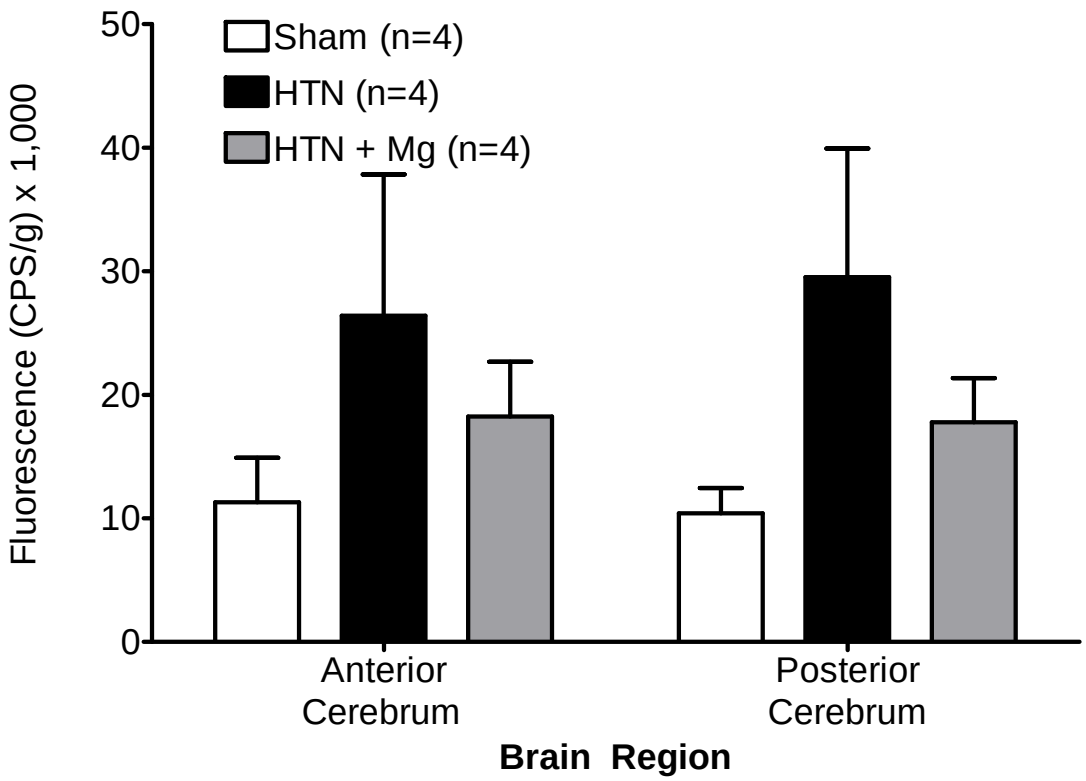

Figure 2: Graph of blood-brain barrier permeability to sodium fluorescein

Graph showing average fluorescence (CPS/g) of sodium fluorescein in the anterior and posterior cerebrum of late-pregnant rats as a measure of blood-brain barrier (BBB) permeability during acute hypertension. Acute hypertension (HTN) increased BBB permeability in both brain regions, though not significantly. Magnesium treatment (HTN + Mg) decreased BBB permeability, however, this also was not statistically significant.

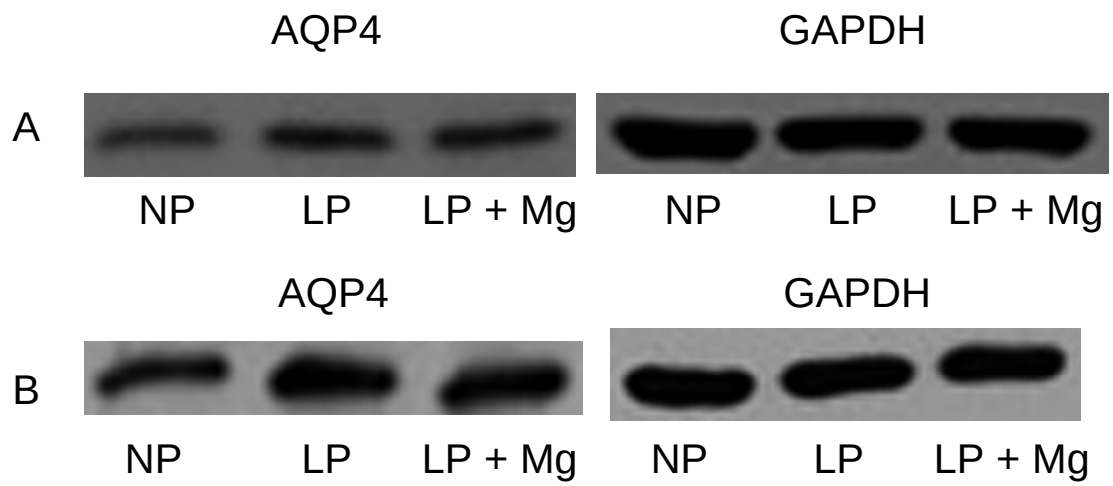

Figure 3: Aquaporin-4 expression in rat brain with magnesium treatment

Representative Western blots of aquaporin-4 (AQP4) expression in the rat brain with and without magnesium treatment. AQP4 expression was up during late-pregnancy (LP) compared to nonpregnant (NP) animals, and treatment with magnesium (LP + Mg) did not have an observable effect. Similar results were obtained in all animals studied (n=3 in each group). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) blots from the same gel are included for comparison of expression levels.

A) Anterior cerebrum

B) Posterior cerebrum

CHAPTER 5: SUMMARY AND CONCLUSIONS

Eclampsia, a hypertensive disorder of pregnancy, is thought to be a form of HTE in which an acute elevation of ABP exceeds the autoregulatory range and causes forced dilatation of cerebral vessels, decreased CVR, hyperperfusion, BBB disruption, and vasogenic cerebral edema formation. During pregnancy, there are many adaptations in the maternal cardiovascular system, however relatively little is known about changes in the cerebrovasculature and CBF during pregnancy. The overall goal of this dissertation project was to understand how pregnancy affects CBF autoregulation and BBB permeability, a principle mechanism of vasogenic edema formation, during acute hypertension.

Using an *in vivo* model of HTE, we found that the upper limit of CBF autoregulation was not different between NP and LP rats. In addition, although BBB solute permeability was not different between NP and LP animals during acute hypertension, cerebral edema formation, indicated by brain water content, was significantly increased only in LP rats. These results suggest that changes during normal pregnancy may predispose women to eclampsia when ABP is acutely elevated above normal levels by potentiating cerebral edema formation. The role of NO in mediating CBF autoregulation and edema formation was also determined. It was found that NOS inhibition dramatically increased the pressure of autoregulatory breakthrough in both NP and LP animals, suggesting that autoregulatory breakthrough is an active process requiring NO. Interestingly, NOS inhibition attenuated cerebral edema formation in LP animals following acute hypertension.

Despite an unclear mechanism of action, $MgSO_4$ is widely used to treat eclampsia. An additional goal of this project was to investigate the effects of $MgSO_4$ on the cerebrovasculature during pregnancy; specifically, the effect of $MgSO_4$ on *in vitro* resistance artery vasodilation and *in vivo* BBB permeability during acute hypertension were studied. It was found that while both cerebral and mesenteric resistance arteries vasodilated to $MgSO_4$, mesenteric arteries were more sensitive than cerebral arteries. Pregnancy further decreased $MgSO_4$ sensitivity in cerebral arteries, but had no effect on mesenteric artery vasodilation, suggesting that changes in cerebrovascular vasodilatory mechanisms occur with pregnancy.

The results from *in vitro* studies demonstrated a limited effect of $MgSO_4$ on cerebral arterial diameter. Therefore, we subsequently hypothesized that in eclampsia $MgSO_4$ may protect the brain by decreasing BBB disruption during acute hypertension. Magnesium treatment has been shown to protect the BBB in other conditions that may cause cerebral edema. Using an *in vivo* model of HTE and *in situ* brain perfusion in LP rats, it was demonstrated that $MgSO_4$ treatment decreased BBB permeability during acute hypertension. Interestingly, the greatest effect was seen in the posterior cerebrum, a region of the brain that is particularly affected in eclampsia and HTE. Magnesium treatment may limit edema formation in eclampsia by attenuating BBB permeability during acute hypertension. A more complete understanding of the effect of magnesium sulfate on the BBB may provide further information regarding the beneficial effect of magnesium in eclampsia treatment and prophylaxis.

Future Directions - The results of these studies lead to further intriguing experimental questions. This dissertation determined the effects of ~10 minutes of acutely elevated ABP. It would be interesting to investigate the effect of a longer period of severe hypertension, on the scale of hours to days, and determine subsequent BBB permeability and cerebral edema formation, perhaps using a continuous infusion of a pressor agent. This may more closely represent the clinical situation in eclamptic women where pressure is likely elevated for some time before neurological symptoms become evident. Because it is thought that $MgSO_4$ may aid the resolution of vasogenic edema formation, it would be of interest to determine the effects of $MgSO_4$ treatment during extended hypertension. It would also be interesting to combine the *in vivo* model of HTE with a rat model of preeclampsia that incorporates systemic endothelial dysfunction, such as the reduced uterine perfusion pressure model. This could possibly represent a more complete spectrum of systemic and cerebral changes in preeclampsia and eclampsia, and may determine if endothelial dysfunction in eclampsia contributes to the onset of seizures at lower blood pressures in eclamptic patients versus HTE patients.

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APPENDIX A: STRUCTURAL AND FUNCTIONAL CHANGES IN CEREBRAL VS. MESENTERIC RESISTANCE ARTERIES DURING GESTATION

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Abstract: It is known that pregnancy induces systemic vascular remodeling; however, how pregnancy affects the cerebral circulation that may predispose to eclampsia is not clear. Third-order branches of the posterior cerebral (PCA) and mesenteric artery (MA) were isolated from non-pregnant (NP, n=6), late pregnant (LP, d19, n=6), and postpartum (PP, d3, n=6) SD rats and studied under pressurized conditions to determine both active (tone) and passive (distensibility) responses to pressure (10-150 mmHg). In all gestational groups, PCAs had greater tone than MAs (21-30% vs. 9-11% at 100 mmHg, $p<0.05$)). In addition, pregnancy influenced how PCAs responded to pressure: LP had greater tone vs. NP and PP at lower pressures (50 mmHg) whereas PP did not develop tone until ≥100 mmHg. MAs were 30-211% more distensible than PCAs, and responded to pregnancy by increasing distensibility in LP and PP animals by 97% and 211% ($p<0.05$). These results demonstrate that gestation caused structural remodeling of the MA that was not present in the PCA. However,

pregnancy-induced changes in myogenic reactivity of the PCA may be an important consideration when blood pressure is elevated as during eclampsia.

Introduction: It is well-known that systemic vascular remodeling occurs during pregnancy to accommodate increases in cardiac output and blood volume, and a decrease in peripheral vascular resistance.[1] However, how this remodeling affects the cerebral circulation vs. the systemic circulation is not clear. Changes in the cerebral circulation during pregnancy may predispose the brain to damage from acute increases in pressure during eclampsia.[2]

Structural and functional changes in the vasculature can be inferred from the characteristic properties of passive distensibility and active tone respectively. Passive distensibility provides an indication of extracellular matrix remodeling, or structural changes in the vessel. Myogenic reactivity is the ability of the vessel, particularly cerebral vessels, to react to changes in intraluminal pressure with changes in diameter. In the present study, we compared gestation-induced changes in structural and functional properties of cerebral and mesenteric arteries.

Methods: Third-order branches (<200 μm) of the posterior cerebral and mesenteric arteries were isolated, both from the same NP (n=6), LP (d19, n=6), or PP (d3, n=6) Sprague Dawley rats. Arteries were mounted on glass cannulas in a dual chamber arteriograph bath, one in each chamber. This system allowed control over intravascular pressure and measurement of lumen diameter. After an equilibration

period at 50mmHg, pressure was increased to 75mmHg, lumen diameter was measured and the amount of spontaneous tone calculated. Passive responses to pressure and distensibility were determined after treatment with papaverine, a smooth muscle contractile inhibitor.

Results: MAs were more distensible than PCAs at each gestational age, and responded to pregnancy by increasing distensibility in LP and PP animals by 97% and 211% respectively ($p<0.05$). There was no difference in distensibility in PCAs between gestational groups.

In all gestational groups, PCAs had greater tone than MAs (21-30% vs. 9-11% at 100 mmHg, $p<0.05$). Additionally, pregnancy influenced how PCAs responded to pressure. PCAs from LP animals had greater tone vs. NP and PP at lower pressures (50 mmHg). PCAs from PP animals did not develop similar levels of tone until ≥100 mmHg.

Discussion: These results demonstrate that gestation caused structural remodeling of the MA that was not present in the PCA. The increase in distensibility in MAs from LP and PP animals found in this study is similar to previously published data on the mesenteric resistance arteries in pregnancy.[3] Our study found no evidence of changes in passive distensibility of the cerebral vessels with pregnancy, a vascular bed of which little is known during pregnancy.

Despite the absence of structural changes observed in cerebral resistance arteries, functional changes with gestation were observed. PCAs from LP animals developed tone at lower pressures, but had less tone than PCAs from NP animals at higher pressures. Pregnancy-induced changes in myogenic reactivity of the PCA may be an important consideration when blood pressure is elevated as during eclampsia.

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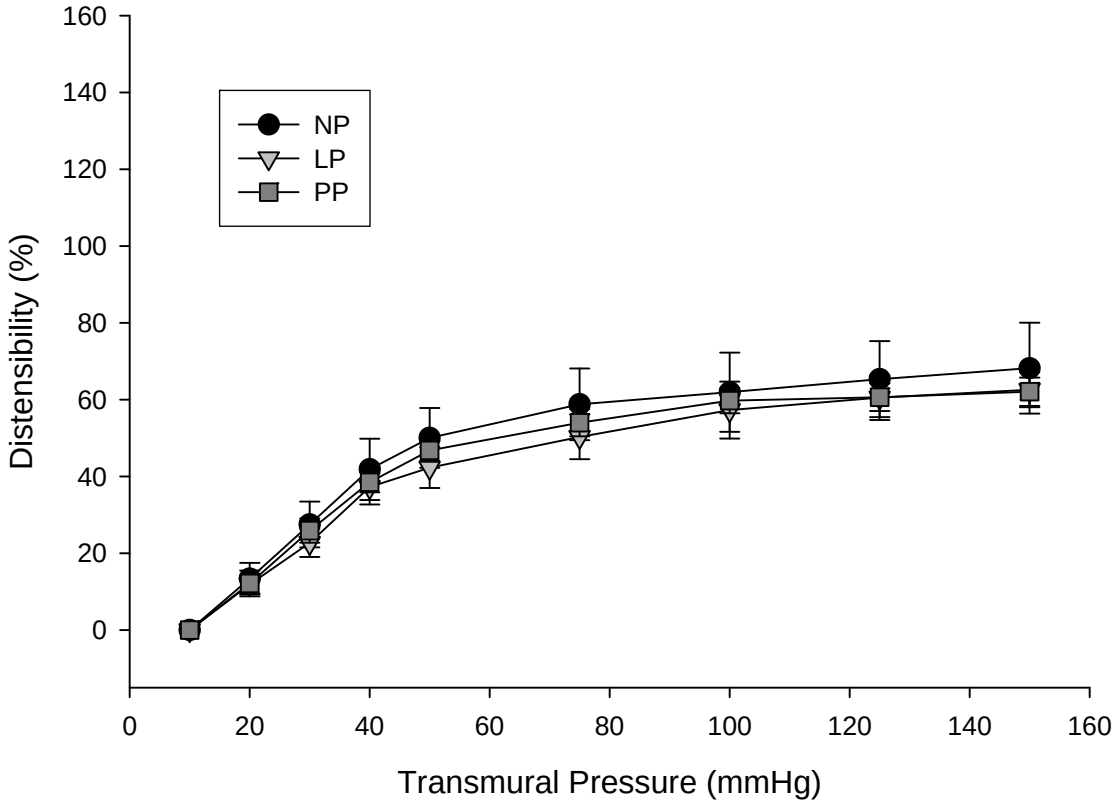

Figure 1: Posterior cerebral artery distensibility over gestation

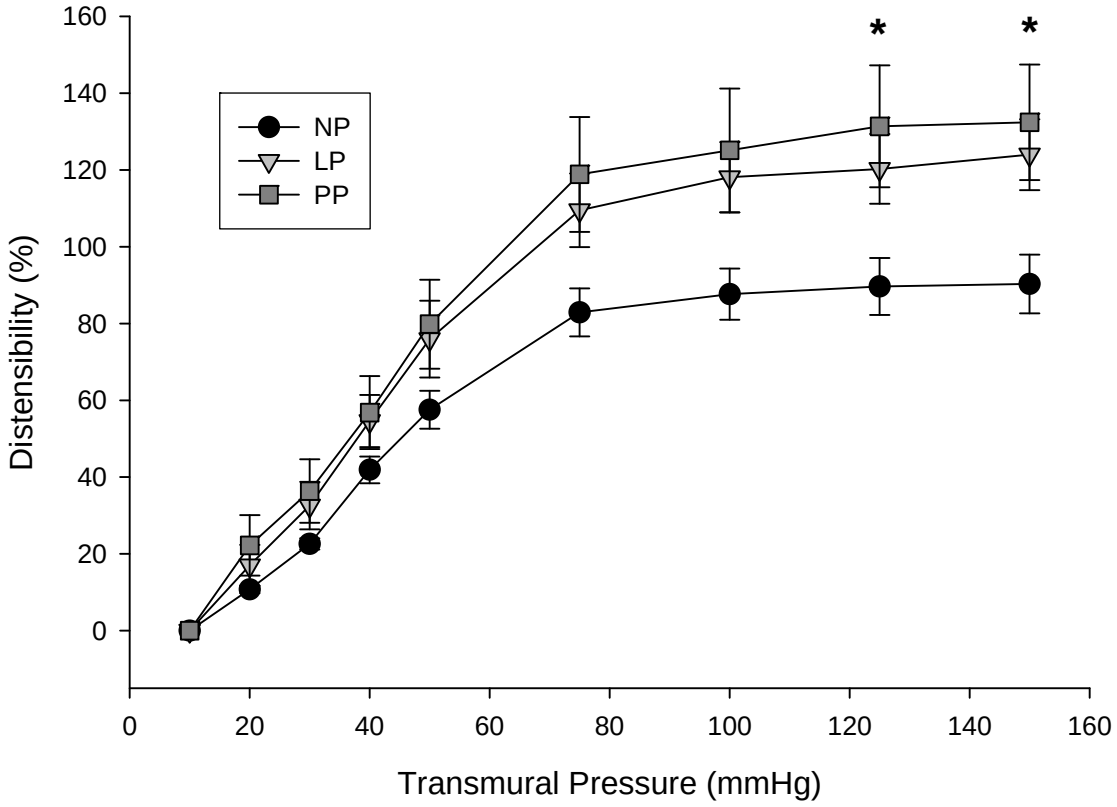

Figure 2: Mesenteric artery distensibility over gestation

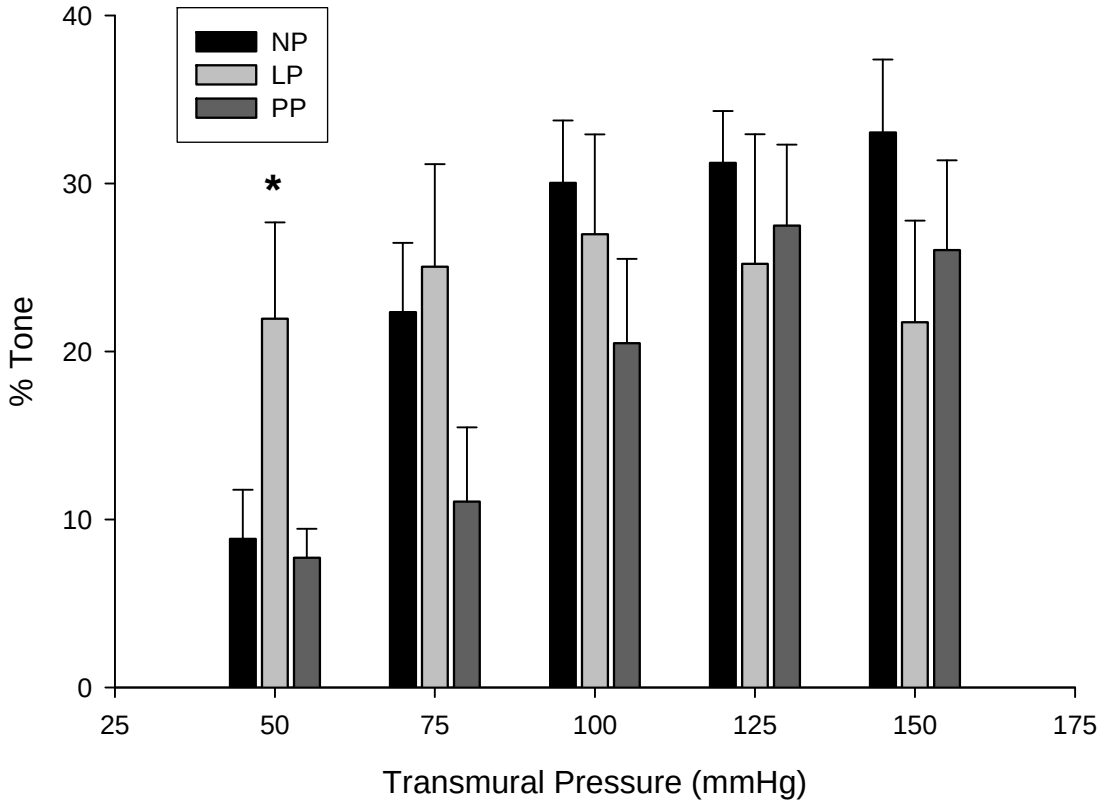

Figure 3: Posterior cerebral artery, percent tone over gestation

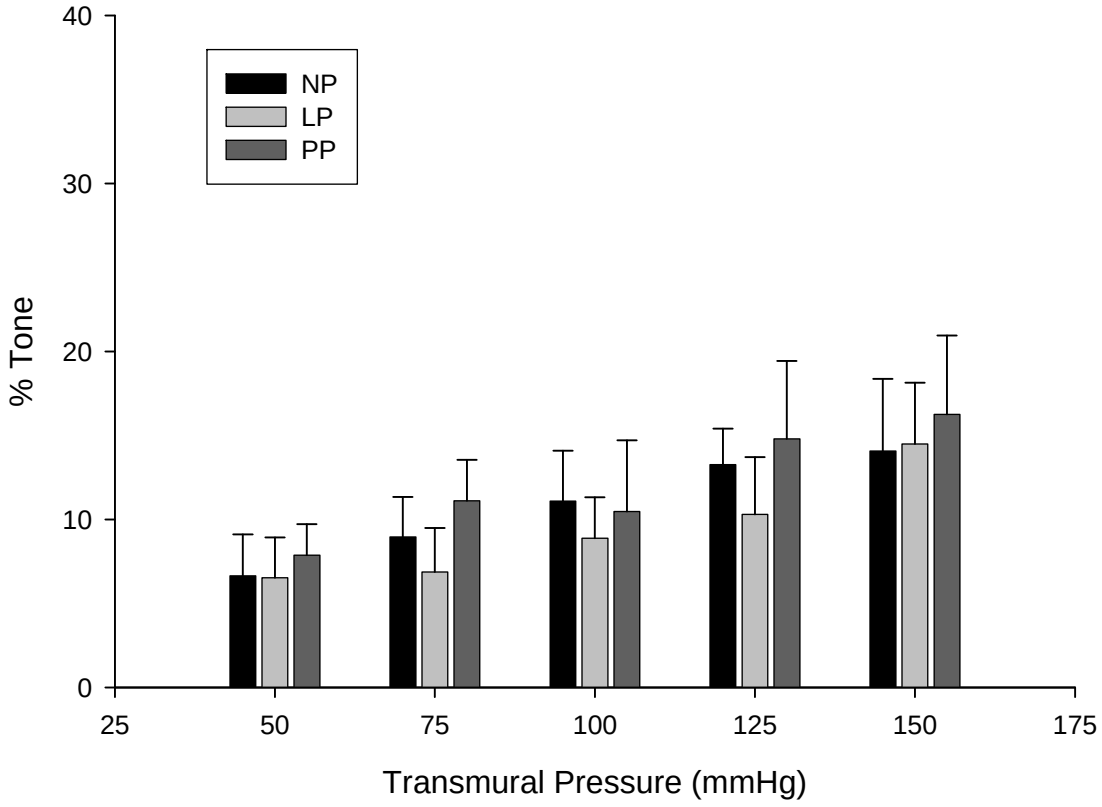

Figure 4: Mesenteric artery, percent tone over gestation