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Magnesium sulphate treatment decreases blood–brain barrier permeability during acute hypertension in pregnant rats

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Eclampsia is associated with increased blood–brain barrier (BBB) permeability and formation of cerebral oedema. Magnesium sulphate is used to treat eclampsia despite an unclear mechanism of action. This study was to determine the effect of magnesium sulphate on *in vivo* BBB permeability and formation of cerebral oedema during acute hypertension and on brain aquaporin-4 (AQP4) protein expression. An *in vivo* model of hypertensive encephalopathy was used in late-pregnant (LP) rats following magnesium sulphate treatment, 270 mg kg^{-1} I.P. injection every 4 h for 24 h. Permeability of the BBB was determined by *in situ* brain perfusion of Evan's Blue (EB) and sodium fluorescein (NaFl), and dye clearance determined by fluorescence spectrophotometry. Cerebral oedema was determined following acute hypertension by measuring brain water content. The effect of magnesium treatment on AQP4 expression was determined by Western blot analysis. Acute hypertension with autoregulatory breakthrough increased BBB permeability to EB in both brain regions studied ($P < 0.05$). Magnesium attenuated BBB permeability to EB during acute hypertension by 41% in the posterior cerebrum ($P < 0.05$) but had no effect in the anterior cerebrum ($P > 0.05$). Treatment with magnesium did not change NaFl permeability, cerebral oedema formation or AQP4 expression. In summary, BBB permeability to Evan's Blue was increased by acute hypertension in LP rats, and this was attenuated by treatment with magnesium sulphate. The greatest effect on BBB permeability to EB was in the posterior cerebrum, an area particularly susceptible to oedema formation during eclampsia.

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Eclampsia is a serious hypertensive disorder of pregnancy associated with increased blood–brain barrier (BBB) permeability and subsequent vasogenic oedema formation (Schwartz *et al.* 1992, 2000; Engelter *et al.* 2000; Zeeman *et al.* 2004). This condition is thought to be a form of hypertensive encephalopathy (HTE; Schwartz *et al.* 1992, 2000; Easton, 1998), and both eclampsia and HTE are causes of posterior reversible encephalopathy syndrome (PRES; Hinchey *et al.* 1996; Lamy *et al.* 2004). Using a model of HTE, we previously showed that acutely elevated arterial pressure with breakthrough of cerebral blood flow (CBF) autoregulation caused a significant increase in cerebral oedema formation in late-pregnant (LP) rats, which was not seen in non-pregnant (NP) control rats despite similar pressures of autoregulatory breakthrough (Euser & Cipolla, 2007). This suggests that pregnancy alone promotes oedema formation under conditions of acute hypertension.

Magnesium sulphate is widely used in North America both to prevent and to treat eclamptic convulsions (Witlin & Sibai, 1998). This treatment has been proven to be more effective than anticonvulsant drugs and placebo (The Eclampsia Trial Collaborative Group, 1995; Altman *et al.* 2002), though the mechanism of action remains unclear. Some studies suggest that magnesium may prevent eclamptic seizures via vasodilatation in the cerebral circulation (Belfort & Moise, 1992; Belfort *et al.* 1993; Naidu *et al.* 1996). However, magnesium treatment has also been reported to have little to no effect on cerebral haemodynamics and CBF (Belfort *et al.* 1999; Sherman *et al.* 2003; Hatab *et al.* 2005). For example, we previously showed that while magnesium

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sulphate has a modest vasodilatory effect on cerebral resistance arteries, the sensitivity of this response is decreased by pregnancy and the postpartum state (Euser & Cipolla, 2005). Furthermore, a randomized controlled trial found that when compared with nimodipine, a calcium channel blocker with specific cerebral vasodilator action, magnesium sulphate was more effective in preventing eclamptic seizures (Belfort *et al.* 2003). Together, these results provide evidence that the primary action of magnesium sulphate in eclampsia is likely not to be the relief of cerebral vasospasm.

Treatment with magnesium sulphate has been reported to decrease BBB permeability and cerebral oedema formation in a variety of brain injury conditions, including traumatic brain injury (Okiyama *et al.* 1995; Feldman *et al.* 1996; Esen *et al.* 2003; Ghabriel *et al.* 2006), septic encephalopathy (Esen *et al.* 2005), hypoglycaemia (Kaya *et al.* 2001) and hyperosmolar mannitol injection (Kaya *et al.* 2004). We therefore hypothesized that the action of magnesium sulphate in the prophylaxis of eclampsia may be related to protection of the BBB during acute hypertension. The goal of this study was to determine the effect of treatment with magnesium sulphate on *in vivo* BBB permeability and formation of cerebral oedema following CBF autoregulatory breakthrough in LP rats. In addition, it has been proposed that magnesium sulphate may limit cerebral oedema formation by decreasing the expression of aquaporin-4 (AQP4; Ghabriel *et al.* 2006), 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 treatment with magnesium sulphate on AQP4 protein expression in LP rats without acute hypertension.

Methods

Animals

All experiments used a rat model of pregnancy in which primiparous Sprague–Dawley rats (Charles River, St Constant, PQ, Canada) were studied on day 19–21 of a 22 day gestation. For each outcome measure, one group of animals was treated with an I.P. injection of magnesium sulphate (270 mg kg^{-1} every 4 h for 24 h) prior to acute hypertension. This dosage of magnesium sulphate has been reported to produce serum magnesium levels in rats within the range (4.2–8.4 mg dl^{-1}, 0.35–0.70 mmol l^{-1}) recommended for eclamptic seizure prophylaxis in pregnant women over 12–24 h (Pritchard, 1955; Hallak *et al.* 1994; Leveno & Cunningham, 1999). For experiments investigating BBB permeability or cerebral oedema formation *in vivo*, three groups of LP rats were studied: animals without acute hypertension or magnesium sulphate treatment (sham, $n = 10$); animals that underwent acute hypertension alone (HTN, $n = 7$); and animals treated with magnesium sulphate prior to acute hypertension (HTN + Mg^{2+}, $n = 7$). Sham rats did not undergo acute hypertension, though experimental length was comparable. For analysis of AQP4 protein expression, separate groups of rats ($n = 3$ per group) were used: LP, LP + Mg^{2+} and non-pregnant (NP). These animals did not undergo acute hypertension, since we were interested in the effect of magnesium treatment on AQP4 protein expression only. 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

An *in vivo* model of HTE was used to determine both BBB permeability and cerebral oedema formation during acute hypertension, as previously described (Euser & Cipolla, 2007). This model of HTE is considered a model of eclampsia (Cipolla, 2007) and has been used previously to study BBB permeability and oedema formation during pregnancy (Euser & Cipolla, 2007). Briefly, anaesthesia was initiated with isoflurane ($\leq 3\%$ in oxygen, inhaled; Abbott, North Chicago, IL, USA) and maintained with I.V. pentobarbital (≤ 60 mg kg^{-1} h^{-1}; Ovation Pharmaceuticals, Deerfield, IL, USA), which was decreased during the surgical preparation, as tolerated, to minimize the effects of anaesthesia on experimental parameters. Adequate anaesthesia was assessed by toe pinch and changes in arterial blood pressure. During the course of each experiment, CBF was measured transcranially using laser Doppler flowmetry with a 1.0 mm probe (Perimed, North Royalton, OH, USA) 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 (Smeda *et al.* 1999). All laser Doppler measurements of CBF were normalized to the flow at baseline (after anaesthesia had been minimized and prior to acute hypertension) to determine a relative CBF (rCBF). The following equation was used:

$$rCBF = (CBF_{max}/CBF_{baseline})$$

where CBF_{max} is the flow in laser Doppler units at maximal pressure and $CBF_{baseline}$ is the flow in laser Doppler units prior to acute hypertension and after anaesthesia had been minimized. Therefore, a rCBF of 2.0 signifies a twofold increase in CBF from baseline.

Catheters were placed in the femoral artery and veins for arterial blood pressure measurement and drug delivery, respectively. Blood pressures were measured via a pressure transducer attached in-line with the catheter. Acute hypertension was induced by i.v. infusion of phenylephrine (0.01 g per 10 ml lactated Ringer's solution; all reagents from Sigma, St Louis, MO, USA unless otherwise specified). Following 10 min of blood pressure 180 mmHg, sufficient to cause autoregulatory breakthrough (Euser & Cipolla, 2007), the experiment was ended with the humane death of the animal by decapitation, and the brain was quickly removed. This model of HTE was used with slight alterations to determine both BBB permeability and cerebral oedema formation during acute hypertension, described in the subsections below.

Blood–brain barrier permeability studies

To determine the extent of BBB permeability, a lactated Ringer solution (100 ml of solution, USP contains: NaCl 600 mg, $NaC_3H_5O_3$ anhydrous 310 mg, KCl 30 mg and $CaCl_2$ dihydrate 20 mg. pH of the solution is 6.6 (6.0–7.5).) Containing two different-sized dye tracers, 0.1% sodium fluorescein (NaFl, 376 Da) and 2% Evan's Blue (EB, 69 kDa), was infused into the left ventricle of the heart via catheter in the carotid artery. This solution was allowed to circulate for 10 min prior to induction of acute hypertension by i.v. infusion of phenylephrine (0.01 g per 10 ml lactated Ringer solution). Following 10 min of blood pressure 180 mmHg, again sufficient to cause autoregulatory breakthrough (Euser & Cipolla, 2007), the animal was perfused with lactated Ringer solution through the central catheter in order to flush the dye from the cerebral circulation. Following decapitation, 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 sections by a coronal cut at the level of the optic chiasm. We chose to compare anterior and posterior cerebrums because of the propensity of oedema to form in the posterior brain regions during eclampsia and PRES (Schwartz *et al.* 1992, 2000; Hinchey *et al.* 1996; Engelter *et al.* 2000; Lamy *et al.* 2004; Zeeman *et al.* 2004; Cipolla, 2007). Any animal in which gross observation revealed inadequate flushing of the cerebrovasculature was excluded from analysis (this occurred in 2 animals). Samples were processed as previously described (Euser & Cipolla, 2007), and the resulting supernatant was analysed by fluorescence spectrophotometry at 460–515 nm for NaFl and 620–680 nm for EB. Data are expressed as average fluorescence counts per second (CPS) per gram brain tissue.

Determination of cerebral oedema

The percentage water content of the brain is a measure of cerebral oedema (Schwab *et al.* 1997). After elevated blood pressure had been maintained for 10 min, animals were quickly decapitated and the brain was then removed for wet and dry weight measurements. The brain was weighed wet immediately after removal and then dried at 100°C for 24 h, at which point the brain was weighed again dry. Brain water content (as a percentage) was determined by the following formula:

$$\text{Brain water content} = [(\text{weight}_{\text{wet}} - \text{weight}_{\text{dry}}) \div \text{weight}_{\text{wet}}] \times 100\%$$

where $\text{weight}_{\text{wet}}$ is the weight of the brain immediately after removal from the animal and $\text{weight}_{\text{dry}}$ is the weight of the brain after drying.

Western blot analysis of AQP4 expression

Following induction of anaesthesia with isoflurane (Abbott, North Chicago, IL, USA) 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 comprising: 50 mM Trizma® hydrochloride, 150 mM NaCl, 10 mM EDTA, 0.25% deoxycholate, 1% nonylphenyl polyethylene glycol detergent (Calbiochem, San Diego, CA, USA), 10% glycerol, 1% sodium dodecyl sulphate, 1 mM dithiothreitol (Bio-Rad, Richmond, CA, USA) and 1% protease inhibitor cocktail. The homogenate was transferred and centrifuged at 3900 g for 10 min 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, USA). Protein samples were incubated in Laemmli sample buffer (Bio-Rad) with 2β-mercaptoethanol at 95°C for 10 min. Protein (10 μg) was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Bio-Rad). Membranes were blocked for 20 min at room temperature in 3% non-fat milk in phosphate-buffered saline containing 0.005% Tween-20 (PBST; Calbiochem), cut vertically, and subsequently incubated overnight at 4°C with two primary antibodies: an affinity purified rabbit polyclonal antibody raised against residues 249–323 of rat aquaporin-4, 1:1000 (Chemicon, Temecula, CA, USA), and a mouse monoclonal antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1:30 000 (Biodesign, Saco, ME, USA). After repeated washing in PBST, membranes were incubated in secondary antibodies conjugated to horseradish peroxidase for 1 h at room temperature. A sheep antirabbit IgG (Abcam, Cambridge, MA, USA), 1:2000, was used for AQP4 and a goat antimouse IgG, 1:3000 (Pierce), was used for GAPDH. Additional washing steps in PBST were

Table 1. Physiological characteristics of animals studied

	Weight (g)	Litter size	Baseline ABP (mmHg)	Maximal ABP (mmHg)	Maximal rCBF
Sham ($n = 10$)	344 ± 4	13 ± 1	118 ± 5	126 ± 5	1.07 ± 0.027
HTN ($n = 7$)	336 ± 11	12 ± 1	116 ± 4	$191 \pm 3^{**}$	$2.41 \pm 0.29^{**}$
HTN + Mg^{2+} ($n = 7$)	342 ± 15	12 ± 1	102 ± 3†	$186 \pm 4^{**}$	$2.21 \pm 0.30^{**}$

Abbreviations: ABP, arterial blood pressure; rCBF, relative cerebral blood flow; and HTN, acute hypertension. $^{**}P < 0.01$ *versus* sham group within same experimental conditions; †$P < 0.05$ *versus* sham and HTN.

followed by chemiluminescence using SuperSignal West Pico Substrate and CL-XPosure Film (Pierce). Films were scanned into Adobe Photoshop CS, and densitometry of AQP4 and GAPDH bands was determined with ImageJ software (version 1.37v, available for download at http://rsb.info.nih.gov/ij/). All experiments were done in duplicate. The ratio of AQP4/GAPDH intensity was calculated for each animal in each group. Values for NP animals were assigned a value of 1.0 and LP and LP + Mg^{2+} values were normalized to the NP group before analysing each group.

Statistical analyses

All data are expressed as means ± s.e.m. Differences in animal characteristics, tissue fluorescence and brain water content between treatment groups were determined by ANOVA with a *post hoc* Student–Newman–Keuls test for multiple comparisons. Differences in tissue fluorescence between the anterior and posterior cerebrum within the same treatment group were determined by Student's paired *t* test. Likewise, differences between baseline and maximal blood pressures within the same treatment group were determined by Student's paired *t* test. Differences in AQP4 expression between LP and LP + Mg^{2+} groups were tested by non-parametric Mann–Whitney *U* test. Differences were considered significant if $P < 0.05$.

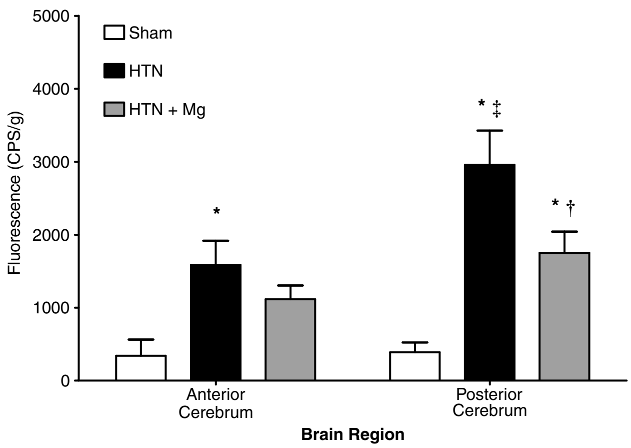

Figure 1. Bar graph showing average fluorescence (in CPS g^{-1}) of Evan's Blue in the anterior and posterior cerebrum of all groups of late-pregnant animals as a measure of BBB permeability

Blood–brain barrier permeability was significantly increased with acute hypertension and autoregulatory breakthrough (HTN) compared with sham control animals ($^{*}P < 0.05$). The increase in BBB permeability was greater in the posterior *versus* anterior cerebrum (‡$P < 0.05$). Magnesium sulphate treatment (HTN + Mg^{2+}) significantly decreased BBB permeability in the posterior cerebrum in response to acute hypertension (†$P < 0.05$ *versus* HTN).

Results

The physiological characteristics of the LP animals studied with the *in vivo* model of HTE are summarized in Table 1. Two animals were excluded from study, one from each group of HTN and HTN + Mg^{2+} animals, owing to insufficient flushing of the dye from the vasculature. There were no significant differences between treatment groups in either animal weight or litter size. Baseline mean arterial blood pressure, measured directly by femoral arterial catheter, was not significantly different between sham and HTN groups, but was significantly lower in animals treated with magnesium ($P < 0.05$). Infusion of phenylephrine to induce autoregulatory breakthrough caused a significant increase in arterial blood pressure in the HTN and HTN + Mg^{2+} groups compared with the sham group ($P < 0.01$). The increase in blood pressure was accompanied by a significant increase in CBF, as represented by the rCBF ≥ 2.0 for both hypertensive groups, indicating autoregulaotry breakthrough ($P < 0.01$ *versus* sham).

Acute hypertension with autoregulatory breakthrough significantly increased BBB permeability to EB in both the anterior and posterior cerebrum (Fig. 1). Interestingly, the increase in BBB permeability was significantly greater in the posterior brain region *versus* anterior (659 *versus* 365%, respectively, $P < 0.05$), a region most susceptible to oedema formation. Magnesium sulphate treatment decreased BBB permeability significantly only in the posterior brain region (Fig. 1). There was a 41% decrease in permeability in the posterior cerebrum ($P < 0.05$). The BBB permeability to NaFl was unaffected by hypertension or magnesium sulphate treatment (Fig. 2). The lack of a significant difference in NaFl permeability probably results from the higher variability in those groups compared with EB.

Brain water content, a measure of cerebral oedema formation (Schwab *et al.* 1997), was not different between any of the groups. The percentage water content was: $78.25 \pm 0.18\%$ for sham group, $78.13 \pm 0.02\%$ for LP

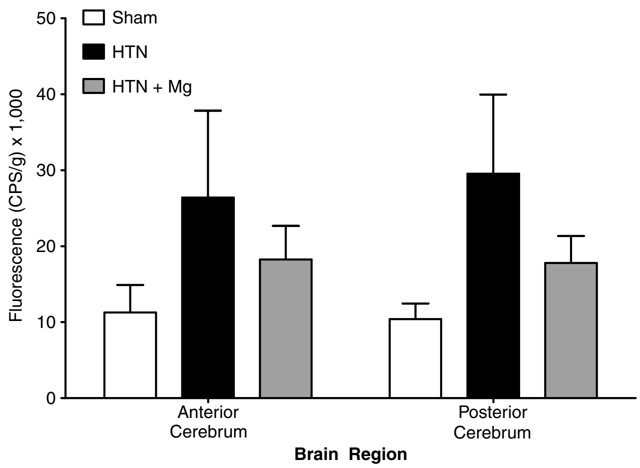

Figure 2. Bar graph showing average fluorescence (in CPS g^{-1}) of sodium fluorescein in the anterior and posterior cerebrum in all groups of late-pregnant rats as a measure of BBB permeability

There was no difference in BBB between animals that underwent acute hypertension (HTN) compared with sham control animals. Magnesium sulphate treatment (HTN + Mg^{2+}) also had no effect on BBB permeability to NaFl compared with HTN.

HTN group and $78.02 \pm 0.07\%$ for LP HTN + Mg^{2+} group (n.s.). In contrast to our previous findings (Euser & Cipolla, 2007), there was no significant difference in cerebral oedema formation between any of the groups.

Aquaporin-4 protein expression was determined in the anterior and posterior cerebrum of normotensive LP and LP + Mg^{2+} groups and compared with NP control animals. Expression of AQP4 was increased in LP and LP + Mg^{2+} compared with NP animals in both the anterior and posterior cerebrum by 3- to 4-fold (Fig. 3), a finding similar to previous results (Quick & Cipolla, 2005). Contrary to previous reports (Ghabriel *et al.* 2006), magnesium sulphate treatment did not have any effect on AQP4 expression.

Discussion

The major finding of this study was that magnesium sulphate treatment significantly decreased BBB permeability to EB in LP rats during acute hypertension, an effect that was significant in the posterior brain region. In fact, BBB permeability to EB varied regionally

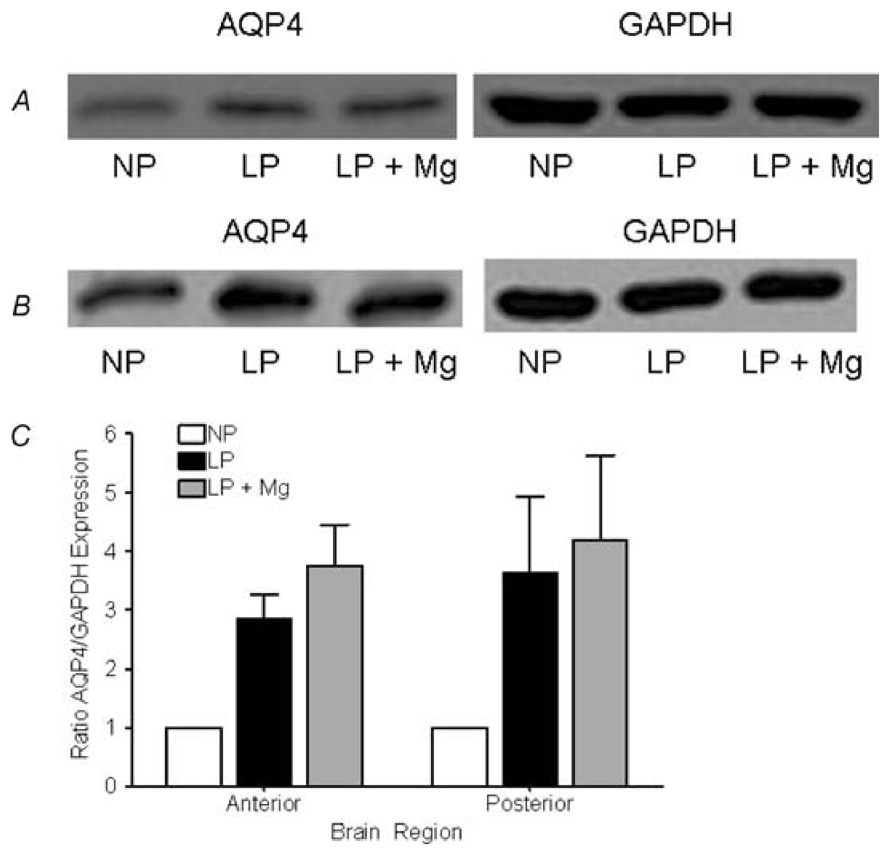

Figure 3. Aquaporin-4 (AQP4) expression in the female rat brain with and without magnesium treatment

Representative Western blots of AQP4 expression in late-pregnant (LP) rats in the anterior cerebrum (*A*) and posterior cerebrum (*B*). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) blots from the same gel were used as an internal control. *C* shows the intensity ratios for untreated (LP) and treated animals (LP + Mg^{2+}) compared with non-pregnant control animals (NP). Aquaporin-4 expression was increased in both LP and LP + Mg^{2+} animals compared with NP control animals, but was unchanged by magnesium sulphate treatment.

in response to autoregulatory breakthrough, such that the posterior cerebrum showed a greater increase in permeability than the anterior cerebrum. Since the posterior brain region is most susceptible to oedema formation during PRES and eclampsia (Schwartz *et al.* 1992, 2000; Hinchey *et al.* 1996; Engelter *et al.* 2000; Lamy *et al.* 2004; Zeeman *et al.* 2004; Cipolla, 2007), these results suggest that one mechanism by which oedema forms preferentially in this region is the enhanced BBB permeability to large proteins such as EB. Together, these results suggest that the beneficial effect of magnesium sulphate treatment during eclampsia may be related to protection of the BBB that limits solute flux into the brain tissue when hydrostatic pressure is elevated to pathological levels.

The mechanism by which magnesium sulphate 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 sulphate is a calcium antagonist (Fawcett *et al.* 1999) 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 (Nag *et al.* 1977, 1979; Westergaard *et al.* 1977). Magnesium may counter this mode of transport and decrease BBB permeability during acute hypertension. In fact, it has been suggested that transport of large molecules across the BBB implicates transcellular *versus* paracellular transport (Mayhan & Heistad, 1985). Since magnesium treatment had a greater effect on a large solute (EB) than on a small solute (NaFl), this suggests that transcellular transport was the primary mode of BBB permeability under these conditions.

Another protective effect of magnesium sulphate on limiting BBB permeability during acute hypertension may be related to its ability to scavenge free radicals. Free radical production during acute hypertension induced by pressor agents has been demonstrated (Zhang & Ellis, 1991). In fact, superoxide dismutase, an oxygen free radical scavenger, was shown to reduce BBB permeability and cerebral oedema during acute hypertension in rats (Zhang & Ellis, 1990). It is therefore possible that magnesium sulphate acts similarly to scavenge free radicals and protect the BBB, since treatment with magnesium sulphate has been shown to decrease lipid peroxidation in pre-eclamptic women, suggesting that it has free radical scavenging properties (Ariza *et al.* 2005).

In contrast to a previous study (Euser & Cipolla, 2007), we did not find that cerebral oedema formation was increased in response to acute hypertension in LP rats, or that magnesium sulphate treatment affected brain water content. This negative and contrary finding may be for several reasons. First, the change in rCBF was considerably less in the present study compared with what we have published previously. The purpose of the previous study (Euser & Cipolla, 2007) was to determine the pressure at which autoregulatory breakthrough occurred between NP and LP rats, and those animals routinely had rCBF > 3.0. This difference would make hydrostatic pressure less in the present group of animals compared with the previous study. Hydrostatic pressure is a primary determinant of brain oedema (Shima, 2003). Therefore, a lower rCBF would suggest a lower hydrostatic pressure and could account for the undetectable changes in brain water content in the present study. It is also possible that the duration of acute hypertension, being only 10 min, was not sufficient to cause a detectable increase in oedema formation with this degree of rCBF change. While this methodology has been commonly used to study BBB permeability in response to acute hypertension, it probably takes some time for oedema to form. Unfortunately, this non-survival model of HTE does not allow pressure to be increased for longer durations (probably due to peripheral vascular dilatation that decreases total peripheral vascular resistance and drops arterial blood pressure). Other methods in which the animals survive for longer periods, such as 2–3 days, would be better for assessment of oedema in response to acute hypertension and magnesium sulphate treatment.

Previous studies have suggested that magnesium sulphate treatment may limit cerebral oedema formation by decreasing the expression of AQP4 (Ghabriel *et al.* 2006). Aquaporin-4 is a transmembrane water channel highly expressed in the brain and shown to be upregulated during pregnancy (Quick & Cipolla, 2005). Owing to its water fluxing capabilities (Papadopoulos *et al.* 2004), a decrease induced by magnesium may limit oedema formation. For this reason, we investigated whether magnesium sulphate treatment affected AQP4 protein expression. Animals were treated with magnesium sulphate for 24 h, a duration consistent with clinical practice, but perhaps not long enough to have an effect on AQP4 protein levels. Figure 3 shows that AQP4 was increased in both pregnant groups compared with NP animals, but magnesium sulphate treatment had no effect on its expression. These measurements were done on normotensive animals because we wanted to determine whether there was any effect of magnesium treatment prior to a hypertensive insult that may limit oedema formation. How AQP4 protein expression changes in response to hypertension is of clear interest; however, animals would probably need to survive for longer time periods.

This study used a model of HTE during pregnancy to investigate how acute hypertension affects BBB permeability. This model has been extensively used to investigate the effect of cerebral haemodynamics on BBB permeability and vasogenic oedema formation (MacKenzie *et al.* 1976; Baumbach & Heistad, 1985;

Hatashita *et al.* 1985, 1986; Mayhan & Heistad, 1985; Mayhan *et al.* 1986; Euser & Cipolla, 2007) and is considered a model of eclampsia (Cipolla, 2007). It is worth noting, however, that we studied the effect of magnesium treatment on BBB permeability during normal pregnancy, not under conditions of oxidative stress or endothelial cell damage which may have a role in eclampsia. However, this model is valuable and has a number of the features relevant to the pathogenesis of eclampsia, including loss of autoregulation, hyperperfusion and BBB disruption. It is also useful for evaluating regional differences in BBB permeability, which are an important feature considering the regional heterogeneity in oedema formation during eclampsia.

To our knowledge, this is the first study to investigate the effect of magnesium sulphate treatment on BBB permeability during acute hypertension in pregnancy. The results demonstrate that treatment with magnesium sulphate decreased BBB permeability following acute hypertension, particularly in the posterior cerebrum. However, magnesium treatment did not affect cerebral oedema formation during the short time course studied. A more complete understanding of the effect of magnesium sulphate on the BBB may provide information regarding the beneficial effect of magnesium in the treatment and prophylaxis of eclampsia.

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