Eclampsia & preeclampsia

Aukes, Annet Maria

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Causes and Long-term Consequences of Maternal Brain Involvement

Annet M. Aukes

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Promotores	Prof. dr. J.G. Aarnoudse
	Prof. dr. M.J. Cipolla
Copromotor	Dr. G.G. Zeeman
Beoordelingscommissie	Prof. dr. A. Franx
	Prof. dr. C.J. de Groot
	Prof. dr. A. van der Lugt

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Introduction

Introduction

Preeclampsia is a pregnancy induced disease, characterized by the occurrence of hypertension (systolic ≥ 140 mmHg and/or diastolic ≥ 90 mmHg) and proteinuria in the second half of pregnancy in a previously normotensive woman. It complicates 5 to 7% of pregnancies in the United States.[1] It is a systemic disease of which the exact pathophysiology remains to be elucidated.

Preeclampsia is a systemic disease that can affect several organs including the liver, the kidneys and the brain. Involvement of the brain can lead to one of the most feared complications of preeclampsia: eclampsia. This is the onset of seizures or coma in a preeclamptic woman not attributable to any other cause. Accompanying symptoms are headache, visual disturbances (cortical blindness, scotoma, photopsia), photophobia, sonophobia, nausea, vomiting and altered mental status.[2] It is a life threatening disease for both mother and child and in the Netherlands cerebral complications such as eclampsia are responsible for most maternal deaths.[3]

The incidence of eclampsia in the Netherlands is 6.2 per 10,000 deliveries. The number of maternal deaths due to eclampsia is 1 in 74, fatality rate 1.4%.[4] Between 1993 and 2005 the maternal mortality rate of (pre)eclampsia in the Netherlands was 3.5 per 100,000 live births.[5] The major mode of maternal death due to hypertensive diseases is a cerebrovascular complication in 71% of the cases.[3] In other European countries the incidence of eclampsia is ranges from 2.4 to 5.0 per 10,000 deliveries[6,7] and ranging from 71 to 173 per 10,000 deliveries in developing countries.[8,9] The mortality rate from eclampsia in the UK is 1.8% [10], in the USA 0.5%[11] and ranging from 6.0-8.0% in developing countries.[9]

Why women with preeclampsia are susceptible to brain involvement is not clear. In addition, how pregnancy affects the brain is just being elucidated. Therefore, in our labs we investigate the brain during pregnancy in animal models and in patient studies we investigate the brain several years after a pregnancy complicated by eclampsia or preeclampsia.

Pathophysiology of eclampsia

There are two major theories on the pathophysiology of eclampsia, both based on failure of the cerebral autoregulation. One is the 'vasculopathy' theory which suggests that there is an 'overregulation' of the cerebral blood flow because previously it was thought that the neurologic symptoms of eclampsia were caused by ischemia. The theory behind this was that in reaction to acute hypertension the cerebral vasculature constricted too severely (overregulation of the cerebral blood flow) causing hypoxia and ischemia.

Evidence favoring this theory was the vasospasm seen on cerebral angiograms in some women with eclampsia.[12,13] Later newer imaging techniques showed presence of vasogenic edema, making ischemia as the only cause unlikely. More recently, Bartynski developed a more subtle theory based on the vasculopathy theory.[14] He hypothesizes that systemic toxicity in patients with (pre)eclampsia such as immune system activation, endothelial cell activation and injury, vascular instability and organ hypoperfusion, are associated with increased vasoconstriction of cerebral vasculature. The presence of systemic hypertension poses an additional trigger for vasoconstriction, leading to cerebral hypoperfusion and hypoxia. Prolonged hypoxia stimulates vascular endothelial growth factor (VEGF) release and activation of endothelial cells. Together, this may result in increased permeability of endothelium followed by vasogenic edema.[14] In support of this theory is the fact that vasogenic edema is typically found in the watershed zones. However, the suggestion that the cerebral vasculature would overregulate and constrict to such an extent to cause ischemia, seems unlikely. Therefore, we consider the second and currently more popular theory that focuses on loss of cerebral autoregulation, more plausible. In this theory it is hypothesized that in the presence of endothelial dysfunction an acute elevation of blood pressure exceeds the upper limit of the cerebral autoregulation leading to forced dilatation of cerebral arteries (Figure 1). Increased cerebral blood flow subsequently results in disruption of the blood brain barrier followed by extravasation of water and plasma solutes and formation of vasogenic edema.[15,16] Once the blood pressure decreases within normal limits of cerebral autoregulation, the vasogenic edema resolves and the neurological symptoms disappear.[17,18] Loss of cerebral autoregulation based on acute hypertension is called hypertensive encephalopathy. Eclampsia may be a form of hypertensive encephalopathy[19-21], however, not all patients with eclampsia have blood pressures high enough to reach the upper limit of cerebral autoregulation and 10% - 16% of eclamptic patients do not even become hypertensive.[10,11] In addition, a clinical and radiological condition similar to eclampsia has been recognized and is thought to be identical to eclampsia although the underlying triggering factor differs. This syndrome was first recognized by Hinchey et al. in 1996 and named 'reversible posterior leucoencephalopathy syndrome'.[17] Later, different names were introduced of which 'posterior reversible encephalopathy syndrome' (PRES) is currently most popular.[22,23]

Several aspects of the cerebral vasculature and its regulating mechanisms play an important role in the pathophysiology of eclampsia or PRES. In the following paragraphs the most important mechanisms of the cerebral vasculature will be discussed. These are cerebral autoregulation, the blood-brain barrier and perivascular innervation. The current knowledge about how these aspects are influenced by pregnancy will be discussed as well.

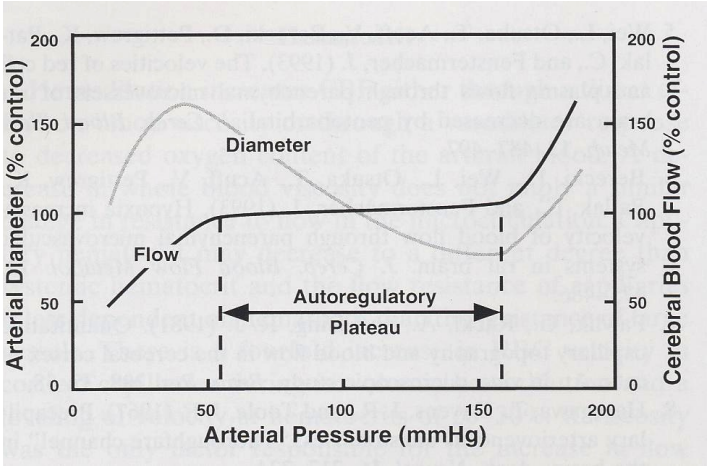

Figure 1 The cerebral autoregulation curve, adapted from Chillion and Baumbach 1997 and used with the permission of Elsevier Limited for Academic Press.[25]

Cerebral autoregulation

The term 'autoregulation' in relation to the cerebral circulation, was first introduced by Lassen in 1959.[24] Autoregulation of blood flow ensures that the blood flow through an organ is maintained at a relatively constant level despite changes in perfusion pressure or arterial blood pressure. Within the limits of cerebral autoregulation this is called the autoregulatory plateau (Fig 1), which ranges from approximately 50-60 to 150-160 mmHg in the healthy human.[25] This plateau is brought about by changes in the arterial diameter with vasoconstriction when blood pressure increases and vasodilatation when blood pressure decreases. When the mean arterial pressure is not within this range, autoregulation may be lost. In case of acute severe hypertension, such as in most cases of eclampsia, the cerebral arterial pressure exceeds the upper limit of autoregulation and cerebral blood flow increases linearly with increase in blood pressure.[26,27] Subsequently, the blood-brain barrier is disrupted and vasogenic edema develops.[15,16] When the blood pressure decreases, the cerebral blood flow normalizes and the edema resolves.[17-19]

Cerebral autoregulation is regulated by several control mechanisms.[28,29] One of those is the myogenic mechanism by which the small vessels react with constriction or vasodilation in response to changes in transmural pressure.[30,31] Secondly, there are metabolic mechanisms by which changes in metabolic demand of local brain areas during neuronal activity alters the cerebral blood flow in this area.[32] Thirdly, endothelium derived factors can regulate cerebral blood flow by exerting a constrictive or relaxing effect on the vascular smooth muscle. Vasodilatory factors are nitric oxide (NO), endothelium derived hyperpolarization factor (EDHF) and prostacylin, constrictor factors are thromboxane A_2,

prostaglandin $F_{2\alpha}$ and endothelin-1.[33] Finally, perivascular nerve activity may control cerebral autoregulation, which will be described below.

Chronic hypertension causes a right-shift of the cerebral autoregulation curve.[34] This is a protective effect, since loss of cerebral autoregulation on the upper end of the curve now occurs at higher pressures.[35,36] This right shift is caused by hypertensive remodeling and vascular hypertrophy, which normalize wall stress by increasing wall thickness and decreasing vascular diameter.[37-39] Stimulation of sympathetic innervation also results in a shift of the upper limit of the autoregulation plateau to higher pressures and has a protective effect on the blood-brain barrier.[40-42] In contrast, acute sympathetic denervation causes lowering of the upper limit of cerebral autoregulation.[43] During normal conditions the effect of sympathetic innervation on resting cerebral blood flow is minimal.[44]

Although progression is being made in this field, whether and how pregnancy influences the cerebral autoregulation has not exactly been elucidated. Some eclamptic women never reach blood pressures considered to be in the hypertensive range and therefore they do not necessarily reach the upper limit of cerebral autoregulation as this has been established in nonpregnant individuals. Thus, it seems likely that pregnancy shifts the upper limit or even the entire curve to the left and that hyperperfusion may occur at lower blood pressures compared to nonpregnant women. In vitro studies in late-pregnant rats showed that myogenic reactivity of posterior cerebral arteries was decreased and that the pressure at which forced dilatation occurs was lower in late-pregnant animals.[45,46] An in vivo study did not show a decrease of the upper limit of autoregulation in pregnant rats; there was no difference between late-pregnant and nonpregnant rats.[47] However, cerebral edema was more extensive in the late-pregnant animals after autoregulation breakthrough[47], suggesting that the blood-brain barrier is more vulnerable to disruption during pregnancy.

Blood-brain barrier

In the cerebral circulation, one of the most obvious and significant specializations is the formation of the blood-brain barrier by cerebral capillary endothelial cells. The endothelial cells in the brain's vasculature form high-resistance tight junctions through which there is no paracellular transport of water or solutes.[48] Also, endocytosis (transcellular transport) is minimal in capillaries of the central nervous system.[48] These features of the cerebral endothelium are considered the blood-brain barrier and protect the brain from formation of vasogenic edema. However, acute increased hydrostatic pressure may disrupt the blood-brain barrier with subsequent vasogenic edema formation.

It is important to understand how pregnancy influences the blood-brain barrier considering the fact that cerebral vasogenic edema is present in eclamptic and some preeclamptic patients.[49,50] In isolated cerebral arteries from rats, the vessels from late pregnant animals show increased permeability of the blood-brain barrier by increased endocytosis and paracellular transport when hydrostatic pressure is increased and forced dilatation occurs.[51,52] In an in vivo study, permeability to Evan's blue was increased after autoregulation breakthrough in late pregnant rats but there was no increase in permeability to sodium fluorescein.[53] Evan's blue permeability was greater in the posterior versus the anterior cerebrum suggesting regional differences in vulnerability of the blood-brain barrier, which may be an explanation for the regional distribution of vasogenic edema in eclampsia. Together, these findings suggest that pregnancy alters the permeability of the blood-brain barrier which may be important in case of acute hypertension.

Perivascular innervation

Cerebral vessels are associated with parasympathetic, sympathetic and sensory or trigeminal fibers.[28] They originate in cranial ganglia and distribute mainly to extraparenchymal vessels.[29] The autonomic neurons run through the adventitial layer of cerebral arteries in pial vessels ending in preterminal axons and terminals proper, which come in close contact with the outer smooth muscle layer of the vessel media.[29] This is the vasomotor innervation of both extracerebral and intracerebral small arteries and arterioles. Under normal resting conditions, perivascular innervation has little or no effect on cerebral autoregulation.[26,54,55] When the steady state of the cerebral autoregulation is altered such as in acute hypertension[55,56] or ischemia/reperfusion[57,58], perivascular innervation may influence the cerebral blood flow. However, the exact role on the regulation of cerebral autoregulation is controversial because of its complex nature.[58]

Cerebral vessels are innervated by an extensive sympathetic nerve supply.[59] In general, anterior vessels in the circle of Willis receive a denser sympathetic nerve supply than those in the posterior circulation.[59] Most of the pial arterioles are extrinsically innervated with sympathetic nerves originating from the cervical sympathetic ganglia.[60-62] Small pial vessels may receive intrinsic noradrenergic nerve fibers from the pons (locus ceruleus).[63] Under resting conditions, experimental manipulation of sympathetic input generally has little or no effect on cerebral blood flow in all the species examined, including humans.[29] However, during acute hypertension sympathetic nerves play a role in protecting the brain from autoregulation breakthrough and loss of integrity of the blood-brain barrier.[64,65] Stimulation of sympathetic nerves during acute hypertension attenuates the increase in cerebral blood flow.[42,55] In chronic hypertension sympathetic nerves exert

a trophic effect on cerebral vessels and contribute to hypertrophy of vascular muscle hereby protecting the downstream vessels.[66] The posterior brain is the primary site where autoregulation is overcome during acute hypertension and thus the primary site of vasogenic edema formation in eclampsia. One hypothesis is that this is because of the lesser sympathetic innervation posteriorly compared to the anterior cerebral vasculature, however, this hypothesis has not been proven.

Parasympathetic innervation plays a less well defined role in cerebral autoregulation. Parasympathetic fibers exert a dilator role, for example in ischemia/reperfusion.[58] In addition, cholinergic nerves interact with presynaptic noradrenergic nerve terminals and modify neurotransmitter release, for example by reducing the constrictor effects of sympathetic stimulation.[58]

Trigeminal innervation is mostly important in nociception, but also plays a role in cerebral vasodilation during cortical spreading depression[67,68], post ischemia/reperfusion[57] and on the lower end of the cerebral autoregulation curve.[69] Because of its role in migraine[70-74] – a disease that shares features with eclampsia[75-77] – and in vascular adaptation during pregnancy, the sensitivity and perivascular innervation of the trigeminal neuropeptide calcitonin gene-related peptide (CGRP) in cerebral arteries during pregnancy are a subject of this thesis.

Calcitonin Gene-Related Peptide

Calcitonin gene-related peptide (CGRP) is a neuropeptide of the sensory or trigeminal nervous system.[29,58,78] It is involved in modulation of peripheral vascular resistance[79-81], attenuation of gastric acid production [82] and nociception.[83] There are two isoforms of CGRP present, which are the α- and β-CGRP. The α-CGRP is a 37-amino-acid peptide produced by tissue-specific alternative splicing of the calcitonin/αCGRP RNA transcript.[78] It is located mostly at the central nervous system.[84] β-CGRP is produced from a different gene exclusive of alternative splicing and is mainly located in the enteric nervous system.[84] CGRP is co-located with substance P and neurokinin A in perivascular nerves where it transmits sensory information to the central nervous system, for example noxious stimuli, and where it serves a regulatory function of the local environment through the release of neurotransmitters.[29] CGRP containing perivascular nerves are found in many vascular beds throughout the entire body. CGRP is the strongest endogenous vasodilator known, in which the α-isoform has a stronger effect compared to β-CGRP.[79,84] The receptor that is identified as the CGRP receptor, is the calcitonin receptor-like receptor (CRLR). It is a G-protein coupled receptor and it requires binding to receptor activity modifying protein 1 (RAMP1) in order to become receptive to CGRP.[84,85] The signaling pathway can be either endothelium-dependent, endothelium-independent or both, differing per tissue bed and

species.[87] In the endothelium-dependent manner, CGRP can activate nitric oxide synthase (NOS), thereby releasing nitric oxide, causing vascular smooth muscle cell (VSMC) relaxation.[73,88] In the endothelium-independent pathway, CGRP binds directly on the CRLR/RAMP1 complex on the VSMC.[73,89] This activates adenyl cyclase, which in turn increases cAMP levels, causing VSMC relaxation.[73]

During pregnancy, levels of plasma CGRP increase to term and drop after delivery[90,91], suggesting that CGRP plays a role in the vascular adaptations that occur during pregnancy, when the plasma volume expands with 40% and blood pressure remains normal. In addition, plasma CGRP levels appear to be lower in the plasma of women whose pregnancy was complicated by preeclampsia compared to normotensive control women.[91] Plasma CGRP levels are also higher in pregnant rats compared to nonpregnant rats and the levels drop after delivery.[92] The vascular sensitivity to CGRP is greater in pregnant rats and ovariectomized rats treated with female sex steroid hormones compared to ovariectomized rats and male rats.[89,93] Administration of CGRP in pregnant rats treated with the NOS inhibitor L-NAME to cause preeclampsia-like features resulted in lowering of blood pressure, less fetal death and increased birth weight.[94] When the CGRP antagonist $CGRP_{8-37}$ was administered in L-NAME treated pregnant animals, blood pressure raised even further while there was no effect on blood pressure of untreated animals.[95] This effect was not associated with an increase of CGRP mRNA in dorsal root ganglia, suggesting that the underlying mechanism is an increased vascular sensitivity to CGRP during pregnancy. Together these findings suggest an important role of CGRP in maternal cardiovascular adaptations during pregnancy and that possibly CGRP or vascular sensitivity to CGRP is involved in the pathogenesis of preeclampsia.

Posterior Reversible Encephalopathy Syndrome

The occurrence of PRES is associated with several different conditions and progressively recognized in more and more different underlying pathologies. Most common patient categories are preeclampsia/eclampsia, patients treated with immunosuppressants or chemotherapy (e.g. cyclosporine and tacrolimus), malignant hypertension, infection/sepsis, solid organ or bone marrow transplantation and several autoimmune diseases.[17,96-98] The syndrome occurs at all ages, including the pediatric population.[99,100] There are no strict criteria, but diagnosis is made based on clinical findings and imaging. Symptoms of this syndrome are similar to eclampsia and include headache, nausea and vomiting, decreased alertness, altered mental functioning, seizures and visual disturbances such as blurred vision or cortical blindness.[17] These symptoms occur usually in conjunction with an acute elevation of the blood pressure.[17] Computed tomography (CT) shows localized hypodense lesions at the gray white matter junction or widespread

diffuse edema. However, occasionally focal areas of edema are beyond the resolution of CT scan, in which case magnetic resonance imaging (MRI) is necessary.[101] Findings on MRI are consistent with cerebral edema and include hypointensities on T1 sequence and hyperintensities on T2 and fluid attenuation inversion recovery (FLAIR) sequences.[17,98] The areas that are hyperintense on T2/FLAIR imaging are iso- or hypointense on diffusion weighted imaging (DWI) and hyperintense on apparent diffusion coefficient (ADC). This pattern of MRI abnormalities is consistent with vasogenic edema. The distribution of the edema on MRI is typically in the subcortical white matter of the parieto-occipital lobes and appears symmetrically. Although the name of the syndrome suggests indeed this kind of distribution, there is a wide variety. Atypical findings include edema in the frontal lobes, the inferior temporal-occipital junction, the cerebellum, the basal ganglia and brainstem.[97,98,102,103] Also, in some cases, the grey matter can be involved, the edema may be asymmetric or accompanied by hemorrhage. Moreover, DWI may demonstrate small areas of cytotoxic edema within lesions of vasogenic edema in patients with eclampsia.[104,105] In those instances it has been suggested that vasogenic edema in PRES can progress to such an extent that regional perfusion pressure decreases and blood flow decreases to ischemic levels leading to cytotoxic edema and infarction.[104,105] Imaging of obstetric patients with PRES (eclampsia and some cases of preeclampsia) is not distinct from other causes of PRES. In one study[106] the obstetric patients demonstrated more often involvement of the basal ganglia, however, this was not found in a larger study by Fugate et al.[98] Except for patients with hypertensive encephalopathy, hypertension is not always clearly present in all patients with PRES; 6 – 16% of PRES patients do not reach blood pressures commonly referred to as being in the hypertensive range.[11,97]

An abrupt rise in blood pressure undoubtedly contributes to the development of PRES but the exact underlying mechanism that causes disruption of the blood-brain barrier in this wide variety of patient categories, has not been elucidated. The conditions associated with PRES are typically systemic processes, which have some degree of endothelial dysfunction and an inflammatory response.[14] Possibly, these conditions (and other unidentified processes) predispose to an increased vulnerability of the cerebral vasculature to loss of cerebral autoregulation. This may be because increased permeability of the blood-brain barrier, a shift of the cerebral autoregulation curve to the left or both.

Animal models of (pre)eclampsia.

Preeclampsia and eclampsia are diseases that do not naturally occur in animals other than primates.[107] The ideal model of preeclampsia should include preferentially as many of the clinical and laboratory features of the disease as possible and progress to eclampsia-like symptoms if severe. However, such a model does not exist and therefore different animal

models of preeclampsia have been designed, some expressing the preeclamptic features more than others. Podjarny et al. gave a nice overview of animal models for preeclampsia including reduction of arterial blood flow to the uterus and placenta by aorta or ovarian artery clipping, chronic NOS inhibition with L-NAME administration or in an endothelial-NOS knock-out model, sympathetic nervous and/or renin-angiotensin system overactivation, inflammatory models with pro-inflammatory cytokine injection or injection of low-dose lipopolysaccharide, insulin resistance and models of angiogenesis antagonism with soluble fms-like tyrosine kinase 1 (sFlt1).[107]

Only a few studies focused on the brain when investigating preeclampsia models. Several models of hypertensive encephalopathy exist and have been used during pregnancy.[45,47] The systemic blood pressure can be increased acutely by administration of a pressor agent which causes loss of cerebral autoregulation. This has been done in our lab while cerebral blood flow was measured indirectly by laser Doppler[47] and with measuring microsphere content in different brain areas after autoregulation breakthrough.[19,108] Kanayama et al. described a rat model in which the celiac ganglion is stimulated with lipopolysaccharide in pregnant animals after which a preeclamptic and HELLP syndrome-like condition develops (Hemolysis, Elevated Liver enzymes, Low Platelets).[109] In addition, seizures occur, cerebral blood flow increases and cerebral edema develops.[110] Another model used in our lab is that of the Dahl salt-sensitive rat.[45] This rat becomes hypertensive when fed with a diet containing a high salt percentage (8%). At high blood pressures the Dahl salt-sensitive rat demonstrates symptoms similar to seizures of hypertensive encephalopathy: rhythmic, abrupt movements of the head in an up-an-down motion often associated with a lateral deflection or repetitive forearm flexion unilaterally.[111] There is evidence for disruption of the blood-brain barrier and edema formation in the brains of these rats as well as linearly decreasing myogenic reactivity with the duration of the high salt diet.[111] These rats also become hypertensive when fed a high salt diet during pregnancy[112] and demonstrate elevated markers of oxidative stress[113,114], both features of human preeclampsia. Furthermore, Dahl salt-sensitive rats suffer endothelial dysfunction, likely due to the salt-induced hypertension.[115] This model has one disadvantage: the Dahl salt-sensitive rats become symptomatic after 2.5 weeks of high salt diet, while the duration of pregnancy in rats is 3 weeks. However, it seems a good model for eclampsia and is used in Chapter 5.

Pressurized arteriograph system

The method that was used to investigate the effect of pregnancy, hypertension and different neurotransmitters on cerebral arteries *in vitro* in Chapters 5 and 6 is the pressurized arteriograph system. This system was developed at the University of Vermont,

United States, and is used extensively throughout the world to investigate vascular structure and function of small arteries and arterioles. In this experimental system, it is possible to investigate the effect of different pharmacological agents and physical forces such as pressure on the vascular smooth muscle and endothelium. The arteriograph that was used was a dual chambered system with two 20mL baths containing physiologic salt solution that is circulated to maintain temperature, oxygen, carbon dioxide and pH at physiologic levels. The carefully dissected vessels are mounted on two glass cannulas with nylon ties. The distal end of both of the cannulas is closed off to maintain pressure that is generated by a servo system that is connected to the proximal cannula. The servo system consists of a miniature peristaltic pump, an in-line pressure transducer and a controller. Through an optical window in the bath, the vessels are imaged by an inverted microscope that is connected to a video camera and a monitor. The video dimension analyzer (VDA) is used to analyze the signal obtained from the video image and to continuously register lumen diameter and wall thickness. The dynamic responses of vessel diameter and the intraluminal pressure are visualized on a computer by a serial data acquisition system that registers the VDA and pressure controller output, similar to a chart recorder. The cerebral autoregulation is subject to many different influences that cannot all be mimicked in an in vitro experiment. However, the use of the arteriograph system gives detailed insight into myogenic activity and passive structural properties of small cerebral arteries in response to some of the control mechanisms of autoregulation such as neurotransmitters and transmural pressure.

The maternal brain following eclampsia

It has long been thought that when a woman with eclampsia survives this condition without the occurrence of cerebral haemorrhage, she will fully recover.[116,117] This is plausible, because when the blood pressure decreases, cerebral vasogenic edema resolves. However, in addition to vasogenic edema also cytotoxic edema has been found during the acute phase of eclampsia.[105,118] Six to eight weeks post partum approximately one fourth of these formerly eclamptic women showed white matter lesions consistent with gliosis. How these lesions in formerly eclamptic women develop over life and their clinical relevance are unknown and are subject of this thesis. Moreover, some women with severe preeclampsia also demonstrated evidence of PRES on cerebral imaging even without experiencing eclamptic seizures[49,50] and an increased risk of stroke in formerly preeclamptic women has been reported.[119] Therefore, also formerly preeclamptic women are subject of neuroimaging and cognitive testing in this thesis.

Aims of this thesis

Part I (patient studies)

- To assess the long term consequences of preeclampsia and eclampsia on daily life with regard to cognitive function (**Chapter 2**)
- To provide insight into the long term consequences of eclampsia on the maternal brain with MR imaging (**Chapter 3**)
- To investigate the prevalence and severity of cerebral white matter lesions several years after preeclampsia and to find factors that are associated to these lesions (**Chapter 4**)

Part II (animal studies)

- To investigate whether decreased myogenic reactivity and the lack of hypertensive remodelling in cerebral arteries that occurs during pregnancy is due to the type of hypertension or to pregnancy (**Chapter 5**)
- To investigate the changes in cerebral perivascular innervation in different pregnancy-related states and the effect of gender on perivascular innervation (**Chapters 5 and 6**)
- To investigate the effect of pregnancy and gender on the sensitivity of cerebral arteries to sympathetic and trigeminal neurotransmitters (**Chapter 6**)

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Part I

Hoe
als je je
met zorgeloosheid
kon omringen
en dat dat
je ruimte
was

(Bert Schierbeek)

2

Self-reported cognitive functioning in formerly eclamptic women

Annet M. Aukes[1,2]
Ineke Wessel[3]
Albertien M. Dubois[1]
Jan G. Aarnoudse[1]
Gerda G. Zeeman[1]

[1]Department of Obstetrics and Gynecology, University Medical Center Groningen, the Netherlands
[2]School of Behavioural and Cognitive Neurosciences,
[3]Faculty of Behavioural and Social Sciences, Division of Clinical & Developmental Psychology, University of Groningen, the Netherlands

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Abstract

OBJECTIVE

Recently, persistent brain white matter lesions were demonstrated in eclamptic women when imaged 6 weeks postpartum. Moreover, many of these women complain about cognitive limitations years after the eclamptic pregnancy. Therefore, in a cohort of such women we assessed cognitive failures in daily life.

STUDY DESIGN

Thirty formerly eclamptic women completed the Cognitive Failures Questionnaire. Scores were compared with those of formerly preeclamptic (n=31) and healthy parous control participants (n=30) using a priori Student t-tests. Groups were matched in terms of current age and years elapsed since index pregnancy.

RESULTS

Formerly eclamptic women scored significantly higher compared with healthy parous controls (43.5 vs. 36.1 respectively, $p < 0.05$).

CONCLUSION

Formerly eclamptic women reported significantly more cognitive failures many years after the index pregnancy. It is hypothesized that this might be due to some degree of cerebral white matter damage. This subjective assessment of cognitive function needs to be confirmed with objective neurocognitive testing and related to neuroimaging findings.

Introduction

The exact pathophysiology of eclampsia has not been elucidated. One of the main theories is that eclampsia is an expression of hypertensive encephalopathy[1], which more recently has been named Posterior Reversible Encephalopathy Syndrome (PRES).[2-4] PRES is also recognized as a complication in various other, nonpregnancy-related disorders, including several of iatrogenic or neurotoxic origin, connective tissue disease, acute glomerulonephritis in children and more.[5] Clinically, PRES is characterized by specific symptoms such as headache, seizures, altered mental status and visual disturbances.[2,6] In PRES, it is thought that in the presence of endothelial dysfunction an acute increase in blood pressure exceeds the upper limit of cerebral autoregulation.[2] In this scheme, forced dilatation of cerebral arteries is followed by blood-brain barrier disruption and formation of vasogenic cerebral edema. The syndrome of PRES is clinically reversible by lowering of blood pressure and/or discontinuation of the offending drug, or in pregnancy, by termination of the pregnancy. Nevertheless, it is hypothesized that when vasogenic edema becomes severe enough, it can result in reduced tissue perfusion and cytotoxic edema due to irreversible ischemic changes leading to brain white matter lesions.[7] Indeed, there is evidence to sustain this concept. Previous studies[2,7,8] using magnetic resonance imaging within days of seizures in eclamptic women showed vasogenic edema mainly in the subcortical white matter of the parieto-occipital lobes and adjacent grey matter. In almost one-fourth of eclamptic patients, persistent white matter lesions, consistent with the appearance of cerebral tissue loss (i.e. gliosis), have been demonstrated with follow-up MRI 6 weeks postpartum.[7,9] Together, these findings contradict the predominant concept that eclampsia is a condition in which women can expect full clinical recovery. Cerebral white matter lesions in the general, mostly aging, population have been associated with a decline in cognitive functioning and dementia.[10,11] To our knowledge, cognitive functioning of previously eclamptic women as well as non-obstetric subjects who experienced PRES has never been investigated. Information about the cognitive abilities in this particular patient population of much younger age is important to either confirm or reject possible harmful effects of persistent white matter lesions due to cytotoxic edema.

In the current study we sought to evaluate subjective cognitive functioning in formerly eclamptic, formerly preeclamptic and healthy parous women by using a validated Cognitive Failures Questionnaire (CFQ).[12,13] We hypothesized that the self-reported cognitive function of formerly eclamptic women is impaired compared with formerly preeclamptic and healthy parous control subjects.

Materials and Methods

Participants

The University Medical Center Groningen (UMCG) Obstetrics department is part of one of the 8 university teaching hospitals in The Netherlands, serving as a tertiary referral center. The annual delivery rate at the UMCG varied from 1600 to 1900 in the last 5 years. The department has used an electronic delivery database since 1988. This database was utilized to identify participants. Seventy-three women diagnosed with eclampsia were admitted to the UMCG between 1988 and 2005. Three women died in the interim, two of which died because of the complications of eclampsia (hepatic rupture with bleeding for several days followed by multiple organ failure and severe cerebral edema which resulted in infratentorial herniation). The third woman died from cervical carcinoma several years after pregnancy. We were unable to contact 28 of these women, resulting in 42 women who were eligible to participate. Each formerly eclamptic woman was matched for age and year of index pregnancy with a formerly preeclamptic woman and a healthy parous control. Controls with epilepsy or other neurological or psychiatric disorders known to influence cognitive functioning were excluded, as were those with a history of alcohol or substance abuse. (Pre)eclampsia was defined according to the definition by the International Society for the Study of Hypertension in Pregnancy.[14] Thus, a total of 126 women were invited to participate in the study and were mailed a questionnaire package. Women in all 3 groups were sent a Dutch translation of the CFQ together with a questionnaire concerning demographic characteristics including employment, education and marital status as well as former and current psychosocial functioning. Participants were also asked about traumatic brain injury and brain surgery in the past, and current use of medication. A total of 92 women returned the questionnaires, resulting in a response rate of 73%. Of the group of formerly eclamptic women 31 completed the questionnaire, as did 31 of the formerly preeclamptic group and 30 of the healthy control group. A few of the formerly eclamptic women declined to participate because they considered the questions too confrontational. All medical records were reviewed to confirm the diagnosis of (pre)eclampsia and to extract clinical information. Through reviewing the medical records, documentation of one woman was insufficient to confirm the diagnosis of eclampsia and therefore she was excluded from the study. This resulted in a total number of 30 formerly eclamptic women participating in this study. The study was approved by the local investigational review board and informed consent for this study was signed by all participants.

Cognitive Failures Questionnaire (CFQ)

The CFQ[12] is a questionnaire assessing the likelihood of committing errors in completing daily tasks, which the participant should be capable of doing, i.e. the routines of every day life. Participants were instructed to complete the items with specific reference to the past six months. The CFQ consisted of 25 items that were scored on a five-point scale (range: 0 = *never* to 4 = *very often*). Thus, the total score ranged from 0 – 100, with higher scores indicating more frequently occurring cognitive failures. A recent factor analytic study[13] confirmed the usefulness of the total score as an index of general cognitive failures as well as four subscales pertaining to more specific areas of cognitive failures. These subscales were Memory (7 items, range 0 - 28) assessing participants' forgetfulness (e.g. "Do you find you forget appointments?"), Distractibility (9 items, range 0 - 36) to assess disturbance of internally focused attention (e.g. "Do you daydream when you ought to be listening to something?"), Blunders (7 items, range 0 - 28) reflecting social blunders and motor control (e.g. "Do you say something and realize afterwards that it might be taken as insulting?", "Do you bump into people?") and Names (2 items, range 0 – 8), (e.g. "Do you find you forget people's names?").

Statistical Analysis

Because of our expectation that cognitive failures would occur specifically in the eclamptic group, planned comparisons (t-tests) were carried out to analyse total CFQ scores as well as scores on the subscales. Demographic characteristics and parameters relevant to psychosocial functioning were analyzed using t-tests or chi-square analyses where appropriate. All tests were two-tailed with alpha set at 0.05.

Results

Relevant characteristics at the time of the index pregnancy are shown in Table I. In the formerly eclamptic groups, no differences were found between participating and non-participating women (Table 1). Formerly eclamptic women scored significantly higher on the CFQ compared with healthy parous controls (Table 2). The difference in total CFQ scores between formerly eclamptic and preeclamptic women showed a nonsignificant trend (p=0.08).

Table 1 Gestational characteristics of index pregnancy.

Group	Eclampsia (n = 30)	Preeclampsia (n = 31)	Controls (n = 30)	Non-participating formerly eclampsia (n = 39)
Percentage primiparous	80	57	52	64
Gestational age (weeks)*	33.2 ± 4.5	34.6 ± 5.0	39.8 ± 1.3	32.3 ± 4.1
Birth weight (grams)*	1849 ± 961	2132 ± 1226	3551 ± 477	1628 ± 818
Percentage Caesarean Section	71	61	8	79
Number of seizures (%):				
- 1	16 (53)			20 (51)
- 2	10 (33)			8 (21)
- 3	4 (13)			7 (18)
- 4	-			3 (8)
- Unknown	-			1 (3)

*Results are given as means ± standard deviations.

Eclamptic women who experienced three seizures had significantly higher CFQ total scores compared with those who only had one seizure (Table 2), suggesting that each seizure had cumulative harmful effects that resulted in increased CFQ scores. As for the CFQ subscales, formerly eclamptic women had higher scores compared with both formerly preeclamptic women and healthy parous controls, however no significant differences were found. Table 3 shows relevant demographic and psychosocial characteristics for all three groups. Current age of the participants in all groups was similar. There was no difference in elapsed time since the index pregnancy between any of the groups.

Table 2 CFQ scores.

Group	Eclampsia (n = 30)	Preeclampsia (n = 31)	Controls (n = 30)
CFQ total score (0-100)	43.5 ± 14.6*	36.9 ± 13.9	36.1 ± 13.9
Memory (0-28)	10.8 ± 5.1	9.0 ± 3.9	9.2 ± 4.7
Distractibility (0-36)	16.6 ± 5.6	14.4 ± 5.3	13.5 ± 5.6
Blunders (0-28)	11.2 ± 4.3	9.4 ± 4.5	8.9 ± 3.6
Names (0-8)	4.8 ± 2.2	4.1 ± 2.3	4.6 ± 1.8
CFQ total score (0-100):			
1 seizure (n = 16)	39.1 ± 10.9		
2 seizures (n = 10)	43.8 ± 16.3		
3 seizures (n = 4)	60.0± 14.5†		

Results are given as means ± with standard deviations.

*p = 0.049, t(58) = 2.0 vs. Controls, †p = 0.005, t(18) = -3.2 vs. Eclampsia with 1 seizure

Table 3 Relevant current demographic, medical and former psychological characteristics

Group	Eclampsia (n = 31)	Preeclampsia (n = 31)	Controls (n = 30)
Current age (years)	38.7 ± 6.6	40.3 ± 5.2	38.7 ± 7.0
Elapsed time since index pregnancy (years)	7.6 ± 5.0	6.8 ± 4.5	5.8 ± 4.1
Education **	8.7 ± 1.5	7.9 ± 1.9	8.6 ± 2.1
Married or cohabited	25 (83)	26 (84)	25 (83)
No work outside of home:	8 (27)	5 (16)	6 (20)
- Of whom receive disability/sickness benefits	4 (50)*	0	2 (33)
Tobacco use	5 (17)	9 (29)	6 (20)
Alcohol use	15 (50)	19 (61)	18 (60)
Antihypertensive medication	2 (7)	8 (26)[†‡]	0
Migraine	9 (30)	14 (45)[§]	5 (17)
Episodes of feeling down and/or lack of interest:	17 (57)	15 (48)	17 (57)
- Of which related to pregnancy or postpartum period	9 (53)[‡]	9 (60)[‡]	2 (12)
Psychological therapy because of pregnancy related problems	8 (27)[‡]	3 (10)	0

Number of women with percentages in parenthesis. Current age, elapsed time since index pregnancy and education are presented as means ± standard deviations. **Education was measured by an increasing scale (1-11) depending on the highest level of completed education. *p≤ 0.05 vs. Preeclampsia, [†]p ≤ 0.05 vs. Eclampsia, [‡]p ≤ 0.01 vs. Controls, [§]p ≤ 0.05 vs. Controls.

There was no difference in the number of women who were currently not working outside the home. However, 4 out of the 8 (50%) formerly eclamptic women reported to be unable to work because of their current health status. Moreover, these women received sickness or disability benefits in contrast to none of the formerly preeclamptic women. In this latter group, 5 women who reported not to be working outside the home stated that this was their personal choice. Significantly more women of the preeclamptic group reported a history of migraine compared with the control subjects (p = 0.02). No participant reported a history of epilepsy, nor any other relevant intercurrent medical condition. Two women in the control group reported a cerebral contusion without any permanent sequelae in the past. There was no difference in level of education between the groups. Use of tobacco and alcohol was similar in all three groups. The current use of antihypertensive medication was significantly higher in the formerly preeclamptic group compared with the formerly eclamptic and control groups (p = 0.040

and p = 0.003, respectively). A similar number of women in each group reported to have experienced past episodes of lack of interest and/or phases of feeling down. Compared with the control subjects, significantly more women in the formerly eclamptic and preeclamptic groups reported that these episodes were specifically related to the index pregnancy or delivery (p = 0.010 and p = 0.004, respectively). Eight of the formerly eclamptic women had received psychological treatment because of these problems, which was significantly greater compared to the control women (p = 0.002). There was no difference between any of the groups in number of women receiving current psychological therapy.

Comment

The main finding of this study is that several years after a pregnancy complicated by eclampsia, women reported impaired cognitive functioning compared with healthy parous women. In addition, women who experienced multiple eclamptic seizures reported greater cognitive impairment compared with those who experienced one seizure. This is a remarkable finding since the predominant view holds that eclampsia concerns a one-time event without any known long-term consequences, provided that intracranial haemorrhage does not precede or follow the acute moment.

The difference in CFQ outcomes may indicate that eclamptic seizures are more harmful than has previously been thought. The concept that the occurrence of eclamptic seizures does not affect maternal outcome as long as the maternal and fetal condition is being monitored closely during a seizure are no longer acceptable.[15,16] The significant difference in CFQ outcomes between formerly eclamptic women and healthy controls likely indicates that formerly eclamptic women function worse in daily life. The CFQ outcomes suggest that this is expressed by more slips of memory, memory for names, more slips of attention and psychomotor function in daily life. Yet, this impaired functioning should be acknowledged as an *indication* of how these women function and more (objective) neurocognitive testing should be conducted. The difference in outcome between the formerly eclamptic and preeclamptic groups, did not reach significance. Yet, the trend towards significant difference together with inspection of Table II, suggests that scores in the preeclamptic group closely resembled those of the healthy parous controls. This lack of significant difference is likely due to a type II error. Future studies with larger groups should better determine differences in functioning between formerly eclamptic and preeclamptic women.

Virtually nothing is known about long-term consequences of PRES in either obstetric or non-obstetric patients regarding their neurocognitive and social functioning. However, there is some evidence that preeclampsia may increase the risk of psychiatric conditions such as depression or Post Traumatic Stress Disorder (PTSD) relative to women with uncomplicated pregnancies.[17,18] The present study employed questions about episodes in the past that were characterised by a loss of interest and/or depressed mood as a crude index of past depression. The finding that formerly eclamptic and preeclamptic women indicated that they had experienced more of such episodes that were specifically tied to the index pregnancy or delivery is consistent with this previous literature on psychiatric sequelae of (pre-) eclamspia.[17,18]

Interestingly, the prevalence of migraine was found to be higher in both formerly preeclamptic and eclamptic women in our study. The association between (pre)eclampsia and migraine has been reported before.[19,20] Both conditions are thought to be characterized by hyperperfusion of the brain[21,22], are related to changes in female sex steroid hormones[23] and have similar symptoms (headache, visual disturbances and nausea).[6] However, the exact pathophysiology of, as well as the association between, migraine and (pre)eclampsia remains unknown. In addition, white matter lesions in the cerebral posterior circulation territory are more frequently demonstrated in the general population in people who suffer migraine, especially in women.[24] This may also be of particular relevance to women with eclampsia.

It should be noted that CFQ scores reflect self-reported difficulties pertaining to everyday slips of attention and action. Although the CFQ has demonstrated satisfactory internal consistency, retest reliability and cross-cultural validity[25,26], additional measures are needed to illuminate the origins of elevated scores. For example, a previous study[27] found elevated CFQ scores in organic (predominantly dementia) and functional (i.e, depression or anxiety) patients. Yet, the authors were unable to identify specific CFQ profiles that discriminated between those organic and functional groups. Thus, although the present findings are potentially consistent with an interpretation in terms of brain white matter lesions, alternative explanations cannot be ruled out. For example, it remains possible that cognitive failures in the eclampsia group were due to non-organic variables such as increased levels of anxiety or depression. Furthermore, it is also possible that higher cognitive failures scores reflect the specific conviction of eclamptic women that they suffer memory and concentration difficulties. That is, it may be that objectively, eclamptic women are comparable to healthy controls, yet interpret the occurrence of cognitive failures as evidence for the negative sequelae of their eclamptic seizures. It is possible that some women attribute a more negative meaning to cognitive lapses than others, resulting in higher estimates of their occurrence over the index period of the prior

6 months as identified in the CFQ instructions. However, the increase of cognitive dysfunction with increased number of seizures and the finding that more women in the eclamptic group were unable to work and received disability benefits may be taken as support for the notion that differences in reported cognitive failures reflected genuine dysfunctions in daily life rather than a self-report bias.

Several additional methodological limitations to this study deserve attention. First, the retrospective nature of the study may have resulted in selection bias. For example, if formerly eclamptic women with more cognitive dysfunction were eager to participate, seeking for recognition of self-perceived cognitive limitations, the cognitive impairment might be overestimated. In addition, the CFQ is a retrospective instrument subject to the limitations of human memory. Secondly, as the CFQ is a subjective measure of cognitive function, neurocognitive examination will be needed to demonstrate objectively cognitive abilities and limitations. A third limitation to this study is that the investigated groups are small. However, in the context of the rare incidence of eclampsia this represents a sizeable study, the results of which may be clinically important.

In conclusion, formerly eclamptic women report some degree of cognitive impairment many years after the index pregnancy. Whether the cognitive impairment found in this study cohort of formerly eclamptic subjects might be due to some degree of permanent cerebral white matter disturbance remains to be seen. Further research is now ongoing in order to confirm our findings, employing objective neurocognitive testing such as functional MRI and neuroimaging to assess the relationship with brain white matter lesions in formerly eclamptic women. The potential for identification (and eventually prevention and treatment) of chronic cognitive and psychosocial impairment in this particular group of young mothers is of important societal relevance and will also draw attention to possible longterm sequelae following PRES in other, nonpregnancy-related patient categories. The paradigm that eclampsia is a condition of which the women can expect full clinical recovery, may need to be revised and the importance of preventing eclamptic seizures should be emphasized.

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Brain lesions several years after eclampsia

Annet M. Aukes[1,2]
Jan Cees de Groot[3]
Jan G. Aarnoudse[2]
Gerda G. Zeeman[1,2]

[1]School for Behavioural and Cognitive Neurosciences, University of Groningen, the Netherlands
Departments of [2]Obstetrics and Gynecology and [3]Radiology, University Medical Center Groningen, the Netherlands

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Abstract

OBJECTIVE

Eclampsia is thought to have no long-term neurological consequences. We aimed to delineate the neurostructural sequelae of eclampsia, in particular brain white matter lesions, utilizing high-resolution 3-tesla MRI.

STUDY DESIGN

Formerly eclamptic women were matched for age and year of index pregnancy with normotensive parous controls. The presence and volume of brain white matter lesions were compared between the groups.

RESULTS

MRI scans of 39 formerly eclamptic and 29 control women were performed on average 6.4 ± 5.6 years following the index pregnancy at a mean age of 38 years. Eclamptic women demonstrated subcortical white matter lesions more than twice as often compared to controls (41 % versus 17 %, OR 3.3, CI 1.05 – 10.61, p = 0.04).

CONCLUSIONS

Cerebral white matter lesions occur more often in formerly eclamptic women compared to women with normotensive pregnancies. The exact pathophysiology underlying these imaging changes and their clinical relevance remains to be elucidated.

Introduction

Eclampsia, a specific neurologic complication of pregnancy, is associated with hypertension and endothelial cell dysfunction.[1,2] Because of its neuroimaging findings and clinical features eclampsia is considered a form of the posterior reversible encephalopathy syndrome (PRES).[3] Besides eclampsia, this syndrome has been recognized in a variety of other disorders which are associated with endothelial dysfunction and some degree of hypertension, including several of iatrogenic or neurotoxic etiology.[4,5] Its pathophysiology is still unclear, but as its name suggests, the syndrome is thought to be reversible. To our knowledge there have been no studies of long-term outcomes in patients with this syndrome. Similarly, eclampsia is considered by most to be a monophasic event with no long-term neurological sequelae. At least two recent studies have documented cytotoxic cerebral edema in up to 25% of women suffering from eclamptic seizures. In these women neuroimaging findings consistent with cerebral infarctions were demonstrated when studied several months following delivery.[6,7] Moreover, there is now evidence that women who suffered eclampsia may develop some degree of neurocognitive dysfunction.[8] This prompted us to investigate whether brain white matter lesions (WML) were more frequently present in formerly eclamptic women compared with women who experienced uneventful pregnancies.

Materials and Methods

Study participants

The University Medical Center Groningen (UMCG) is a tertiary referral and academic teaching hospital in The Netherlands that serves as a perinatal referral center for high-risk pregnancies. A small percentage of healthy women without complicated pregnancies chooses to deliver in the UMCG as well. The annual delivery rate averages 1,600. The population in the Northern part of the Netherlands is predominantly Caucasian. The department works with an electronic admission and delivery database since 1988. From 1988 until 2005, 73 women were diagnosed with eclampsia. Eclampsia was defined as new onset of seizures after 20 weeks gestational age and within 1 week postpartum in women with preeclampsia. Preeclampsia was defined according to internationally agreed standards. [9,10]

Of these 73 women the medical records were reviewed for accuracy of diagnosis of eclampsia. Upon reviewing the medical records, one formerly eclamptic participant was excluded because the diagnosis eclampsia could not be confirmed. Two had died from cerebral complications (both due to hypoxic encephalopathy secondary to severe cerebral edema) as a result of eclampsia and one woman died of cervical cancer several years after pregnancy. The remaining 69 women were invited by mail to participate in this MRI study. Forty-six (67%) formerly eclamptic women were reached and willing to participate. Exclusion criteria included preexistent epilepsy or other neurological disorders including a known cerebrovascular accident, intracranial infections, a history of any neurosurgical procedure, current pregnancy or claustrophobia. Furthermore, because of the use of a 3 Tesla magnet women with metallic implants including some dental inlays as well as heavy metallic tattoos were excluded as well. Of the formerly eclamptic participants 4 appeared to have general contra-indications for MRI scanning. Each of the remaining formerly eclamptic women was matched for age (within 1 year) and year of index pregnancy (within 2 years) with a parous control whose pregnancy had been uncomplicated and normotensive. These controls were recruited either through the department's electronic delivery database or recruited amongst hospital/department employees and their family members. Forty such women were willing to participate. Their records were evaluated to confirm that the pregnancy was indeed uneventful.

One control participant was subsequently excluded because she was diagnosed with gestational hypertension during the index pregnancy and a second control participant because she suffered epilepsy. Three of the normotensive controls had general contra-indications for MRI.

Blood pressure was measured manually with aneroid sphygmomanometer in sitting position after a resting period. Blood pressure of $\geq$ 140/90 mmHg was used for the diagnosis of hypertension in this group of women. The project was approved by the UMCG Institutional Review Board and all women signed informed consent.

MRI protocol

All studies were performed on a 3 Tesla MRI system (Philips Intera) at the Neuroimaging Center of the School for Behavioral and Cognitive Neurosciences in Groningen using 5 mm slices with a 20% gap. Used sequences include T1 (repetition time [TR] 700 ms, echo time [TE] 4.7 ms, α=65°), Proton Density (TR 3000 ms, TE 26.7 ms, α=90°),T2 (TR 3000 ms, TE 120 ms, α=90°), and FLAIR (TR 11000 ms, TE 100 ms, α=90°). An experienced neuroradiologist, blinded to participant's category and clinical data, rated the presence, size and number of white matter lesions. White matter lesions were considered present if

hyperintense on proton density-weighted and T2-weighted image (Figure 1) and not hypointense on a T1-weighted image. A WML severity score was used to asses the extent of increased white matter signal intensity on FLAIR images for the subcortical area as described previously [11,12]. Briefly, for subcortical WML an index for their total volume was approximated (based on number and size of all subcortical lesions (range 0 - 0.4 mL). The size of subcortical WML were rated according to their largest diameter in categories of small (< 3 mm), medium (3-10 mm), or large lesions (> 10 mm). Considering them spherical with a fixed diameter per size category, a total approximated volume of subcortical WML was calculated. No periventricular WML were demonstrated except for one patient who demonstrated lesions suggestive of demyelinating disease and who was excluded from the analysis.

Data analysis

Demographic data were compared using Chi-square or Student t-test where appropriate. The presence of WML was compared between groups using Chi-square. The severity of WML between the groups was analyzed by using the Mann-Whitney test. The relation between the number of seizures and the presence of WML was analyzed using regression analyses methods. For the test for trend the variables were entered as continuous measures in the regression model. The relation between the number of seizures and the severity of WML was analyzed using the Kruskal-Wallis test. A P value of < 0.05 was considered statistically significant. SPSS version 14.0 (SPSS Inc, Chigaco, IL) was used for data analysis.

Results

For this study we evaluated the MRI data of 41 formerly eclamptic women and 31 healthy parous control women with a history of normotensive pregnancies. Two MRI scans of formerly eclamptic women were not useable due to extensive movement artifacts. One of the controls was excluded because of the incidental finding of a brain tumor during the MRI scan. A second control was excluded because she demonstrated brain WML suggestive of a demyelinating disorder. This resulted in 39 eligible MRI scans in the formerly eclamptic group and 29 in the control group. All women were Caucasian except for two formerly eclamptic participants who were of African-American and Indonesian descent. Of the eclamptic participants 29 of 39 (74%) were nulliparous versus 15 (52%) of

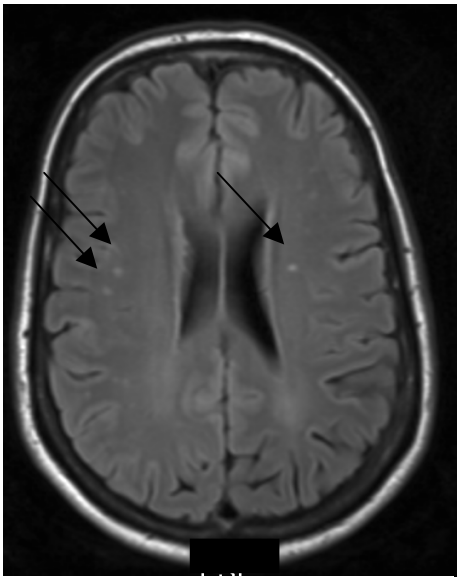

Figure 1 Magnetic Resonance Image (FLAIR) of a formerly eclamptic woman, demonstrating several white matter lesions (arrows).

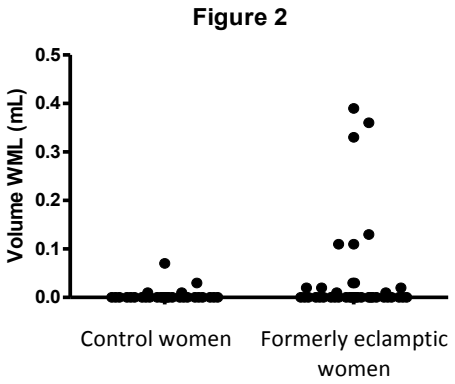

Figure 2 White matter lesion volume load in healthy control women compared to formerly eclamptic women. Mann-Whitney test p = 0.025.

controls. Nineteen participants had experienced a single seizure, 10 women had 2 seizures, 8 experienced 3 seizures and 2 women had 4 seizures. One formerly eclamptic woman was known with insulin-dependent diabetes mellitus and one with systemic lupus erythematosus. None of the other formerly eclamptic women nor any of the controls had a known underlying medical disorder at time of pregnancy nor at follow-up. Six of the formerly eclamptic participants (15%) as well as 4 women in the control group (14%) were currently hypertensive and/or using antihypertensive medication. Current age, elapsed time since index pregnancy, current blood pressure as well as the gestational age at delivery and birth weight of the index pregnancy are shown in Table 1. As may be expected, the estimated gestational age and birth weight were significantly different between the groups. Current age and elapsed time since the index pregnancy were similar in the two groups.

Of the formerly eclamptic women cerebral WML were found in 16 of 39 (41%), compared with 5 of 29 (17%) women in the control group (p = 0.03). As shown in figure 2, there was a large variation of WML volume within the eclampsia group. Mean WML volume in the formerly eclamptic participants was 0.041 mL (SE 0.016) vs. 0.004 mL (SE 0.002) in the control group. However, there was a significant difference in WML lesion volume between the groups (mean rank 29.4 and 38.3, respectively, p = 0.025). Five of

the 6 (83%) women with the largest WML volume had experienced multiple seizures compared with 16 of 33 (48%) eclamptic women with lesser WML volume (p = 0.11). Four of the 6 (67%) women with the largest WML volume also had HELLP syndrome compared with 16 of 33 (48 %) eclamptic women with lesser WML lesion load (p = 0.41). $MgSO_4$ was administered in 26 of 39 (67%) of women, of which 5 received it prior to the first seizure. Of these 26 women 12 (46%) had WML on MRI. This is not significantly different from the group that did not receive $MgSO_4$ (p=0.36) and the number of seizures was not different between the group and the group without $MgSO_4$ administration.

The maximum blood pressure at the time of seizures was not different between the formerly eclamptic women with or without WML (188±7 vs. 199±7 mmHg diastolic respectively, p-value 0.29 and 109±4 vs. 116±3 mmHg systolic, p-value 0.123). The presence of HELLP and the parity at the time of seizures were not associated with the presence of white matter lesions in the formerly eclamptic group (p-values 0.24 and 0.50 respectively).

As shown in Figure 3, there was a positive correlation between the number of seizures and the presence of WML (test for trend, p = 0.009). Women who had experienced 3 or more seizures (n = 10) demonstrated WML more than 3 times as often as compared to controls (p = 0.01). The Kruskal-Wallis test showed a significant p-value of 0.042 when applied for the WML volume in these groups. The mean rank for the control group was 29.4, for formerly eclamptic women with 1 seizure (n = 19) 34.2, 2 seizures (n = 10) 39.1 and 3 or more seizures (n = 10) 45.4. These numbers, together with Figure 4, suggest that there is a significant relation between the number of experienced seizures and the severity of WML.

In the formerly eclamptic group two participants demonstrated evidence of cortical infarction and one woman demonstrated lacunar infarcts. None of the healthy control participants demonstrated such lesions.

Table 1 Relevant characteristics of participants.

	Controls (n = 29)	**Eclampsia** (n = 39)	**P-value**
Current age (years)	38 ± 6.9	38 ± 6.2	0.79
Current SBP (mmHg)	119 ± 14	126 ± 13	0.10
Current DBP (mmHg)	75 ± 9.5	76 ± 17	0.96
Elapsed time since index pregnancy (years)	5.3 ± 4.3	7.1 ± 4.7	0.88
EGA at delivery (weeks)	39.7 ± 1.3	33.6 ± 4.3	< 0.001
Birth weight (grams)	3511 ± 423	1898 ± 915	< 0.001

Results are presented as means ± standard deviations. EGA = Estimated Gestational Age, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure.

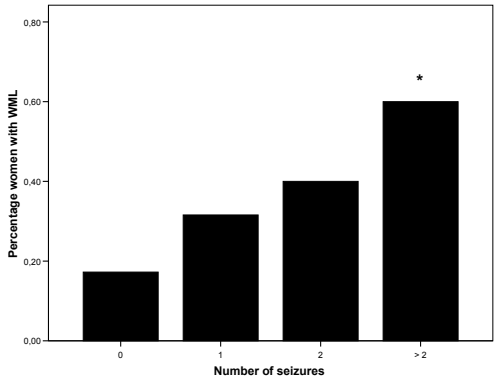

Figure 3 Percentage of women in both groups who demonstrated WML according to number of seizures. *Chi-square test p = 0.01, Test for trend p = 0.01.

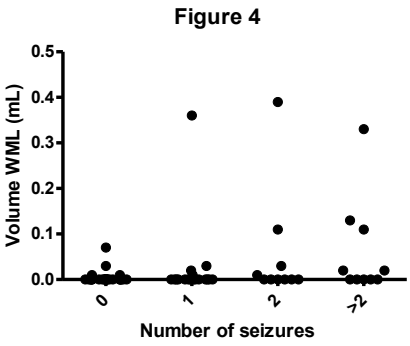

Figure 4 White matter lesion volume load in all subjects according to number of seizures. Kruskal-Walls test p = 0.04, test for trend p = 0.02.

Comment

Data now presented are compatible with long-term presence of brain white matter lesions possibly incurred at the time of eclamptic convulsions. Specifically, when studied with magnetic-resonance neuroimaging at a mean of 7.1 years following a pregnancy complicated by eclampsia, 41% of women demonstrated WML compared with only 17 % of control women on average 5.3 years following a contemporaneous non-eclamptic pregnancy. Moreover, the volume of these lesions was significantly larger in the women who had experienced eclampsia, and in addition, the presence and volume of the WML appeared to be associated with the number of seizures. These findings are in concert with recent observations that document MRI evidence of persistent WML in up to 25 % of eclamptic women at short term follow up of 2 months.[6,7] Another indication that formerly eclamptic women face long-term neurological sequelae comes from Aukes et al.[8] who describe more self-reported cognitive dysfunction in eclamptic women compared with a non-eclamptic cohort of contemporaneous parous controls. Also in this study an association between the number of seizures and the degree of self-reported cognitive dysfunction seems to be present.[8] Together these findings may indicate that eclamptic seizures are more harmful than is known at the present time. We, therefore, counter the position by some in the field of high risk obstetrics that the occurrence of eclamptic

seizures is benign and should not be prevented as long as the maternal and fetal condition is being monitored closely during a seizure.[13]

Currently, two concepts regarding the pathophysiology of eclampsia have been presented. According to the `vasculopathy` concept it is suggested that in response to acute severe hypertension cerebral `overregulation` leads to vasospasm.[14] This presumption is based on the angiographic appearance of diffuse or multifocal segmental narrowings suggestive of vasospasm of the cerebral vasculature in women with severe preeclampsia and eclampsia.[15] In that concept, diminished cerebral blood flow is hypothesized to result in ischemia, cytotoxic edema and, eventually, tissue infarction. According to the alternate, and nowadays preferred, concept eclampsia is likely the most common condition described underlying the posterior reversible encephalopathy syndrome (PRES).[3] Similar to non-pregnant patients with PRES, MRI demonstrates subcortical cerebral edema in nearly all women with eclampsia when imaged within the first 36 hours after the seizure(s).[6,7,16] We, therefore assume that also the cohort of formerly eclamptic women now described would have demonstrated this during the acute phase. The current concept of the development of PRES is related to breakthrough of the brain's well-developed autoregulatory capacity.[17] From clinical observations it seems that in the presence of endothelial dysfunction, sudden, even minute, elevations in systemic blood pressure may result in failure of autoregulation.[18-20] It is hypothesized that forced vasodilatation, increased hydrostatic pressure and hyperperfusion result in disruption of the blood-brain barrier.[2,20] Subsequent extravasation of plasma and opening of the endothelial tight junctions (blood-brain barrier) is followed by formation of vasogenic edema and the resulting manifestations of the clinical syndrome and accompanying neuroimaging findings.[3,4,21] Typical neuroimaging lesions in PRES, which are thought to be transient and indicative of vasogenic edema, predominate in the posterior cerebral white matter and cortex.[21] However, the name PRES appears to be inappropriate for the imaging findings in eclampsia because upon repeating MRI several weeks after delivery, the cerebral edema had resolved but cerebral WML were visible in almost a fourth of formerly eclamptic women.[6,7] We suggest that severe vasogenic edema in PRES can reduce tissue perfusion which results in ischemia and the development of cytotoxic edema.[5,22] In this scheme, such areas of poorly perfused brain may ultimately be at risk for ischemia and even infarction, all of which may give rise to the development of brain WML.[23]

Alternatively, some raise the question whether an underlying vascular condition predisposed to both the eclamptic seizures as well as the WML. Obviously, this study was not designed to answer that question. It is now recognized that women with (pre) eclampsia are at increased risk of hypertensive disorders and other cardiovascular and cerebrovascular disorders following pregnancy.[24,25] It is possible that an underlying

vascular condition gives rise to the development of WML in the years following the eclamptic seizure, rather than WML being a direct consequence of eclampsia. Also, it is possible that the WML preexisted prior to the eclamptic seizures. Nevertheless, our findings that women who experienced multiple seizures more often demonstrated WML and also larger WML volumes are suggestive of a cause and effect relationship between eclamptic seizures and the development of WML. The only way to answer the question whether the WML were preexistent, caused by the eclamptic seizures or by an underlying vascular disease is to perform serial MRI prior to and following eclampsia. Additionally, such women should be considered at risk for development of chronic vascular disorders. Interestingly, there was no difference in the rate of chronic hypertension between formerly eclamptic women and their age-matched controls. This finding is consistent with the work of Chesley[26] that primiparous women who experience eclampsia do not demonstrate increased blood pressure years after pregnancy.

The presence of WML in healthy individuals accrues with age. Indeed, more than half of healthy elderly persons demonstrate some degree of WML on MRI.[27,28] Although the prevalence of WML in younger age categories, such as our study participants, has not been reported, it should not be surprising that in our study 17% of otherwise healthy control women had evidence for such WML late in their fourth decade. The exact pathophysiology underlying these imaging changes and their clinical relevance remain so far unknown.[22] Concerning is the relation of WML with several vascular risk factors and the risk for stroke in the general population.[29-31] Epidemiological data suggest that preeclamptic subjects can face long-term cerebrovascular consequences such as the more than 3- to 5-fold increased risk for death from stroke.[24,25] Together, this suggests a prominent vascular etiology for brain WML.

Besides the tragic cerebrovascular endpoints such as stroke, intriguing information has recently become available regarding the cognitive and affective consequences of brain WML.[11,32,33] Obviously, aforementioned research pertains to a much older population unlike our much younger formerly eclamptic cohort. However, it is interesting to speculate about the possible clinical consequences of brain WML. Self-reported impaired cognitive function in women years after experiencing eclampsia[8] resembles the self-reported impaired cognitive function in elderly being related to WML burden.[33] Future research needs to determine whether the appearance, number and location of brain WML in formerly eclamptic women are associated with cognitive dysfunction and structural brain disease.

Taken together with these other studies[7,8], our current findings suggest that there may be permanent cerebral sequelae of eclamptic seizures. However, the magnitude of symptomatic neurological disability is yet unknown. These data are also supportive of

prevailing obstetrical practices to give magnesium sulfate for seizure prophylaxis to prevent seizure in women with preeclampsia.

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Preeclampsia and the risk of cerebral white matter lesions

Annet M. Aukes[1,2]
Jan C. De Groot[3]
Marjon J. Wiegman[1,2]
Jan G. Aarnoudse[2]
Gwendolyn S. Sanwikarja[2]
Gerda G. Zeeman[1,2]

[1]School for Behavioural and Cognitive Neurosciences, University of Groningen, the Netherlands
Departments of [2]Obstetrics and Gynecology and [3]Radiology, University Medical Center Groningen, the Netherlands

Submitted

Abstract

INTRODUCTION

Several years after pregnancy women with a history of eclampsia have cerebral white matter lesions more often compared to parous controls. The presence of these lesions is hypothesized to be the long-term result of the posterior reversible encephalopathy syndrome (PRES). Preeclamptic women may also experience PRES in the absence of eclamptic seizures. The aim of this study was to assess presence and severity of white matter lesions in formerly preeclamptic women.

METHODS

Cerebral MR imaging was performed on 73 formerly preeclamptic women and 75 healthy parous controls. Presence and severity of white matter lesion were determined by a neuroradiologist blinded for patient category.

RESULTS

Formerly preeclamptic women had white matter lesions significantly more often (37%) and more severely (mean 0.11, median 0.00, range 0-2.34ml) compared to controls (21%, p=0.04, mean 0.015, median 0.00, range 0-0.13ml, p=0.02). Average age (37±6 years) and elapsed time since index pregnancy (5.1±3.7 years) were similar in both groups. Within the formerly preeclamptic women current hypertension and a history of early-onset preeclampsia (<37 weeks) were independently associated with presence of white matter lesions.

CONCLUSIONS

Cerebral white matter lesions were present more often and more severely in formerly preeclamptic women compared to age-matched controls. Although, the predisposition of formerly preeclamptic women to cardiovascular disease, especially those with early-onset preeclampsia, may be an important cause of cerebral white matter lesions, a history of PRES is possibly an additive risk factor for the development of these lesions. Our findings indicate that preeclampsia might be a risk factor for early cerebrovascular damage.

Introduction

Women who had a pregnancy complicated by eclampsia, have cerebral white matter lesions (WML) several years later more often compared to women with normotensive pregnancies.[1] In addition, women with a history of preeclampsia have an increased risk of cardiovascular disease including stroke.[2,3] It has been suggested that the WML in formerly eclamptic women are a long term result of the posterior reversible encephalopathy syndrome (PRES)[1]. PRES is characterized by neurologic symptoms such as headache, altered mental functioning, seizures and loss of vision, together with bilateral vasogenic subcortical edema mainly in occipital and parietal lobes on cerebral computed tomography (CT) or magnetic resonance (MR) imaging.[4] It has been hypothesized that severe vasogenic edema can cause compression of cerebral tissue leading to reduced perfusion followed by ischemia, hypoxia and cell death.[5] Although its pathophysiology is still unclear, PRES has been recognized in eclampsia as well as in a variety of other disorders including several of iatrogenic or neurotoxic etiology.[6,7] These disorders are associated with endothelial dysfunction and hypertension in most cases.[5,6] Tonic-clonic seizures are not mandatory for the diagnosis of PRES and preeclamptic women may demonstrate signs, symptoms and imaging features of PRES in the absence of an eclamptic seizure.[8-10] The incidence and possible sequelae of PRES in women with preeclampsia are unknown, because cerebral imaging in such women is not standard practice. The aim of this study was to assess the presence and severity of WML in formerly preeclamptic women.

Materials and Methods

Study participants

The project was approved by the University Medical Center Groningen (UMCG) Institutional Review Board and all participants signed informed consent. The UMCG is a tertiary referral and academic teaching hospital in The Netherlands that serves as a perinatal referral center for high-risk pregnancies. A small percentage of healthy women without complicated pregnancies chooses to deliver in the UMCG as well. The annual delivery rate averages 1,600. The population in the northern part of the Netherlands is predominantly Caucasian. The department works with an electronic admission and delivery database since 1988. Women who were admitted with preeclampsia between 1988 and 2005 were selected and matched for age and year of index pregnancy to women

who experienced eclampsia of whom we reported in previous studies.[1,11] These formerly preeclamptic women were invited to participate in the current study by mail. In addition to this group, all women admitted to the obstetric high care with severe preeclampsia and/or HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelets) over a 2-year period (January 1, 2005 and December 31, 2006) were invited by mail to participate. Five formerly preeclamptic women who delivered in other hospitals than the UMCG had heard about this study and requested to participate in our study, which was allowed. Preeclampsia and gestational hypertension were defined according to internationally agreed standards.[12,13]

Only those women who were without contraindications for MR imaging (i.e. presence of metallic objects, tattoos, current pregnancy or claustrophobia) were eligible. When eligible, medical records were reviewed for accuracy and severity of diagnosis of preeclampsia and for the presence of neurological symptoms (visual disturbances, headache, increased tendon reflexes, myoclonia, nausea and vomiting) and HELLP syndrome during their admission. Exclusion criteria, besides mentioned MRI contraindications, included preexistent epilepsy, demyelinating disorders, a known cerebrovascular accident, intracranial infections or a history of any neurosurgical procedure. Formerly preeclamptic women were subsequently matched for age (within 2 years) and year of index pregnancy (within 2 years) with parous control women whose pregnancies had been uncomplicated and normotensive. These controls were recruited either through the department's electronic delivery database or recruited amongst hospital/department employees and their family members. Their records were evaluated to confirm that the pregnancy was indeed uneventful and normotensive.

Physical examination

On the day of MR imaging, body weight and blood pressure were measured. Blood pressure was measured manually with an aneroid sphygmomanometer. Participants were in sitting position with their arm resting on the chair-arm. Blood pressure was measured after a resting period of > 5 minutes and repeated after one hour if blood pressure was high. Blood pressure of ≥ 140/90 mmHg, or known hypertension with current antihypertensive medication use was used for the diagnosis of current hypertension in this group of women. The lowest measured blood pressure was used for analysis. Measurements were done by well-trained final year medical students and physicians.

MRI protocol

All studies were performed on a 3 Tesla MRI system (Philips Intera) at the Neuroimaging Center of the School for Behavioural and Cognitive Neurosciences in Groningen using 5 mm slices with a 20% gap and a matrix: 256 x 256. Used sequences include T1 (repetition time [TR] 700 ms, echo time [TE] 8.4 ms, α=65°, number of averages = 1), Proton Density (TR 3000 ms, TE 26.7 ms, α=90°), T2 (TR 3000 ms, TE 120 ms, α=90°) and fluid attenuation inversion recovery (FLAIR) (TR 11000 ms, TE 100 ms, TI 2800 ms, α=90°, number of averages = 2). For all women that underwent imaging in the second half of the study additional venous BOLD scans were preformed in order to detect microbleeds (volume scan with a voxel size of 0.45 x 0.45 x 1.00 mm. TR 34.9, TE 49.9, α=15°, matrix: 512 x 512). These were performed in 36 formerly preeclamptic women and 46 control women. An experienced neuroradiologist, blinded to participant's category and clinical data, rated the presence, size and number of white matter lesions. White matter lesions were considered present if hyperintense on FLAIR, proton density-weighted and T2-weighted image and not hypointense on a T1-weighted image. A WML severity score was used to asses the severity of WML for the subcortical area as described previously.[14,15] Briefly, the size of subcortical WML were rated according to their largest diameter in categories of small (< 3 mm), medium (3-10 mm), or large (> 10 mm). Considering them spherical with a fixed diameter per size category, a total volume-index of subcortical WML was calculated (range 0 – 0.4 mL). Having less than 3 possible small lesions and no confluent lesions was considered as no lesions to adjust for possible misclassifications. WML were considered periventricular lesions if the largest diameter was adjacent to the ventricular lining.

Data analysis

Demographic data were compared using Chi-square or Student t-test where appropriate. The presence of WML was compared between groups using Chi-square. The severity of WML between the groups was analyzed by using the Mann-Whitney (2 groups) or Kruskal-Wallis (3 groups) test. When the Kruskal-Wallis test was used, the Mann-Whitney was used as a post-hoc test. Univariate and multivariate binary logistic regression analysis were used for identify variables related to the presence of WML. Univariate regression analysis was used to identify variables related to WML severity. Considered covariables for the preeclampsia and control groups are listed in Table 1, estimated gestational age and birth weight were not included. Covariables for the preeclampsia subgroups are listed in Table 2. A P-value of < 0.05 was considered statistically significant. SPSS version 16.0 was used for data analysis.

Results

Three control participants were excluded because they had been diagnosed with gestational hypertension during the index pregnancy and a fourth control participant because she suffered epilepsy. One of the controls was excluded because of the incidental finding of a brain tumor during the MRI scan and one control was excluded because she demonstrated brain WML suggestive of a demyelinating disorder. One formerly preeclamptic woman was excluded because the diagnosis of preeclampsia could not be confirmed. The remaining number of participating women was 73 in the formerly preeclamptic group and 75 in the control group. In the formerly preeclamptic group 3 women had hypertension without proteinuria but with HELLP syndrome during the index pregnancy. One of the control women was of Asian origin, all other participants were Caucasian.

The participant's characteristics are shown in Table 1. As may be expected, the estimated gestational age and birth weight were significantly different between the groups. Current age and elapsed time since the index pregnancy were similar for both groups. Current systolic and diastolic blood pressure as well as current body weight was significantly higher in the formerly preeclamptic group. In the preeclamptic group 8 (11%) women had pre-existent hypertension prior to the index pregnancy and 10 (14%) women had suffered preeclampsia in more than one pregnancy.

Table 1 Participant's characteristics. Values are ± standard deviation.

	Controls (n = 73)	**Preeclampsia** (n = 75)	**P-value**
Age (years)	36.9 ± 6.0	36.6 ± 6.2	0.76
Elapsed Time since Index Pregnancy (years)	5.0 ± 3.3	5.3 ± 4.1	0.61
Birth Weight of Child (grams)	3464 ± 462	1842 ± 1176	0.00
Estimated Gestational Age at Delivery (weeks)	39.9 ± 1.2	33.0 ± 5.1	0.00
Current Systolic BP (mmHg)	116 ± 12	127 ± 12	0.00
Current Diastolic BP (mmHg)	74 ± 9	82 ± 11	0.00
Current Weight (kg)	71.0 ± 11.7	76.1 ± 18	0.03
Currently Hypertensive	4 (5%)	18 (25%)	0.00
Currently Smoking	14 (19%)	13 (18%)	0.88
History of Migraine	16 (21%)	24 (33%)	0.09

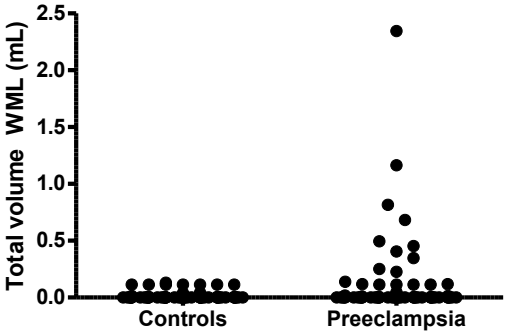

Figure 1 The total volume of white matter lesions in the formerly preeclamptic group and the control group. Mann-Whitney test, p = 0.02.

Significantly more women in the formerly preeclamptic group had WML compared to women in the control group (p=0.04, table 2). In addition, WML were more severe in the formerly preeclamptic women compared to the controls (p = 0.02, Figure 1, Table 2). In the formerly preeclamptic group, age (β = 0.09, P = 0.04) and current hypertension (β = 1.34, p = 0.02) were associated with the presence of WML. Adjusting for age revealed that current hypertension was independently associated with the presence of WML (β = 1.18, P = 0.04) in the formerly preeclamptic women. Current hypertension was not associated with severity of WML and none of the other women's characteristics (Table 1) were independently associated with neither presence nor severity of WML. In the control group no associations were found with the presence of WML.

The subgroup analyses within the preeclamptic group are shown in Table 2. The presence of HELLP syndrome or neurological symptoms during the index pregnancy in formerly preeclamptic women was not related to the presence or severity of WML. However, women who delivered prior to a gestational age of 37 weeks (early-onset preeclampsia) had significantly more often WML in comparison to those with late-onset preeclampsia. Also, the WML were significantly more severe in the formerly early-onset preeclamptic women compared to late-onset preeclampsia (Table 2) and controls (p<0.01, Figure 2). Formerly preeclamptic women had WML more often when they were currently hypertensive (11/18, 61%) compared to non-hypertensive formerly preeclamptic women (16/55, 29%, p = 0.02). The WLM were more severe in the currently hypertensive women, however, this did not reach significance (p = 0.07). There was no difference in presence or severity of WML between preeclamptic women with or without preexistent hypertension, preeclampsia during subsequent pregnancies, use of magnesiumsulphate and diastolic blood pressure above 110 mmHg during preeclampsia (an indicator for severe preeclampsia, Table 2). Of these factors, only early-onset preeclampsia was associated with the presence of WML (β = 1.73, p = 0.01).

Table 2 Presence and volume of white matter lesions (WML) in formerly preeclamptic women, control women and preeclampsia subgroups.

	Preeclampsia and Control groups		**P-value**
	Controls (n=75)	***Preeclampsia (n=73)***	
WML present	16 (21%)	27 (37%)	0.04
Mean volume	0.015 (0.00 – 0.13)	0.11 (0.00 – 2.34)	0.02
	Preeclampsia subgroups		
	Late-onset PE (>37 weeks, n=22)	***Early-onset PE (<37 weeks, n=51)***	
WML present	3 (14%)	24 (47%)	0.01
Mean volume	0.01 (0.00 – 0.11)	0.15 (0.00 – 2.34)	0.01
	Mild or no diastolic hypertension (n=47)	***Severe diastolic hypertension (n=25)***	
WML present	10 (14%)	16 (64%)	0.62
Mean volume	0.22 (0.00 – 2.34)	0.06 (0.00 –0.49)	0.42
	No neurologic symptoms (n=25)	***Neurologic symptoms (n=48)***	
WML present	10 (40%)	17 (35%)	0.70
Mean volume	0.14 (0.00 – 1.16)	0.97 (0.00 – 2.34)	0.59
	No HELLP syndrome (n=34)	***HELLP syndrome (n=39)***	
WML present	14 (41%)	13 (33%)	0.49
Mean volume	0.09 (0.00 -1.16)	0.13 (0.00 – 2.34)	0.80
	No $MgSO_4$ (n=49)	***$MgSO_4$ (n=24)***	
WML present	20 (41%)	7 (29%)	0.33
Mean volume	0.10 (0.00 - 1.16)	0.13 (0.00 – 2.34)	0.40

Volume is in ml with the range in parenthesis. The median was 0.00 ml in all (sub)groups. Severe diastolic hypertension is defined as ≥ 110 mmHg.

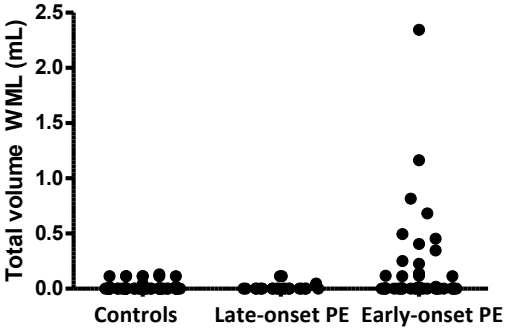

Figure 2 Total volume of white matter lesions in the control group, late-onset preeclamptic group and early-onset preeclamptic group. Kruskal-Wallis test over all: $p < 0.01$. Post-hoc: early-onset PE vs. late-onset PE: $p = 0.01$ and vs. controls: $p < 0.01$. PE = preeclampsia.

In the formerly preeclamptic group 2 women had a lacunar infarct and 1 woman had a cortical infarct. None of the control women had cerebral infarcts. All of the three women who had infarcts had several WML in addition. One had recurrent preeclampsia in three pregnancies and diabetes mellitus type 2, another had hereditary renal dysfunction, two had early-onset preeclampsia, two were currently hypertensive, and two had hypothyroidism.

Five formerly preeclamptic women had periventricular WML versus none of the controls (p=0.02). These five women also had subcortical WML in addition and all had early-onset preeclampsia. Microbleeds were seen in one control woman and in one formerly preeclamptic woman.

Comment

In this study we found that several years after the index pregnancy formerly preeclamptic women had cerebral WML more often and more severe compared to control women with normotensive pregnancies. This appeared predominantly in women with early-onset preeclampsia. In addition, the current blood pressure of formerly preeclamptic women was significantly higher compared to controls and in the formerly preeclamptic women current hypertension was independently associated with the presence of WML. Since a relationship with neurologic symptoms at the time of preeclampsia could not be demonstrated in our study, PRES may not be the single factor associated with these WML.

The current concept of the development of PRES is related to breakthrough of the brain's well developed autoregulatory capacity.[16] From clinical observations it seems that in the presence of endothelial dysfunction, sudden, even minute, elevations in systemic blood pressure may result in failure of cerebral autoregulation.[17] It has been hypothesized that in this syndrome forced vasodilatation, increased hydrostatic pressure and hyperperfusion result in disruption of the blood-brain barrier.[18] Subsequent extravasation of plasma and opening of the endothelial tight junctions (blood-brain barrier) is followed by formation of vasogenic edema and results in manifestation of the clinical syndrome with accompanying neuroimaging findings.[4,7,16]

Eclamptic women demonstrate vasogenic edema on CT and MRI at the acute moment.[4,9,10] Using diffusion weighted imaging (DWI) in a subset of women areas with cytotoxic edema can also be demonstrated.[5,19] It has been suggested that vasogenic edema in PRES/eclampsia can progress to such an extent that regional perfusion pressure

and blood flow decrease causing ischemia and cytotoxic edema.[5,20] Vasogenic edema resolves as the blood pressure normalizes [4], however, at two months follow-up approximately one fourth of eclamptic women demonstrate areas of gliosis or infarction within and around the area of previously restricted diffusion as seen on DWI.[5,19] In addition, several years after the index pregnancy formerly eclamptic women have WML more often and more severe compared to parous controls, in a linear relationship with the number of tonic-clonic seizures.[1] These findings suggest a causal relationship between severe cerebral vasogenic edema during the acute phase of PRES and the subsequent development of infarction and WML.

Preeclamptic women do not usually demonstrate cerebral abnormalities on radiologic imaging during the acute moment, although some women with severe preeclampsia have cerebral edema consistent with PRES.[8-10,21,22] We found that the presence of neurologic symptoms during the index pregnancy was not associated with the presence of WML several years after the index pregnancy which may suggest that PRES is not solely responsible for WML in the long term. Because of the reported inconsistency in presence of imaging abnormalities during the acute phase in women with preeclampsia[21,22], it is possible that some of our participants did suffer PRES during the index pregnancy. Whether or not they had subtle signs or symptoms of PRES may have been missed in our study due to its retrospective nature.

An additional explanation for the high percentage of women with WML in our group of formerly preeclamptic women, is the high prevalence of current hypertension in this group. In general, cardiovascular risk factors, especially hypertension, are related to the presence and progression of WML. The presence of hypertension is a risk factor for the development of WML in elderly populations[23,24] and younger cohorts.[25] Age is the predominant determinant for the presence of WML, however, this is a less important risk factor in younger (<55 yrs) populations.[25] The exact clinical importance of the presence of WML in a young cohort as in our study, is not clear. However, in the elderly there is evidence that the presence and particularly the severity of WML are important risk factors for the development of cognitive impairment, vascular dementia, Alzheimer's disease and stroke.[26]

A recent meta-analysis revealed that formerly preeclamptic women, especially those with early-onset preeclampsia, have an increased risk of ischemic and hemorrhagic stroke, both fatal and non-fatal, in later life.[3] Women who suffered preeclampsia appear to be at increased risk of hypertension, ischemic heart disease, stroke and venous thromboembolism later in life.[3] The results from our study are in line with these epidemiologic findings. In formerly preeclamptic women we found a high percentage of current hypertension and WML and in women with early-onset preeclampsia also more

often and more severe WML. A current concept is that pregnancy is a vascular and metabolic 'stress test' for a woman's health later in life.[27] If a woman develops preeclampsia – especially early-onset preeclampsia – she 'fails' to adapt to the cardiovascular and metabolic challenges of pregnancy. She has a higher risk to develop cardiovascular disease later in life. The exact underlying mechanism of the increased risk for cardiovascular disease following a pregnancy complicated with preeclampsia remains unknown but risk factors for atherosclerosis such as chronic hypertension, dislipidemia, obesity and glucose intolerance are likely to play a role.[28] This stresses the importance of decreasing the number of risk factors by changing life style and checking blood pressure regularly post partum in these young women.

Several methodological limitations to this study deserve attention. First, this study is a retrospective study and there are no imaging data of our participants prior to their index pregnancy. Whether WML were present prior to the index pregnancy, is therefore unknown, but considered unlikely. Second, cerebral imaging was not performed during the index pregnancy. This makes it impossible to identify those women who may have had cerebral edema due to PRES and to relate this to the current presence of WML. Third, we retrieved information on neurological symptoms retrospectively from medical records. This may be unreliable if used to determine retrospectively which women may have had signs and symptoms of PRES at the acute moment during the index pregnancy.

Perspectives

To our knowledge this study is the first to report cerebral imaging and cerebral white matter lesions in the long term in a considerable group of women with a history of preeclampsia. The exact cause of WML as well as the clinical implications of our findings are so far unknown, but seem ominous considering the higher incidence of stroke in later life in women who suffered preeclampsia. In older populations an association between WML and cognitive impairment has been found.[24,29] However, self-reported cognitive impairment was not obvious in a younger cohort with a history of preeclampsia.[11,30] Presence of WML is associated with the development of vascular dementia and Alzheimer's disease later in life. In addition to cognitive impairment and dementia, the baseline severity of WML is associated with the risk of stroke.[31,32] Therefore, the finding of WML in formerly preeclamptic women could indicate a precursor of cerebrovascular disease later in life. Future research should determine the clinical importance and development throughout the years of these WML in this young group of women.

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Part II

Er is
nog nooit
een mens geweest
die een korrel aarde
heeft bezeten

(Jan Arends)

Pregnancy prevents hypertensive remodeling and decreases myogenic reactivity in posterior cerebral arteries from Dahl salt-sensitive rats: a role in eclampsia?

Annet M. Aukes[1,2]
Lisa Vitullo[1]
Gerda G. Zeeman[2]
Marilyn J. Cipolla[1]

[1]*Departments of Neurology, Obstetrics & Gynecology and Pharmacology, University of Vermont, Burlington, VT, USA*
[2]*Department of Obstetrics & Gynecology, University Medical Center Groningen, the Netherlands*

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Abstract

Previous studies have demonstrated that pregnancy prevents protective hypertension-induced remodeling of cerebral arteries using nitric oxide synthase (NOS) inhibition to raise mean arterial pressure (MAP). In the present study we investigated whether this effect of pregnancy was specific to NOS inhibition by using the Dahl salt-sensitive rat (Dahl SS) as a model of hypertension. Nonpregnant (n=16) and late-pregnant (n=17) Dahl SS rats were fed either a high salt diet (8% NaCl) to raise blood pressure, or a low salt diet (<0.7% NaCl). Third-order posterior cerebral arteries were isolated and pressurized in an arteriograph chamber to measure active responses to pressure and passive remodeling. Several vessels from each group were stained for protein gene product 9.5 to determine perivascular nerve density. Blood pressure was elevated in both groups on high salt. The elevated mean arterial pressure was associated with significantly smaller active and passive diameters ($p<0.05$) and inward remodeling in the nonpregnant hypertensive group only. While no structural changes were observed in the late-pregnant hypertensive animals, both late-pregnant groups had diminished myogenic reactivity ($p<0.05$). Nerve density in both the late-pregnant groups was significantly greater compared to the nonpregnant groups, suggesting that pregnancy has a trophic influence on perivascular innervation of the posterior cerebral artery. However, hypertension lowered the nerve density in both nonpregnant and late-pregnant animals. It therefore appears that pregnancy has an overall effect to prevent hypertension-induced remodeling regardless of the mode of hypertension. This effect may predispose the brain to autoregulatory breakthrough, hyperperfusion and eclampsia when MAP is elevated.

Introduction

Hypertension is one of the most common complications of pregnancy and is a life-threatening disease for both mother and fetus.(27) Several organs are affected by pregnancy induced hypertension, including the brain manifesting itself as eclampsia with classical neurological features such as headache, nausea, cortical blindness, loss of consciousness and seizures.(35) Eclampsia is one of the leading causes of maternal death (12, 23), yet little is known about how hypertension in pregnancy affects the cerebral circulation and causes the symptoms of eclampsia.

The primary explanation for the pathogenesis of eclampsia is that it is thought to be a form of hypertensive encephalopathy.(11, 22, 36, 37) This syndrome is characterized by an acute rise in blood pressure that overcomes cerebral artery myogenic tone causing forced dilatation, autoregulatory breakthrough and hyperperfusion.(21) As a consequence, blood-brain barrier disruption occurs followed by cerebral edema formation.(18) Because of the significant involvement of the cerebrovasculature in mediating these symptoms, investigating how pregnancy and hypertension during pregnancy affect the cerebral circulation seems critical to understanding and treating eclampsia.

While there is evidence to suggest that the cerebral circulation is altered during normal pregnancy (9), how hypertension during pregnancy affects the cerebral circulation is largely unknown, but may have a unique influence compared to normal pregnancy. One study in which nitro-L-arginine (L-NAME) was used to raise mean arterial pressure (MAP) in nonpregnant (NP) and late-pregnant (LP) Sprague-Dawley rats demonstrated significant remodeling and medial hypertrophy of the posterior cerebral artery (PCA) from NP animals, a response that was absent in the LP animals.(7) In addition, LP animals underwent forced dilatation at significantly lower pressures compared to NP animals, regardless of the presence of hypertension. These findings suggest that pregnancy prevents protective hypertensive remodeling of cerebral arteries and may make hypertension in pregnancy a vulnerable state for autoregulatory breakthrough and the complications of eclampsia.

Because cerebral artery remodeling may be influenced by the type of hypertension, we investigated whether or not inducing hypertension by another means would have a similar effect on remodeling and those parameters that influence cerebrovascular resistance and cerebral autoregulation. The Dahl salt-sensitive (Dahl SS) rat is a genetic model of hypertension that has been shown to spontaneously develop the

neurological complications of hypertensive encephalopathy, including loss of autoregulation, BBB disruption and edema formation when fed a high salt diet.(33) In addition, this model of hypertension has been shown to have significant oxidative stress(2, 20) and is one of the few hypertensive strains in which MAP remains elevated during pregnancy.(10) We therefore investigated the effect of pregnancy on the structure and function of PCAs from Dahl SS rats made hypertensive by feeding high salt, compared to animals on a low salt diet.

We investigated the effect of hypertension during pregnancy on the active response to pressure (myogenic activity) and passive structural changes. This included lumen diameter, wall thickness and wall:lumen ratio because these properties are known to contribute to cerebrovascular resistance and autoregulation of cerebral blood flow.(16) In addition, because perivascular innervation has been shown to be involved in medial hypertrophy in other genetic models of hypertension(19), we also determined changes in nerve density of the PCA. To our knowledge, this is the first study to examine cerebrovascular changes in female Dahl rats during hypertension and pregnancy.

Materials and Methods

Animal Model

A rat model of pregnancy and hypertension was used for all experiments. All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Seven-week-old virgin female Dahl SS nonpregnant (Dahl SS NP, n=16) rats were compared to late-pregnant Dahl SS (Dahl SS LP, n=17) rats of the same age. Both groups were split in two groups on either a high salt diet, 8% NaCl (NP n=8, LP n=8) to raise their blood pressure or on a low salt diet, <0.7% NaCl (NP n=8, LP n=9). The LP animals received this high salt food in the last 12-14 days of gestation when eclampsia occurs most often.(27) The NP controls received the high salt diet in the last 12-14 days before the experiment. The rats on low salt had been on this diet since they were born and remained on it until the day of the experiment.

Blood Pressure Measurements

Blood pressures were measured every day by a tail cuff method using the Coda 6 System (Kent Scientific), as previously described.(7) The volume and pressure recording technique allowed noninvasive measurements of 6 blood pressure parameters: systolic blood

pressure, diastolic blood pressure, mean arterial pressure, heart rate, tail blood volume, and tail blood flow.

Preparation of the Vessels

After the animals were anesthetized with isoflurane in oxygen, they were decapitated. The brain was quickly removed and placed in cold physiological salt solution (HEPES solution). A third-order branch of the PCA was carefully dissected and cleared of connective tissue. The vessel was then placed in an arteriograph chamber (Living Systems, Burlington, Vermont) for isolated vessel experiments, described below. The PCA was used because the symptoms of eclampsia most commonly occur in the posterior circulation.(22) For pregnant animals, the abdomen was opened and the number of pups counted in utero.

Pressurized Arteriograph System

Dissected arteries were mounted on two glass cannulas in the arteriograph chamber, attached with nylon ties and pressurized, as previously described.(9) The whole chamber was placed on an inverted microscope connected to a video camera and monitor to measure lumen diameter and wall thickness of the arteries using video microscopy and video dimensional analysis (VDA). The chamber was attached to a heat exchanger that continuously recirculated the HEPES solution and kept the temperature at 37.0 ± 0.5 ºC. The pH was continually measured and maintained at 7.40 ± 0.05.

Experimental Protocol

After equilibration for 45 minutes at 50mmHg, the HEPES was replaced with new HEPES and equilibrated for another 15 minutes. To obtain active pressure vs. diameter curves, pressure was increased to 200mmHg in steps of 25mmHg and the lumen diameter and wall thickness were measured at each step, once stable after about 10 minutes. At 200mmHg papaverine (0.1mmol/L) was added to the bath to completely relax the vessel, so that passive pressure-diameter curves could be obtained and passive distensibility calculated. The pressure was lowered in steps of 25mmHg to 50mmHg and to 10mmHg in steps of 10mmHg. Again, lumen diameter and wall thickness were measured at each pressure.

Perivascular Nerve Staining and Deterimination of Nerve Density

Immunohistochemical staining of perivascular nerves and quantification of nerve density was accomplished as previously described.(8) Briefly, the entire left PCA segment was removed from each animal and placed in fixative consisting of 2% paraformaldehyde and 0.2% picric acid. Vessels were incubated overnight with Rabbit anti-PGP 9.5 (Chemicon

Int., Temecula, CA), a pan-neuronal stain at a concentration of 1:3000 in blocking media, followed by incubation in Goat anti-rabbit Cy-3 at 1:400 for 2 hours. Imaging was performed on an Olympus microscope at 20x magnification using a filter for Cy-3 and captured using Magnifire software. Images of the 3rd order branch of the PCA were used to determine nerve density and area, measured using Metamorph software. Determination of nerve density was performed in Metamorph by a standard square lattice pointing grid (30μm^2) over each image and counting the number of intersect points where perivascular nerves crossed the grid.(8) Nerve density was then calculated as the number of points divided by the area.

Data Calculations

Percent tone was calculated as the percent decrease in diameter from the fully relaxed diameter in papaverine at 75mmHg by the equation $[1-(\Phi_{tone}/\Phi_{papav})]\bullet 100\%$, where Φ_{tone} is the diameter of the vessel with tone and Φ_{papav} is the diameter of the vessel in papaverine.

Slope of the active pressure vs. diameter curves was determined at pressures within the myogenic pressure range from 75 to 125mmHg and calculated by the equation $m = \Delta_{\Phi}/\Delta_{pressure}$, where Δ_{Φ} is the difference in diameter of the myogenic active vessel at the two different pressures and $\Delta_{pressure}$ the difference in pressure.

Statistical Analysis

Results are presented as mean±SEM. Differences in blood pressures, body weights, lumen diameter, slope, percent tone, wall thickness and wall:lumen ratio between groups were determined by one-way ANOVA followed by a posthoc Student-Neuman Keull's test for multiple comparisons, where appropriate. Differences in diameter within each group at 75mmHg vs. 125mmHg were determined by repeated measures ANOVA. Differences were considered significant at $p<0.05$.

Results

Table 1 shows the characteristics of the animals used in this study. Blood pressure, including systolic and mean arterial, of the NP rats on high salt was significantly elevated compared to the low salt controls ($p<0.05$). The blood pressure of the LP rats on high salt was also elevated to within a hypertensive range compared to LP rats on low salt ($p=0.06$), but not as much as in the NP group. Hypertension had no effect on the outcome of

pregnancy, as was demonstrated by similar body weights of the LP animals and a similar number of pups. Hypertension did have an effect on the body weight of the NP animals; the body weight of the rats on high salt was significantly lower than those on low salt.

Figure 1 shows the active pressure vs. diameter curves of the PCAs within the myogenic pressure range from 75 to 125mmHg. Both of the NP groups demonstrated myogenic reactivity (Figure 1A), as demonstrated by the small or negative slope of the curves and no statistical difference in diameter between 75 and 125mmHg ($p>0.05$). The NP hypertensive animals had significantly smaller diameters at 75mmHg vs. the normotensive animals. This may have been partly due to increased myogenic tone in the hypertensive animals since these animals had increased tone, but this was not statistically significant. The percent tone in the NP normotensive and hypertensive animals was, respectively: 30.2 ± 6.0% vs. 40.7 ± 4.6% ($p>0.05$). Regardless of the presence of hypertension, myogenic reactivity was significantly diminished in both LP groups (Figure 1B). This was demonstrated by the significantly steeper slope of the pressure vs. diameter curves in the LP animals compared to the NP animals ($p<0.05$) and the significantly larger diameters at 125mmHg vs. 75mmHg ($p<0.01$). Both the LP groups had similar percent tone. The percent tone in the LP normotensive and hypertensive animals was, respectively: 36.3 ± 5.2% vs. 40.0 ± 5.8% ($p>0.05$).

Figure 2 shows the passive pressure vs. diameter curves of the PCAs in papaverine. The NP animals (Figure 2A) responded to hypertension by decreased inner diameters, demonstrating structural remodeling to decrease the lumen diameter. This effect was not due to changes in passive distensibility, since there was no difference

Table 1 Characteristics of Dahl salt-sensitive rats.

	SBP (mmHg)	**DBP** (mmHg)	**MAP** (mmHg)	**Body weight** (gram)	**Pups**(#)
Nonpregnant					
Low salt (n=8)	130 ± 6	91 ± 5	104 ± 5	194 ± 6	
High salt (n=8)	152 ± 5*	105 ± 5	120 ± 5*	180 ± 3†	
Late-pregnant					
Low salt (n=9)	129 ± 3	93 ± 4	104 ± 3	283 ± 4‡	9 ± 1
High salt (n=8)	142 ± 6§	100 ± 6	113 ± 6	286 ± 9‡	10 ± 1

Values are means ± SE for n rats. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. * $p < 0.01$ vs. nonpregnant low salt group; † $p < 0.05$ vs. nonpregnant low salt group; ‡ $p < 0.01$ vs. nonpregnant group; § $p = 0.06$ vs. late-pregnant low salt group.

Figure 1

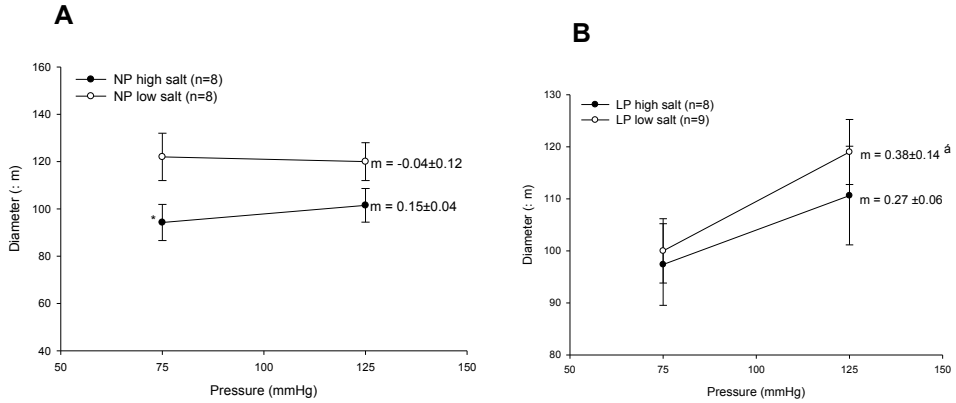

Figure 1 Active pressure vs. diameter curves with slope (m) for posterior cerebral arteries (PCA) from nonpregnant (NP) and late-pregnant (LP) rats. A: both groups of NP animals responded to pressure myogenically, as demonstrated by the small slope. NP hypertensive animals (closed circles) had significantly smaller diameters at 75mmHg compared with the normotensive control animals (open circles). B: LP animals had significantly diminished myogenic reactivity as demonstrated by significantly larger diameters at 125mmHg compared to 75mmHg and the significantly steeper slopes. *$p < 0.05$ vs. NP hypertensive; $^{\dagger}p < 0.05$ vs. 75mmHg; $^{\ddagger}p < 0.05$ LP vs. NP hypertensive.

Figure 2

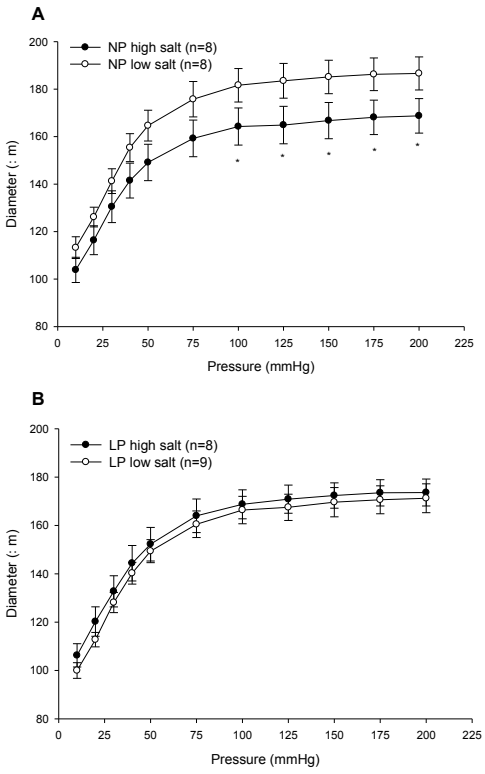

Figure 2 Passive pressure vs. diameter (in μm) curves for PCAs from NP and LP animals. A: the diameters of PCAs in NP hypertensive animals were smaller compared to normotensive control animals. B: the diameters of PCAs in LP hypertensive and normotensive animals were similar. *$p = 0.06$ vs. NP low salt.

between the groups (data not shown). The passive inner diameters of the two LP groups (Figure 2B) did not differ and were similar to the inner diameters of the NP hypertensive animals, suggesting that pregnancy alone causes remodeling to decrease the inner diameters independently of the hypertension. The changes in inner diameters were not due to alterations in passive distensibility, since there was no significant difference between the hypertensive or normotensive groups (data not shown).

Table 2 shows the passive measurements of unpressurized PCAs. There was no significant difference between the inner and outer diameters between groups; however, the wall thickness of PCAs from the NP hypertensive group was smaller than from the NP normotensive group ($p<0.05$), demonstrating structural remodeling in response to hypertension. There was no significant difference in wall:lumen ratio between any of the groups, although the NP hypertensive group had smaller ratios.

Staining of perivascular nerves with the pan-neuronal stain PGP 9.5 revealed that these vessels were densely innervated with vericose fibers within the adventitial layer (Figure 3A). The nerve density of PCAs from both groups of LP animals was significantly greater than both groups of NP animals, demonstrating that pregnancy has a trophic influence on perivascular nerves from the posterior cerebral circulation (Figure 3B). However, both NP and LP hypertensive animals had significantly fewer perivascular nerves compared to normotensive animals, suggesting that either a high salt diet or elevated MAP influences the extent of innervation.

Table 2 Passive measurements of unpressurized posterior cerebral arteries.

	Inner Diameter (μm)	**Outer Diameter** (μm)	**Wall thickness** (μm)	**Wall:Lumen ratio**
Nonpregnant				
Low salt (n=8)	108 ± 5	142 ± 5	17.1 ± 0.8	0.16 ± 0.01
High salt (n=8)	101 ± 5	128 ± 7	13.6 ± 1.3*	0.14 ± 0.01
Late-pregnant				
Low salt (n=9)	97 ± 3	130 ± 3	15.9 ± 0.4	0.16 ± 0.01
High salt (n=8)	98 ± 6	130 ± 6	16.0 ± 1.0	0.17 ± 0.02

Values are mean ± SE for n rats. * $p < 0.05$ vs. nonpregnant low salt group.

Figure 3

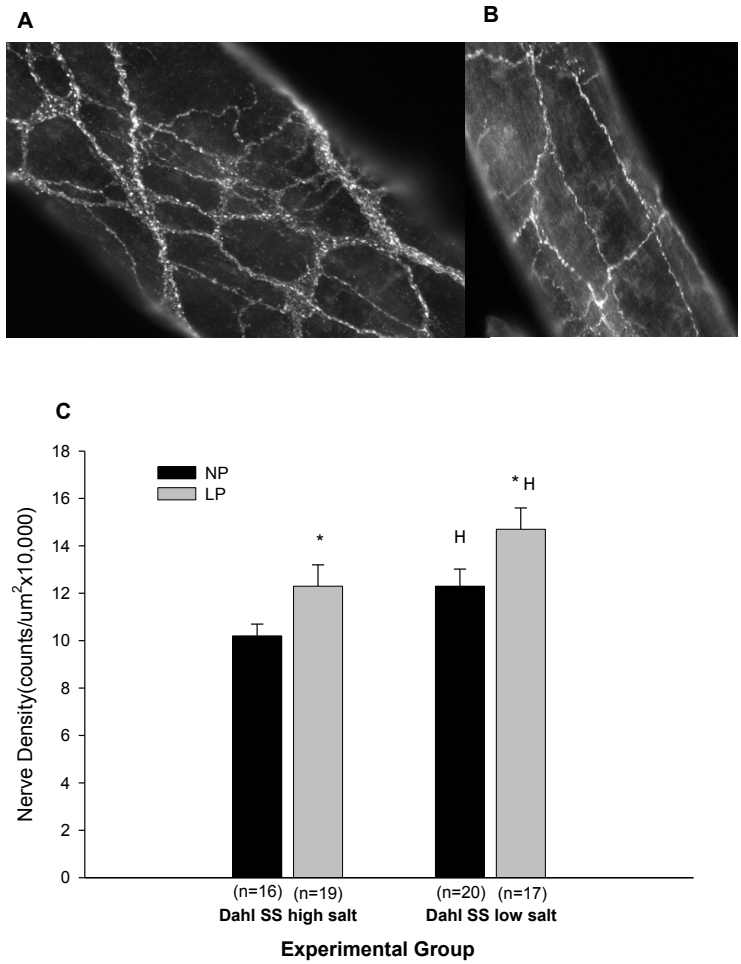

Figure 3 Perivascular nerve fibers and their density in pial PCAs of NP and LP Dahl salt-sensitive rats. A and B: photomicrographs of PCAs stained for perivascular nerves with the pan-neuronal stain, protein gene product 9.5. The arteries were imaged using a fluorescent microscope for Cy3. A: photomicrograph of perivascular nerves on the PCA from a LP normotensive animal. B: photomicrograph of perivascular nerves on the PCA from a NP hypertensive animal. C: perivascular nerve density of PCAs for all groups of animals. Nerve density is expressed per square micrometer of vascular wall. The nerve density was significantly higher in both LP groups when compared with that in the NP groups; however, both hypertensive groups had nerve density that was significantly less when compared with the normotensive groups. *$p < 0.05$ vs. NP; $^{\dagger}p < 0.05$ vs. hypertensive.

Discussion

In the present study, we used Dahl SS rats to investigate how pregnancy and hypertension during pregnancy affected the structure and function of the posterior cerebral circulation. The major finding was that regardless of the presence of hypertension, pregnancy diminished myogenic activity of PCAs (Figure 1B) and prevented protective hypertension-induced remodeling of PCAs. These results are comparable to what was found in a similar study using NOS inhibition with L-NAME to induce hypertension in female Sprague-Dawley rats that showed a decrease in the pressure of forced dilatation in LP animals regardless of the presence of hypertension and showed significant remodeling only in NP animals.(9) Thus, the lack of remodeling in reaction to hypertension in the LP animals is not specific to NOS inhibition, but appears to be an effect of pregnancy.

While the PCAs from LP animals had significantly diminished myogenic activity, this was not the case in NP animals. In fact, PCAs from NP animals had considerable myogenic tone and reactivity to pressure (Figure 1A). In addition, NP animals responded to the hypertension with smaller diameters both actively and passively (Figures 1A and 2A). This inward remodeling of cerebral arteries has been shown in numerous other studies using genetically hypertensive male animals(1, 16) and during L-NAME hypertension in female animals.(7, 24) To our knowledge, this is the first study to specifically show this response to hypertension in cerebral arteries from female Dahl SS animals.

To more accurately assess passive remodeling of cerebral arteries, the unpressurized diameter and wall thicknesses were measured. This eliminated any differences in distensibility that may contribute to those measurements. While there was no significant difference in inner or outer diameter of PCAs between groups, LP animals had diameters more similar to NP hypertensive animals (Figure 2 and Table 2). In fact, there was no difference in diameters between control and hypertensive LP animals either passively or actively, again suggesting that pregnancy prevents any response to hypertension, similar to L-NAME hypertension. Also similar to L-NAME hypertension, PCAs from NP hypertensive animals underwent inward remodeling, as demonstrated by the significantly smaller wall thickness and lumen diameter. However, unlike the response to L-NAME hypertension in which the arterial wall became thicker, the decrease in wall thickness in the Dahl rat suggests that the site of remodeling has shifted towards upstream arteries. This difference in remodeling in response to hypertension may be due to several factors, including the presence of nitric oxide and oxidative stress in the Dahl animals that is not present in the L-NAME treated animals.(2, 20) Also genetic factors may play a role in this difference. Chillon and Baumbach found that in L-NAME treated Wistar-

Kyoto male rats pial arterioles undergo inward remodeling, whereas pial arterioles of L-NAME treated Sprague-Dawley rats undergo outward remodeling.(6) Therefore different genetic strains of rats can have different reactions to chronic hypertension.

To our knowledge, vascular remodeling in the cerebral arteries of Dahl hypertensive rats has not been reported before. It is also worth mentioning that most studies on the rat cerebral vasculature were done in chronic hypertension (e.g. 3 months). Hypertension during pregnancy is not quite chronic hypertension, but more a unique form of hypertension, as it is somewhere between chronic and acute. Yet, vascular remodeling occurs quickly after the onset of hypertension; after only 7 days of L-NAME treatment the pregnant Sprague-Dawley rats PCAs showed significant vascular remodeling.(7)

In the present study, we chose to use the Dahl SS rat to investigate how hypertension during pregnancy affected the structure and function of cerebral arteries because in male rats this model of hypertension has been shown to cause hypertensive encephalopathy, a similar condition to eclampsia, including loss of autoregulation, hyperperfusion and cerebral edema formation.(33) In addition, this model of genetic hypertension has been shown to have considerable oxidative stress(2, 20), another feature of (pre)eclamptic women(15), and to remain hypertensive during pregnancy.(10) Dahl SS rats were fed a high salt diet for the last 14 days of a 22 day gestation (or for 14 days prior to experimentation) because late gestation is when eclampsia occurs most often.(27)

The Dahl SS rat was also chosen for this study because it is thought to develop hypertension distinct from NOS inhibition, although treatment of Dahl SS rats with L-arginine, prevented the development of salt induced hypertension, suggesting a role for nitric oxide deficiency in the development of hypertension.(17) A primary mechanism by which the Dahl SS rat develops hypertension when fed a high salt diet is due to a lack of suppression in proximal tubular renin in this strain of rats, which may cause increased levels of angiotensin II and increased sodium absorption.(34) A restriction fragment length polymorphism (RFLP) in the renin gene was found in the Dahl SS rat (25), confirming the suggestion of the involvement of renin angiotensin system in causing hypertension. While it is beyond the scope of this study to determine the mechanism by which pregnancy prevents hypertension-induced remodeling of cerebral arteries, it does appear to be an overall effect of pregnancy since the response was similar in two different models of hypertension.

It is worth mentioning that we measured active and passive responses in only one vessel within the PCA territory. While it is possible that pregnancy has an overall effect on the cerebrovasculature that may promote forced dilatation at lower pressures, autoregulatory breakthrough and hyperperfusion, all complications that could lead to the

neurologic complications of eclampsia, it cannot be discerned from this study. However, both clinical and animal studies have found that normal pregnancy is associated with an increased cerebral perfusion pressure, a decreased cerebral vascular resistance and hyperperfusion.(3, 14) In addition, (pre)eclamptic women have been shown to have an increased cerebral perfusion pressure, a further decreased CVR and a further increased CBF.(26, 36) Therefore, the findings of the current study support these hemodynamic changes during pregnancy and (pre)eclampsia.

One interesting finding of this study was that pregnancy had a trophic influence on the perivascular innervation of the PCA, regardless of the presence of hypertension (Figure 3). These nerve fibers are extrinsic in origin (i.e., have ganglion outside the CNS)(4, 9) and contain neurotransmitters from parasympathetic, sympathetic and trigeminal systems.(13) Numerous studies have shown that the sympathetic innervation of cerebral arteries contributes to medial hypertrophy in response to genetic hypertension.(19, 29) In the present study, a pan-neuronal stain was used to visualize all nerve fibers and therefore we cannot distinguish which specific fibers, if any, were influenced by pregnancy.

It is unlikely that the growth of these nerve fibers contributed to arterial remodeling since there was no correlation between nerve fiber density and diameter or wall thickness. Again, while distinction of specific fibers is not possible from this study, understanding how these extrinsic nerves may be affecting the cerebral pial arteries may provide valuable insight into the neurologic symptoms of eclampsia. For example, the trigeminovascular system has been shown to have a prominent role in mediating migraine, a condition with similar symptoms to eclampsia (headache, nausea, vomiting, visual disturbances).(31) In fact, headache is the most common symptom of eclampsia.(32) In addition, these disorders both involve nocicepetive fibers and are hormonally sensitive.(5, 28) It is therefore possible that pregnancy affects the trigeminal fibers or the response of the cerebral arteries to trigeminal neuropeptides in a way that promotes the neurologic symptoms of eclampsia, similar to migraine (e.g., vasodilatation and increased BBB permeability).

In summary, this study demonstrates that 1) pregnancy diminished myogenic reactivity in the PCA regardless of the presence of hypertension. While we cannot rule out the possibility that pregnancy shifts the site of the myogenic activity to upstream or downstream vessels, it is possible that the posterior circulation is more vulnerable to autoregulatory breakthrough and hyperperfusion when blood pressure rises, as in eclampsia; 2) hypertension in nonpregnant female Dahl SS rats caused inward remodeling with a decrease in wall thickness suggesting that the state at which hypertension affected the cerebral arteries was upstream from these pial vessels; and 3) pregnancy enhanced growth of perivascular nerve fibers of the PCA. While it is unclear which specific nerve

fibers are influenced by pregnancy (e.g., sympathetic, parasympathetic or trigeminal), it is possible that changes in perivascular innervation of the cerebral pial arteries during pregnancy play part in the symptoms of eclampsia. For example, sympathetic nerves protect the blood-brain barrier against disruption during chronic hypertension,(30) whereas trigeminal nerves are nociceptive and are involved in migraine, a state with similar neurologic symptoms to eclampsia.(5) The effect of pregnancy on perivascular innervation and its role in mediating the pathophysiology of eclampsia deserves further study.

Grants

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The influence of pregnancy and gender on perivascular innervation of rat posterior cerebral arteries

Annet M. Aukes[1,2]
Nicole Bishop[1]
Julie Godfrey[1]
Marilyn J. Cipolla[1]

[1]*Departments of Neurology, Obstetrics and Gynecology, and Pharmacology, University of Vermont, Burlington, VT, USA*
[2]*School of Behavioral and Cognitive Neurosciences, University of Groningen, and Department of Obstetrics and Gynecology, University Medical Center Groningen, the Netherlands*

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Abstract

The autors investigated the influence of pregnancy and gender on the density of trigeminal and sympathetic perivascular nerves in posterior cerebral arteries (PCA) and the reactivity to norepinephrine and calcitonin gene-related peptide (CGRP). PCAs were isolated from nonpregnant, late-pregnant, postpartum and male rats, mounted and pressurized on an arteriograph chamber to obtain concentration-response curves to norepinephrine and CGRP. Arteries were immunostained for CGRP-, tyrosine hydroxylase- and protein gene product 9.5 (PGP 9.5)-containing perivascular nerves, and nerve density was determined morphologically. Pregnancy had a trophic effect on trigeminal perivascular innervation ($p<0.01$ vs. male); however, this was not accompanied by a change in reactivity to CGRP. Sympathetic and PGP 9.5 nerve densities were not altered by pregnancy or gender and there were no differences in reactivity to norepinephrine. Together, these results suggest that the increase in trigeminal innervation during pregnancy is more related to nociception than in controlling resting cerebral blood flow.

Introduction

Cerebral pial arteries are extrinsically innervated by perivascular nerves[1]; that is, these nerves have their origin outside the central nervous system. The perivascular innervation consists of sympathetic, parasympathetic and sensory (trigeminal) nerves originating from the superior cervical ganglion, the otic and sphenopalatine ganglia, and the trigeminal ganglion, respectively.[2] Under physiologic conditions, these perivascular nerves do not seem to have a major effect on resting cerebral blood flow.[3,4] However, under pathologic conditions, their role appears to be more important. For example, sympathetic nerve activity has a protective function during acute hypertension where it limits hyperperfusion and decreases blood-brain barrier permeability.[5,6] Trigeminal nerves have a role in the pathophysiology of migraine, causing vasodilation in dural and pial arteries and hyperemia in these regions. The trigeminal afferents from dural and pial vessels also sense noxious stimuli and signal to the pain center in the brainstem.[7-9]

In a previous study, we found that pregnancy had a trophic effect on perivascular innervation of posterior cerebral arteries (PCAs) from Dahl salt-sensitive rats, with a significant increase in nerve density in PCAs from late-pregnant rats compared to nonpregnant rats.[10] In that study, a pan-neuronal stain, protein gene product 9.5 (PGP 9.5) was used to visualize and determine nerve density. Therefore, it was not possible to distinguish the type of nerve fibers that was affected by pregnancy (eg, sympathetic, parasympathetic, trigeminal). In the current study, we investigated the effect of pregnancy and the postpartum state on specific perivascular nerve fibers of the PCA, including calcitonin gene-related peptide (CGRP)- and tyrosine hydroxilase (TH)-containing perivascular nerves. These trigeminal and sympathetic nerve fibers are of particular interest because of the significant cardiovascular adaptation that occurs during pregnancy and the postpartum state that may include perivascular innervation. While cardiovascular adaptations are well-studied in the peripheral vascular system[11-13], the effect of gestation on the cerebral circulation is largely unknown, but may have a significant role in the development of pathologic conditions including severe preeclampsia and eclampsia where neurological symptoms are noted.

CGRP is a neurotransmitter of the trigeminal nervous system and a potent vasodilator both peripherally and centrally.[14,15] In addition to its prominent role in the pathophysiology of migraine, CGRP is thought to contribute to the vascular adaptation that occurs during pregnancy when plasma volume expands without an increase in blood pressure.[16,17] While the effect of pregnancy on CGRP containing nerves in cerebral pial

arteries is unknown, it is interesting that headache is the most common complaint of eclamptic women.[18] Being the only nociceptive fiber in the brain, we hypothesized that pregnancy has a trophic effect on CGRP-containing perivascular nerves in rat PCAs. In addition, because of the somewhat gender-specific nature of migraine[19], we further hypothesized that there would be an effect of gender such that PCAs from male animals would have less CGRP innervation than any of the female groups investigated.

The effect of sympathetic perivascular nerve activity on cerebral pial arteries has been well-studied.[5,6,20-22] Sympathetic perivascular nerve activity contributes to protective medial hypertrophy of the pial artery wall during chronic hypertension, a consequence that is thought to cause a rightward shift in the cerebral blood flow autoregulation curve.[23] However, pregnancy has been shown to prevent this type of hypertensive remodeling,[10,24] suggesting an influence of pregnancy on sympathetic innervation. We therefore hypothesized that sympathetic nerve density decreases in pregnancy-related states. We further hypothesized that while PCAs from male animals would have the least amount of trigeminal innervation, they would have the greatest sympathetic innervation. To determine the specific contribution of each nerve fiber to the total innervation, a separate set of arteries from each group was stained with the pan-neuronal stain, PGP 9.5 and compared to both CGRP- and TH-containing nerve density. Lastly, because both trigeminal and sympathetic activity can affect cerebral artery reactivity, we also compared the reactivity of isolated and pressurized PCAs to CGRP and norepinephrine (NE) between nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats.

Methods

Animals

Virgin NP (n = 23), LP (day 19-20, n = 22), PP (day 3-4, n = 23) and male (n = 22) Sprague-Dawley rats were obtained from Charles River (Canada) and housed at the University of Vermont Animal Care Facility, an American Association for Accreditation of Laboratory Animal Care (AAALAC) facility. Food and water were provided ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Preparation of Vessels

The animals were anesthetized with isoflurane in oxygen and decapitated, after which the brain was quickly removed and placed in cold physiological salt solution (HEPES PSS). Both

right and left PCAs were dissected from each brain and carefully cleared of connective tissue. A third-order branch of one PCA was used for isolated vessel experiments, described below. The other PCA was fixed in 2% paraformaldehyde and 0.2% picric acid for perivascular nerve staining, also described see below.

Pressurized Arteriograph System

After dissection, a third-order PCA was mounted on 2 glass cannulas with nylon ties and pressurized in an arteriograph chamber (Living Systems, Burlington, Vermont, USA) as previously described.[10,25] Intraluminal pressure was set and maintained by a pressure controller and a miniature peristaltic pump connected to an in-line pressure transducer attached to the proximal cannula. The distal cannula was closed off so there was no flow through the vessel. HEPES PSS was continuously circulated through the chamber and a heat exchanger to maintain the temperature at 37.0 ± 0.3°C and the pH 7.40 ± 0.05. The entire arteriograph chamber was placed on an inverted microscope, attached to a video camera and monitor. Through an optical window on the bottom of the chamber, lumen diameter and wall thickness were measured with use of video microscopy and video dimensional analysis.

Experimental Protocol for Arteriograph Studies

After mounting, the vessels were equilibrated for 45 minutes at 75 mmHg after which the HEPES PSS was replaced with fresh HEPES PSS. Pressure was increased up to 125 mmHg in steps of 25 mmHg until stable myogenic tone had developed and lumen diameter was measured. Pressure was then decreased to 75 mmHg for the rest of the experiment. To obtain reactivity curves to CGRP and NE, either α-CGRP was cumulatively added to the bath from 10^{-11}M to $3x10^{-8}$M or NE from 10^{-8}M to 10^{-5}M and lumen diameter was measured at each concentration. Because some vessels dilated at higher concentrations of NE, the nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-L-arginine (L-NNA, 10^{-4}M), and the nonselective cyclooxygenase inhibitor, indomethacin (10^{-5}M), were added to the HEPES PSS prior to performing the NE concentration-response curves. A separate set of vessels were given L-NNA only prior to performing the CGRP concentration-response curves to assess the role of the endothelium in mediating this dilation. Papaverine (10^{-4}M) was added to the bath after the last dose of α-CGRP or NE to fully relax the vessel and obtain the passive diameter at 75 mmHg.

Perivascular Nerve Staining and Determination of Nerve Density

Immunohistochemical staining of perivascular nerves and quantification of nerve density was done as previously described.[10,26] Briefly, the entire PCA segment was fixed in 2%

paraformaldehyde and 0.2% picric acid for 24 hours, after which the vessels were incubated overnight with either the primary antibody rabbit anti-PGP 9.5 at a concentration of 1:4000, or with primary antibody rabbit anti-α-CGRP at a concentration of 1:15,000, or with the primary antibody rabbit anti-TH at a concentration of 1:500, all in blocking media. The PCA segments for CGRP staining were incubated with the secondary antibody Alexa Fluor 647 donkey anti-goat IgG at a 1:500 dilution in blocking media for 1 hour. The PCA segments for PGP 9.5 and TH were incubated with the secondary antibody goat anti-rabbit Cy3 at a concentration of 1:500 in blocking media for 1 hour. The entire PCA segment was whole mounted on a glass slide with Aqua Poly/Mount (Molecular Probes, Eugene, OR). The vessels that were stained for CGRP were imaged on a Zeiss LSM 510 confocal with a 10x magnification, excitation 650 nm and emission 668 nm. The vessels stained for PGP and TH were imaged on an Olympus microscope at 10x magnification using a filter for Cy3 and captured using Magnifire software. Nerve density was determined with the aid of Metamorph software. Briefly, a 30μm^2 square lattice pointing grid was placed over the image and the points where the nerves crossed the grid were counted. Nerve density for each segment was determined by dividing the number of intersect points by the area of the vessel segment. In addition to determining nerve density for each segment of PCA, we also determined the nerve density of the entire PCA segment for each neurotransmitter by adding all the intersect points and dividing by the total area.

Data Calculations

The reactivity of the PCA to α-CGRP was calculated as the percent dilation to α-CGRP normalized to the maximum diameter in papaverine by the equation $[(\varnothing_{conc} - \varnothing_{start})/(\varnothing_{papav} - \varnothing_{start})] \bullet 100\%$, where $\varnothing_{conc}$ is the diameter of the vessel at the specific concentration of α-CGRP, $\varnothing_{start}$ is the diameter at the lowest concentration of α-CGRP used (10^{-11}M) and $\varnothing_{papav}$ is the diameter of the vessel fully relaxed by papaverine. Similarly, the reactivity to NE was calculated as the percent constriction to NE normalized to the maximum diameter in papaverine by the equation $[(\varnothing_{papav/} \varnothing_{conc})/(\varnothing_{papav} - \varnothing_{max})] \bullet 100\%$, where $\varnothing_{conc}$ is the diameter of the vessel at the specific concentration of NE and $\varnothing_{max}$ is the diameter at the maximum concentration of NE.

Statistical Analysis

All the data are presented as mean ± standard error. To determine differences in nerve density and sensitivity to CGRP or NE, a one-way analysis of variance (ANOVA) was used, followed by a post hoc Bonferroni test for multiple comparisons. A p-value < 0.05 was considered statistical significant.

Drugs

The physiologic salt solution (HEPES PSS) was made fresh daily and composed of: NaCl (142 mM), KCl (4.7 mM), $MgSO_4$ (1.71 mM), EDTA (0.50 mM), $CaCl_2$ (2.8 mM), HEPES (1.0 mM), KH_2PO_4 (1.2 mM) and glucose (5.0 mM). L-NNA, indomethacin and papaverine were prepared once a week in 10^{-2}M stock solution and stored at 4°C. The α-CGRP was diluted in double distilled H_2O and stored at -20°C in 10^{-8}M, 10^{-6}M, 10^{-5}M and 10^{-4}M aliquots. The NE was prepared once a week in 10^{-3}M stock solution and stored at 4°C. All the components of the HEPES PSS, N^{ω}-nitro-L-arginine, indomethacin, papaverine, NE and α-CGRP were obtained from Sigma (Saint Louis, MO, USA). Primary antibody solution for staining rabbit anti α-CGRP was obtained from Chemicon Int. (Temecula, CA, USA) and the secondary antibody Alexa Fluor 647 donkey anti-goat IgG from Invitrogen (Chicago, IL, USA). Primary antibody solutions for staining rabbit anti-TH or rabbit anti-PGP 9.5 were obtained from Chemicon Int. (Temecula, CA, USA) and the secondary antibody Cy3 goat anti-rabbit IgG from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA, USA).

Results

Figure 1 shows the nerve density of CGRP containing perivascular nerves from the PCA in NP, LP, PP and male rats. The nerve density was determined in three different locations of the PCA: primary (1'), secondary (2') and tertiary (3') branches, designated by the first branch of the communicating artery of the Circle of Willis (primary) and subsequent branches as secondary and tertiary. The same pattern of nerve density was found in all the female groups: the perivascular nerve density decreased as branching increased in the smaller vessels, although this difference was not statistically significant. Importantly, the nerve density increased during pregnancy and decreased in the postpartum period, however, no significant differences were found between the female groups. It is remarkable that only a few nerve fibers were seen on the PCAs from the male rats compared to PCAs from the female animals. All female animals had PCAs with higher density of innervation than the male animals with the PCAs from pregnant animals having the greatest density, however, this difference was only significant between the male and the LP rats. The nerve density of the primary, secondary and tertiary branches from the LP animals were significantly higher compared to those of the males ($p<0.05$). Photomicrographs of PCAs stained for CGRP containing nerve fibers are shown in Figure 2.

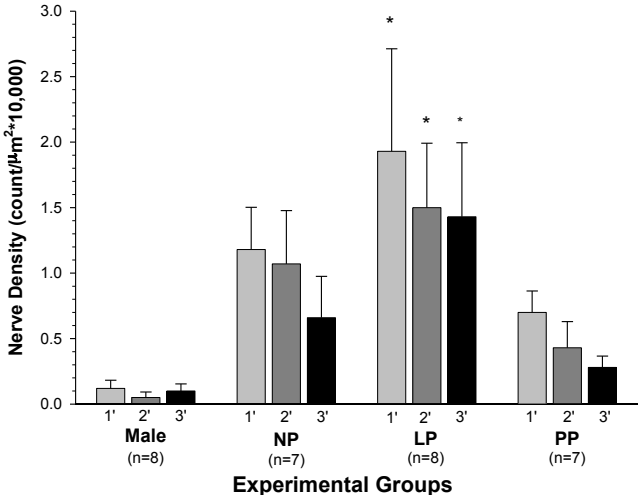

Figure 1 Nerve density of perivascular calcitonin gene-related peptide (CGRP)-containing nerve fibers of posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micron of vascular wall for each arterial segment. * $p < 0.05$ vs. male.

The nerve density of TH containing nerves in PCAs is shown in Figure 3. The same pattern as in CGRP innervation was found in all the female groups where density decreased in the smaller vessels. In the LP group, both the secondary and tertiary branches had significantly lower densities compared to the primary. There were no differences in nerve density of TH-containing nerves between any of the groups.

Figure 4 shows the PGP 9.5-containing nerve density in PCAs per segment. All groups showed less staining in 2' and 3' branches compared to 1'. In the PP group, the nerve density in the 3' branch was significantly lower compared to the primary branch, but there were no differences between any of the groups in PGP 9.5-containing nerves.

Figure 5 compares the nerve density of the entire PCA segment for PGP 9.5, TH and CGRP between groups. PGP 9.5 and TH nerve density was similar in all groups, without any change during pregnancy. In all groups, total CGRP nerve density was significantly less compared to total PGP 9.5 and TH nerve density. The difference between trigeminal and sympathetic innervation of the PCAs is remarkable, especially in the male rats in which trigeminal innervation comprised only 2% of total nerve density (PGP 9.5) whereas sympathetic innervation comprised 95%.

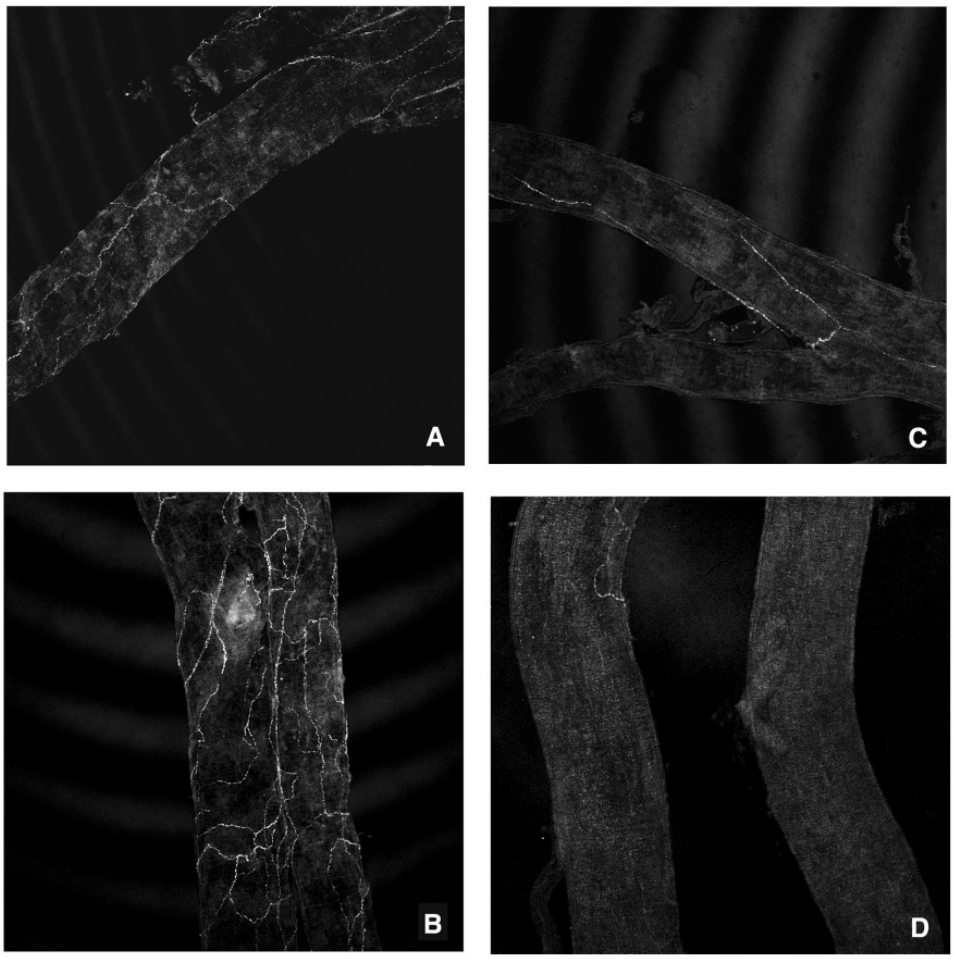

Figure 2 Photomicrographs of posterior cerebral arteries stained for calcitonin gene-related peptide (CGRP)-containing nerve fibers from nonpregnant (**A**), late-pregnant (**B**), postpartum (**C**) and male (**D**) rats. The innervation of CGRP-containing nerve fibers in posterior cerebral arteries was considerable in LP animals, whereas arteries from male rats had very few fibers containing CGRP.

While pregnancy had a trophic effect on CGRP containing nerve fibers, there was no effect of pregnancy on dilation to this neuropeptide. CGRP caused dilation of tone in all groups of animals and Figure 6 shows the reactivity of the PCA to CGRP dilation from NP, LP, PP and male rats. Spontaneous tone developed in all groups and was similar (percent tone: NP 30.8 ± 2.8, LP 33.6 ± 5.8, PP 37.9 ± 2.5, male 39.6 ± 1.6, $p>0.05$). Addition of low doses CGRP caused instability of tone followed by more stable dilation at higher concentrations, the sensitivity of which was not different between any of the groups (Figure 6).

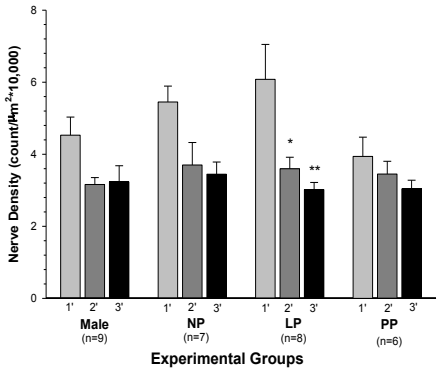

Figure 3 Nerve density of perivascular tyrosine hydroxylase (TH)-containing nerve fibers of posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micrometer of vascular wall for each arterial segment. *p < 0.05 vs. LP 1', **p ≤ 0.01 vs. LP 1'.

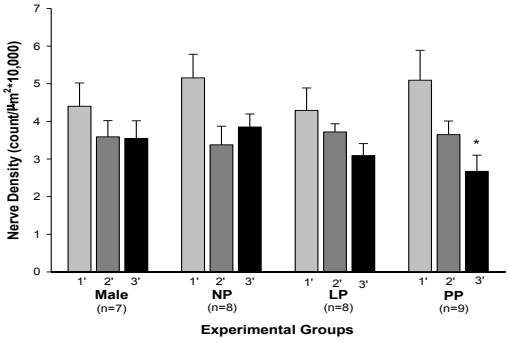

Figure 4 Nerve density of perivascular protein gene product 9.5 (PGP 9.5)-containing nerve fibers of posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micrometer of vascular wall for each arterial segment. *p < 0.05 vs. PP 1'.

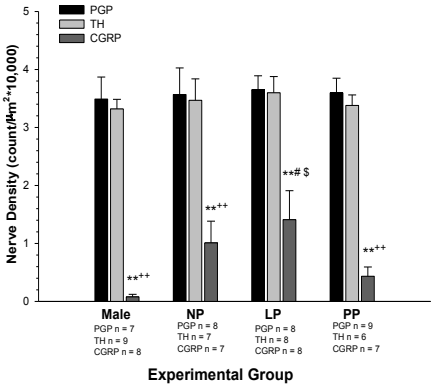

Figure 5 Nerve density of perivascular calcitonin gene-related peptide (CGRP)-, protein gene product 9.5 (PGP 9.5)- and tyrosine hydroxilase (TH)-containing nerves of the entire posterior cerebral artery segment from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micrometer of vascular wall. *p ≤ 0.01 vs. PGP, ++p ≤ 0.01 vs. TH, §p < 0.05 vs. TH, #p < 0.05 vs. male CGRP.

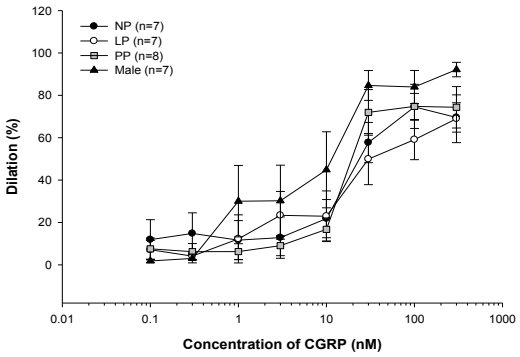

Figure 6 Reactivity curves to α-calcitonin gene-related peptide in posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. There was no difference in reactivity to CGRP between groups (p > 0.05).

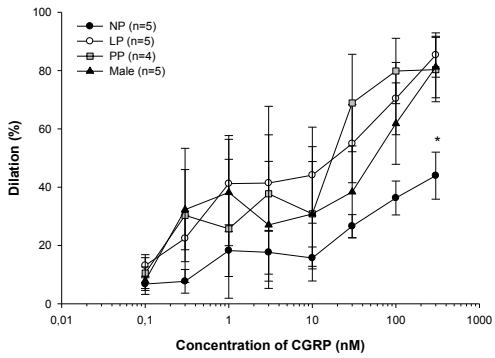

Figure 7 Reactivity curves to α-calcitonin gene-related peptide (CGRP) in posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats in presence of nitro-L-arginine (L-NNA). There was no difference in reactivity to CGRP between groups except for the NP group at the highest concentration; *p < 0.05 vs. male.

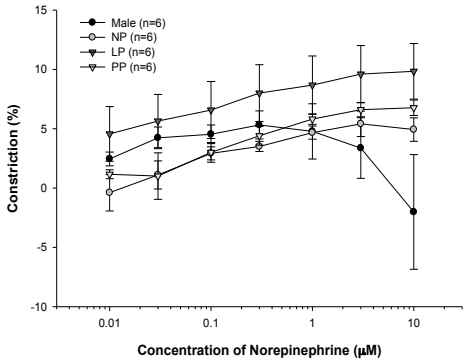

Figure 8 Reactivity curves to norepinephrine (NE) in posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats in presence of nitro-L-arginine (L-NNA) and indomethacin. Reactivity to NE was considerably less compared with CGRP. There was no difference in reactivity between any of the groups (p > 0.05).

Because of the instability in tone, the concentration-response curves to CGRP were repeated in the presence of L-NNA using a separate set of vessels. Figure 7 shows the reactivity of PCAs from NP, LP, PP and male animals to CGRP in presence of L-NNA. Again, all vessels developed tone, which was similar in all groups (percent tone: NP 36.8 ± 3.3, LP 37.0 ± 2.4, PP 25.3 ± 2.2, males 34.0 ± 6.3, p>0.05) and all vessels dilated in reaction to CGRP. NOS inhibition attenuated the dilation in PCAs from NP animals with only a significant difference compared to male rats at 300 nM, suggesting that the dilation to CGRP was partially mediated by NO in this group. However, it seems that the pregnancy- related states changed the role of the endothelium in the action of CGRP since NOS inhibition had no effect on the reactivity to CGRP in either the LP or PP group. The percent reactivity to the highest concentration of CGRP (300 nM) was 44.0 ± 8.1 for NP, 85.3 ± 7.6 for LP, 80.3 ± 11.0 for PP and 81.2 ± 10.5 for males (p<0.05).

Figure 8 shows the reactivity of PCAs from all four groups to NE in presence of L-NNA and indomethacin. All vessels developed tone, which was similar in all groups (percent tone: NP 39.3 ± 3.3, LP 37.9 ± 3.7, PP 39.9 ± 4.2, males 39.9 ± 2.3, p>0.05). All vessels in the female groups constricted to NE, however, at higher concentrations, vessels from male rats dilated. No significant differences in reactivity were found between any of the groups. The percent reactivity to highest concentration of NE (10μM) was 4.9 ± 1.0 for

NP, 9.8 ± 2.3 for LP, 6.7 ± 0.7 for PP and -2.0 ± 4.8 for males, which was considerably lower in comparison to reactivity to CGRP.

Discussion

There were several major findings in this study. First, pregnancy had a trophic effect on perivascular trigeminal CGRP containing nerves in the posterior cerebral circulation in rats, however there was no effect of pregnancy on sympathetic perivascular innervation. Second, trigeminal innervation in PCAs from male rats was remarkably low compared to the female groups. Third, differences in nerve density were not accompanied by an increase in sensitivity to CGRP dilation in any of the groups. These findings demonstrate that pregnancy has a significant effect on perivascular trigeminal innervation, but not on sensitivity to CGRP, suggesting that alterations in perivascular innervation during pregnancy do not have a major role in regulating cerebral blood flow.

During normal pregnancy, the density of CGRP-containing nerves was increased in PCAs. The importance of this finding is unclear, however CGRP-containing perivascular nerves have been shown to play an important role in the pathophysiology of migraine by signaling noxious stimuli from pial and dural vessels to the nucleus trigeminal caudalis in the brainstem and triggering headache.[7-9] One possible consequence of the increase in nociceptive perivascular nerve fibers during pregnancy is that it is related to the appearance of headache during eclampsia since the most common symptom of eclampsia is headache[18], of which the exact origin in unknown. In fact, eclampsia occurs most often in women who were healthy prior to pregnancy and are asymptomatic during the first half of pregnancy.[18] The increased density of nociceptive fibers on PCAs during late-pregnancy might cause a susceptibility of pregnant women to the headache that occurs during eclampsia. Alternatively, it should be noted that in women suffering from migraine, the symptoms most often improve or disappear during pregnancy[19], which again may be related to the significant effect of pregnancy on these nociceptive fibers.

The innervation of CGRP-containing perivascular nerves in PCAs from male rats was considerably less than all female groups studied. This suggests that the difference is associated with the levels of female sex steroid hormones. For example, innervation of PCAs from LP animals was the highest, whereas the innervation was lowest in males. Levels of female sex steroid hormones follow this pattern and are high during pregnancy, drop in the postpartum period and are low in male animals. In support of this, the synthesis of CGRP mRNA in the dorsal root ganglia has been shown to be increased under the influence of female sex steroid hormones.[27,28] While we do not know the mechanism

by which pregnancy enhances the trigeminal nerve fiber growth, the high levels of hormones could release several growth factors (e.g. nerve growth factor) and stimulate growth of perivascular trigeminal nerves as well as synthesis of CGRP. Together these findings suggest that gender-specific factors, such as female sex steroid hormones, influence the cerebral CGRP-containing perivascular innervation, and may be related to the fact that the incidence of migraine is higher in women than in men and also is associated with female sex steroid hormones.[19,29]

In contrast to our hypothesis, there was no effect of pregnancy on sympathetic innervation suggesting that the lack of hypertensive remodeling of cerebral arteries during pregnancy noted in previous studies was not due to changes in this type of innervation.[10,24] In addition, reactivity to NE was minimal in the cerebral circulation, as has been shown previously.[26]

The sensitivity of PCAs to CGRP-induced dilation was not altered by pregnant state or gender, regardless of the increased CGRP perivascular nerve density in these vessels. However, the reactivity of PCAs to CGRP in the NP animals was significantly diminished with NOS inhibition, while in the LP and PP animals' reactivity to CGRP was comparable to the male group. This suggests that pregnancy and the postpartum state alter the role of endothelium in CGRP-induced vasodilation in the PCA. CGRP has been shown to cause vasodilation by both endothelium-dependent and -independent mechanisms.[30,31] In the endothelium-dependent manner, CGRP can activate NOS, thereby releasing nitric oxide, causing vascular smooth muscle cell (VSMC) relaxation.[30,31] In the endothelium-independent pathway, CGRP binds directly on the CGRP receptor calcitonin receptor like receptor (CGLR) on the VSMC, which is co-expressed with receptor activity-modifying protein 1 (RAMP 1).[30,32] This activates adenyl cyclase, which in turn increases cAMP levels, causing VSMC relaxation.[30] The fact that only PCAs from NP animals showed diminished reactivity to CGRP dilation after NOS inhibition suggests that pregnancy changes the role of vasodilators and that pregnancy shifts the role of the endothelium in mediating CGRP dilation to the VSMC in cerebral arteries. The cause of pregnancy affecting vasodilator production in the cerebral circulation is unknown, but may relate to the high levels of hormone production during this state.

The nerve density of the entire PCA segment was determined independently of individual segments. It was remarkable that there was a considerable difference between sympathetic and trigeminal innervation, especially in the male rats where sympathetic innervation was high and trigeminal innervation comprised only 2% of total innervation (PGP 9.5). The difference is even more remarkable when one considers that the reactivity to CGRP (Figure 7) was much higher in all groups than the reactivity to NE (Figure 8). It has been reported previously that CGRP is a potent dilator[30,33], while NE causes a moderate

constriction of cerebral arteries.[26] This suggests a difference in receptor density of α-adrenoceptors and CRLR/RAMP1 and/or a difference in affinity of these receptors to NE and CGRP, respectively. Nevertheless, there was no difference in reactivity to either CGRP or NE between groups, suggesting there is little role for trigeminal and sympathetic perivascular nerves on controlling CBF under normal conditions. However, it is possible that during hypertension a change in nerve density during pregnancy causes a change in the control of blood flow, since it is known that both sympathetic and trigeminal nerves have a more distinct role during hypertension.[15,23]

In summary, we found that pregnancy increased the density of perivascular CGRP-containing nerve fibers in PCAs from rats, but no influence of pregnancy on sympathetic innervation was found. The difference in CGRP-containing innervation appeared to be gender-related with lowest nerve density in male rats. The change in trigeminal innervation was not accompanied by an increase in sensitivity to CGRP-mediated vasodilation, suggesting that CGRP-containing nerves in the PCA are more likely to be involved in nociception than in controlling cerebral blood flow. However, it is possible that both trigeminal and sympathetic perivascular innervation have a more prominent role in controlling cerebral blood flow if blood pressure increases during pregnancy, such as during preeclampsia and eclampsia.

Acknowledgements

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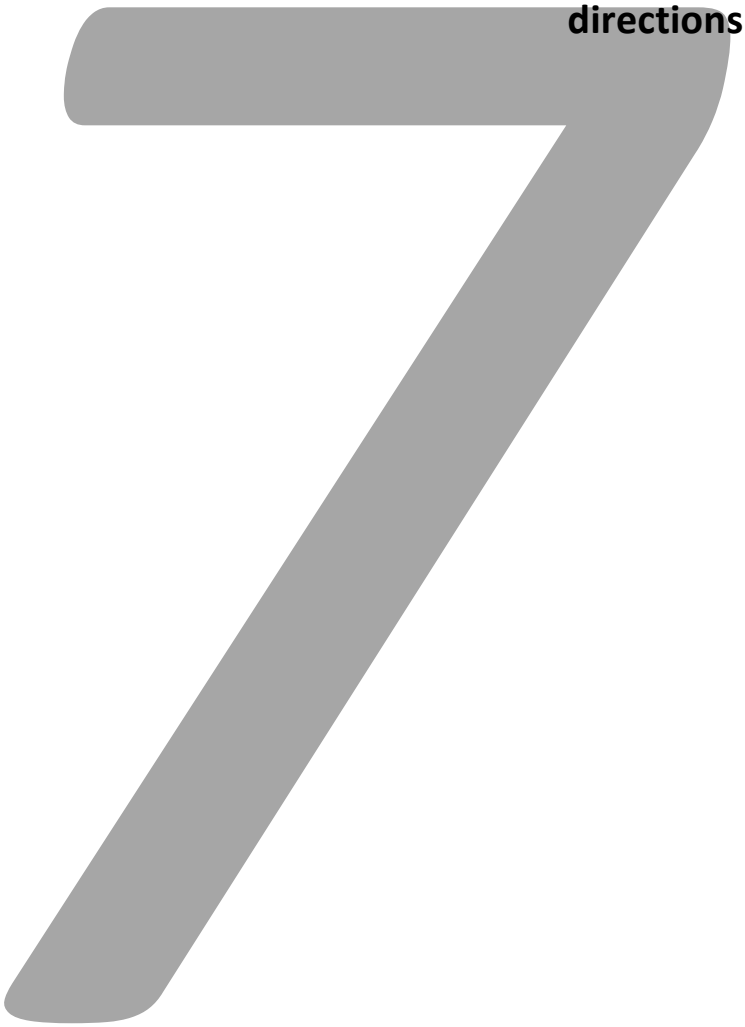
Summary, general discussion, conclusions and future directions



Summary

General discussion

- **The causes of eclampsia**
- **The long term consequences of eclampsia and preeclampsia on the maternal brain**
    - *The brain after eclampsia*
    - *The brain after preeclampsia*
    - *White matter lesions in young cohorts of women*

Conclusions

Future perspectives

References

Summary

In the **first part** of this thesis the long-term consequences of eclampsia and preeclampsia on the maternal brain are presented. This part describes the result of MR imaging and cognitive testing in women several years after pregnancies that were complicated by eclampsia or preeclampsia.

In **Chapter 2** outcome of the Cognitive Failures Questionnaire (CFQ) in 30 formerly eclamptic, 31 formerly preeclamptic and 30 healthy parous control women were compared. The scores on this test were significantly worse in the group of women who had had a pregnancy complicated by eclampsia (43.5 ± 14.6) compared to control women (36.1 ± 13.9), p = 0.049. Formerly preeclamptic women had a similar score to control women but this was not significantly different from the formerly eclamptic women (36.9 ± 13.9). The more seizures formerly eclamptic women had suffered, the worse their score on the CFQ. The women who had experienced more than two eclamptic seizures scored 60.0 ± 14.5 versus 39.1 ± 10.9 in the women who suffered a single seizure (p = 0.005). The CFQ is a subjective test; the participant answers questions about memory and concentration. Therefore, the outcome of this study only signifies an indication of the cognitive functioning of these women in daily life. However, the fact that women with multiple seizures reported most cognitive impairment seems to be an indication that eclamptic seizures may have long-term sequelae and are more harmful than was previously thought. In **Chapter 3** more data are presented to support this hypothesis. Thirty-nine formerly eclamptic women underwent MR imaging on average 7 years after the index pregnancy. Twenty-nine healthy parous control women, on average 5 years after the index pregnancy, were matched for age and elapsed time since the index pregnancy and also underwent MR imaging. We found that 41% of the formerly eclamptic women had white matter lesions on the MRI scans, while this was the case in 17% of the control women (p = 0.03). The volume of the white matter lesions was significantly larger in the formerly eclamptic group (0.041 mL ± 0.016) compared to the control group (0.004 mL ± 0.002, p = 0.025). With increasing number of eclamptic seizures the prevalence and severity of white matter lesions increased (test for trend for prevalence p = 0.009, Kruskal-Wallis for volume p = 0.042). Together, these findings suggest that the paradigm that eclamptic women fully recover after eclamptic seizures, provided that haemorrhage does not occur, should be revised. The prevention of seizures by administering magnesium sulphate should be emphasized in this regard. In **Chapter 4** the presence and volume of

cerebral white matter lesions in 73 formerly preeclamptic women and 75 healthy parous control women was assessed. Of the formerly preeclamptic women 37% had white matter lesions on MR imaging, which was a significantly more compared with 21% of the control women ($p = 0.036$). Formerly preeclamptic women also had more severe lesions (volume-index: 0.11 ± 0.33 mL) compared with the controls (0.015 ± 0.04 mL, $p = 0.019$). The white matter lesions were not related to neurologic symptoms at the time of pregnancy, the concomitant presence of HELLP syndrome, magnesium sulphate administration or diastolic blood pressure > 110 mmHg at the acute moment. Preeclampsia with an onset and delivery prior to 37 weeks was associated with increased prevalence and severity of cerebral white matter lesions. Formerly preeclamptic women who were currently hypertensive were associated with presence of white matter lesions. Presence of current hypertension, absolute current blood pressure values and current weight were significantly higher in the formerly preeclamptic group. Retrospectively, it was impossible to determine which women had posterior reversible encephalopathy syndrome (PRES) during their preeclamptic pregnancy, simply because in general such women do not undergo cerebral imaging. Therefore, in our group we can only suggest contribution of cerebral edema to the development of white matter lesions, but we are unable to proof this. Recent data have emerged to indicate that women with a history of preeclampsia are predisposed to develop cardiovascular disease later in life, including stroke. The white matter lesions found in our study could be an early expression of cardiovascular disease manifesting it self as cerebrovascular disease. Most vulnerable are women who suffered early onset (<37 weeks) preeclampsia. Emphasis should be placed on ameliorating risk factors of cardiovascular disease in this young group of women.

In the **second part** of this thesis animal studies are presented that give insight in the cerebral vasculature during pregnancy and hypertension.

In **Chapter 5** a rat model of hypertensive encephalopathy was used to mimic eclampsia. Two groups of nonpregnant (n = 8 in both groups) and two groups of late-pregnant (n = 8 in high salt, n = 9 in low salt group) Dahl salt-sensitive rats were fed either a high salt containing diet (8% NaCl) or a low salt containing diet (<0.7% NaCl). After two weeks of this diet, for the pregnant animals at the end of gestation, their posterior cerebral arteries were used to determine the effects of hypertension and pregnancy on the reactivity and remodeling of pial arteries. We found that pregnancy prevented hypertensive remodeling because there were no structural differences between cerebral arteries from the hypertensive and normotensive pregnant rats, while remodeling was present in the nonpregnant hypertensive animals. Also, the passive and active diameters were similar in

both pregnant groups. At higher pressures there was diminished myogenic reactivity in pregnant animals regardless of hypertension, suggesting that pregnancy makes the brain more susceptible to loss of autoregulation when blood pressure increases. Since this was also found in a previous study where animals were made hypertensive with a nitric oxide synthase inhibitor, we concluded that the lack of remodeling and the diminished myogenic reactivity is an effect of pregnancy independent of the method by which hypertension was induced. In this study, we also stained perivascular nerves of the posterior cerebral arteries with a pan-neuronal stain. We found that in the presence of hypertension perivascular innervation was significantly decreased, while during pregnancy the nerve density was significantly increased regardless of the presence of hypertension. The type of perivascular nerves could not be distinguished due to the use of a pan-neuronal stain and the cause and consequence of the changes remained unclear. Therefore, in **Chapter 6** we investigated the posterior cerebral arteries of nonpregnant (n = 23), late-pregnant (n = 22), post partum (n = 23) and male (n = 22) Sprague Dawley rats. We determined the nerve density in the posterior cerebral arteries not only with a pan-neuronal stain; we also measured the nerve density of sympathetic (tyrosine hydroxilase) and trigeminal (calcitonin gene-related peptide, CGRP) perivascular nerves. In this rat strain the total perivascular nerve density was unchanged during pregnancy, probably due to genetic differences. The sympathetic nerve density was similar in all groups, but the trigeminal nerve density increased during pregnancy and was significantly higher compared to the male animals. The sensitivity to CGRP was not altered by pregnancy or gender, neither was the reactivity to norepinephrine. The significance of the increased CGRP-containing perivascular nerves of cerebral arteries in the pregnant animals is unclear, but in this chapter we speculate about its importance in pain signalling and cerebral hyperperfusion. Especially in migraine, a disease that shares features with eclampsia and in which CGRP is known to play a major role.

General discussion

The causes of eclampsia.

A classic thought is that a patient with mild preeclampsia progresses through a phase of severe preeclampsia and eventually develops eclampsia. It appears, however, that a significant number of eclamptic women never reach blood pressures generally considered to be hypertensive prior to developing tonic-clonic seizures. This appears similar for non-obstetric patients who develop PRES.[1] We raised the question whether pregnancy in and

of itself could cause a left shift of the cerebral autoregulation curve which would make the brain susceptible to loss of autoregulation at lower blood pressures. Two studies from our lab have shown that isolated posterior cerebral arteries from pregnant rats have diminished myogenic reactivity at increasing pressure independent of hypertension.[2,3] This may indicate a left shift of the autoregulation curve, however isolated arteries lack several factors that influence the cerebral autoregulation such as perivascular innervation. In vivo experiments indicated that the blood pressure at which autoregulation breakthrough occurred did not differ between pregnant and nonpregnant animals [4], which contradicts the hypothesis of a pregnancy-induced left shift of the autoregulation curve. The brains of these pregnant animals, however, did contain more edema after cerebral autoregulation breakthrough in comparison to nonpregnant animals, especially in the posterior part of the brain.[4] This suggests that the blood-brain barrier is more susceptible to disruption in pregnancy. Pregnancy prevents hypertensive remodeling of posterior cerebral arteries in rats [2,5] and even reverses remodeling that is present in chronically hypertensive rats.[6] In addition, penetrating arterioles undergo outward remodeling during pregnancy.[7] This may contribute to increased wall stress and failure of the usual protective mechanisms for the downstream vasculature in case of acute hypertension and cause subsequent vulnerability of the blood-brain barrier. Posterior cerebral arteries from late pregnant rats demonstrate increased permeability of the blood-brain barrier as indicated by increased endocytosis and paracellular transport when hydrostatic pressure is increased and when forced dilatation occurs [8,9], but pregnancy does not appear to alter mRNA expression of key tight junctions.[7]

The primary site of blood-brain barrier disruption in acute hypertension is the cerebral veins.[10] During acute hypertension cerebral venular pressure increases and the blood-brain barrier becomes leaky in venules and veins.[11] Cerebral veins from virgin female rats have an increased hydraulic conductivity when they are exposed to plasma from pregnant women compared to normal physiologic salt solution.[12] In addition, when veins were exposed to plasma from women with early onset preeclampsia, hydraulic conductivity is increased significantly in comparison to exposure to plasma from normotensive pregnant women. There was no difference in myogenic activity or vascular tone in posterior cerebral arteries between normal pregnant plasma and preeclamptic plasma.[12] It seems therefore that an important factor in the development of cerebral vasogenic edema during pregnancy and in eclampsia, is the vulnerability of the blood-brain barrier.

What causes vulnerability of the blood-brain barrier during pregnancy or during (pre)eclampsia? In pregnant rats cerebral vascular resistance is more severely decreased and cerebral blood flow more severely increased compared to nonpregnant rats during

acute hypertension.[7] Together with an increase in capillary density in the posterior part of the brain[7] and outward remodeling of penetrating arteries, it may well be that the blood-brain barrier is more susceptible to breakthrough during acute hypertension because of relatively increased hydrostatic pressure and cerebral blood flow. Furthermore, in the pregnant cerebral vasculature vascular endothelial growth factor (VEGF) appears to play a role, although it is not the only factor that influences the blood-brain barrier.[12] In experiments on traumatic brain injury, ischemia/reperfusion or infection several factors play a role in the regulation of blood-brain permeability.[11] Examples of these factors are bradykinin, histamine, nitric oxide and cytokines such as tumor necrosis factor, VEGF and interleukins. In addition, oxidative stress influences the permeability of the blood-brain barrier.[13] Endothelial dysfunction, oxidative stress and pro-inflammatory cytokines are known to be important in the pathophysiology of preeclampsia [14,15] and PRES [16] and it is reasonable to suggest that some of these factors are associated with increased permeability of the blood-brain barrier in this patient category.

Whether or not there is a pregnancy-related change of the cerebral autoregulation curve is an important question. Based on the experimental data mentioned above, it seems that pregnancy may cause a modest left shift of the curve and a greater increase in cerebral blood flow when blood pressure increases compared to nonpregnant state which make the brain more susceptible to autoregulatory breakthrough. In addition, circulating factors and endothelial dysfunction in preeclampsia cause vulnerability of the blood-brain barrier resulting in formation of vasogenic edema even at relatively low blood pressures. Much more future research in both humans and animal models is needed to elucidate the adaptations of the cerebral autoregulation curve in pregnancy and in (pre)eclampsia. Once the main factors responsible for this vulnerability have been identified, focus should be on the development of blood-brain barrier protecting agents.

The long term consequences of eclampsia and preeclampsia on the maternal brain.

The brain after eclampsia.

Several years after a pregnancy complicated by severe preeclampsia, a large group of women complain about suboptimal physical and mental well being.[17] A high percentage of such women complain about recurrent headache, visual disturbances, fatigue, decreased concentration and mental problems.[17] Most often, no pathological substrate is found in these women to explain their complaints. The incidence of post-traumatic stress disorder is also increased in formerly preeclamptic women.[18,19] In Chapter 2 we found that several years after eclampsia women report to perform worse in daily life with regard to cognitive

function compared to healthy parous controls, whereas formerly preeclamptic women had the same score as controls on this subjective test. Objective neurocognitive tests in formerly preeclamptic women show conflicting results.[20-22] Brussé et al. showed that formerly severe preeclamptic women have impaired short and long term memory [20], Baecke et al. found mental slowing and affected working memory after preeclampsia but no impaired concentration or self-reported cognition [21], while Postma et al. found no difference in executive function and sustained attention between formerly preeclamptic women and healthy parous controls.[22] The number of participants in all of these studies is relatively small, however, the findings suggest that there is a clear correlation between mental well being and a history of preeclampsia but that cognitive functioning after preeclampsia may not be largely impaired. In formerly eclamptic women however, the number of seizures is related to both self-reported cognitive impairment and decreased sustained attention.[22,23] The incidence of post-traumatic stress disorder following eclampsia is unknown, but these findings suggest that there may be a difference in etiology of the reported impaired cognitive function between preeclampsia and eclampsia. Although there are no studies on cognitive functioning following an episode of PRES in non-obstetric patient categories, it seems likely that vasogenic edema and/or the seizures during an episode of PRES in eclampsia can explain the impaired cognitive functioning several years later. In Chapter 3 we found that the prevalence and volume of white matter lesions in the brains of young women several years after eclampsia were higher compared to healthy parous control women. The prevalence and severity increased linearly with the number of seizures, comparable to the findings of decreasing cognitive function and sustained attention with increasing number of seizures.[22,23] This strengthens our hypothesis of the relationship between PRES and subsequent impaired cognitive functioning.

What is the pathogenesis of the white matter lesions that we found in formerly eclamptic women? A plausible scenario, that has been suggested before in humans [24,25] and in animal studies [26], is that cerebral vasogenic edema develops to such an extent that cerebral microvasculature is compressed in the narrow space of the skull. This leads to hypoxia, ischemia and eventually to microinfarction. The fact that the burden of lesions increases with increasing severity of eclampsia, supports this theory. On the other hand, the seizure activity in and of it self may cause neuron damage to the brain, since patients with epilepsy also have a high incidence of white matter lesions.[27,28] Alternatively, women who experience eclampsia may well be predisposed to develop brain white matter lesions in later life independent of the eclamptic seizures. One way or the other, it appears that eclamptic seizures are more harmful to the maternal brain than was previously thought. Eclampsia is not a one-time event of which the patient can expect full clinical recovery.

Therefore, it should be emphasized that prevention of eclamptic seizures and prevention of recurrence of seizures is very important. This can be established by magnesium sulphate administration as has been shown in the Magpie trail.[29] In addition, serious attention should be paid to symptoms of post-traumatic stress disorder in women following a pregnancy complicated by preeclampsia, eclampsia and HELLP syndrome.

The brain after preeclampsia.

Not only women with a history of eclampsia (i.e. with a history of PRES) demonstrate an increased prevalence of white matter lesions. Also formerly preeclamptic women do. To what degree the presence of PRES contributed to the subsequent development of white matter lesions in formerly preeclamptic women, is unknown, because MR imaging was not performed at the acute moment of preeclampsia and the women who may have had imaging features of PRES could obviously not be identified retrospectively. Significantly more formerly preeclamptic women were currently hypertensive and the presence of white matter lesions was associated with current hypertension. The presence and development of white matter lesions in middle-aged and older adults are associated with vascular risks and there is a strong correlation between white matter lesions and hypertension in particular.[30,31] It is therefore possible that the development of white matter lesions in formerly preeclamptic women is initiated by two factors which may be independent from each other: PRES and/or the increased cardiovascular risk in this patient group. There is solid epidemiologic evidence that the risk for cardiovascular events later in life is increased after a preeclamptic pregnancy.[32] The risk of fatal and non-fatal coronary heart disease, hypertension, stroke and venous thromboembolism is significantly increased. Early-onset preeclampsia (<37 weeks) is associated with an even greater risk of future cardiovascular disease. Several years after a preeclamptic pregnancy, markers for cardiovascular disease are increased, especially in women with early onset preeclampsia.[33-36] Although the exact underlying mechanism of the increased risk for cardiovascular disease following a pregnancy complicated by preeclampsia remains unknown, preeclampsia and atherosclerosis share risk factors such as chronic hypertension, dislipidemia, obesity, metabolic syndrome and insulin resistance. A current concept is that pregnancy is a vascular and metabolic 'stress test' for a woman's health later in life.[33,37] She carries a modest risk of cardiovascular disease prior to pregnancy and when she becomes pregnant, this is a scenario of metabolic and vascular stress. She develops preeclampsia because she 'fails' to adapt to the cardiovascular and metabolic challenges of pregnancy. This is associated with an increased risk of cardiovascular disease and cardiovascular events will become manifest at an earlier age compared to women

with an uncomplicated pregnancy. It may well be that the white matter lesions that we found in our formerly preeclamptic women did not necessarily develop during their pregnancy. It is possible that because of their constitutional cardiovascular make-up these women develop preeclampsia during pregnancy and, independently, cerebrovascular lesions - in this case white matter lesions - later in life. In this scheme, early-onset preeclampsia differs from late-onset preeclampsia in that it carries a higher risk. That early-onset and late-onset preeclampsia are different entities has been suggested by others.[38,39] Recently, it has become apparent that postpartum lifestyle intervention improves several risk factors for cardiovascular disease in women with a history of preeclampsia, intra uterine growth restriction or gestational hypertension.[40] In this study weight, hip-circumference, waist-circumference, total cholesterol, LDL cholesterol, HDL cholesterol and heart rate decreased significantly. Blood pressure and fasting glucose and insulin levels did not significantly change.[40] This is a very interesting and important finding which provides an opportunity to develop lifestyle intervention programs for women with a history of preeclampsia in order to establish primary prevention of cardiovascular events.

White matter lesions in young cohorts of women

In the elderly population the prevalence and volume of white matter lesions are correlated with development of cognitive impairment, stroke and dementia.[41] Therefore, it is tempting to immediately conclude that the white matter lesions found in our cohorts will lead to cognitive impairment, stroke and dementia. However, currently it is unknown how the white matter lesions in our young (average 38 years) cohorts will develop over time. In formerly preeclamptic women the risk of stroke later in life is increased [42], however, it is unknown which of these women had white matter lesions prior to stroke. The correlation between dementia and a history of preeclampsia has not been demonstrated either, although the preeclampsia STOX1 gene is upregulated in preeclamptic placentas and also in brain tissue of late-onset Alzheimer's disease patients.[43] Long term follow up studies (20 – 30 years) of cognitive function and cerebral imaging would provide insight in the relationship between white matter lesions and (pre)eclampsia earlier in life and neurodegenerative or cerebrovascular disease later in life.

Conclusions

1. Eclampsia is not a one-time event of which a woman can expect to recover undoubtedly if she survives and the seizures are not complicated by cerebral hemorrhage. Each seizure may carry a risk for cerebral damage and increasing impairment of cognitive function.

2. The importance of preventing eclamptic seizures and prevention of recurrence of seizures in an eclamptic patient should be emphasized.

3. The prevalence and severity of cerebral white matter lesions is increased in women with a history of (pre)eclampsia and with an average age of 38 years. The prevalence and severity of these lesions is most pronounced in preeclamptic women who delivered before 37 weeks of gestation.

4. Formerly eclamptic and formerly early-onset preeclamptic women are particularly vulnerable with regard to the long-term consequences on the maternal brain. Since these consequences are probably vascular in origin and because these women are known to be predisposed to develop cardiovascular and cerebrovascular disease later in life, early and frequent follow-up of cardiovascular risk factors is warranted post partum.

5. In animal models pregnancy prevents hypertensive remodeling in posterior cerebral arteries. Pregnancy also diminishes myogenic reactivity at higher transmural pressures in these arteries, independently of the presence of hypertension. These findings suggest increased vulnerability of the cerebral vasculature during pregnancy, which predisposes to the blood-brain barrier disruption when blood pressure increases acutely.

6. Cerebral perivascular innervation is altered by pregnancy. Which type of nerves are subject of alteration (sympathetic, parasympathetic or trigeminal) seems to be dependent on rat or animal species. In Sprague-Dawley rats pregnancy has a trophic effect on CGRP-containing trigeminal nerves, but because this is not accompanied by altered myogenic reactivity of posterior cerebral artiers to CGRP, there is no changed influence on cerebral blood flow regulation. Possibly, the increased trigeminal

perivascular innervation is related to nociception. However, how pregnancy alters the cerebral perivascular innervation, is largely unknown. How changes on perivascular innervation during pregnancy influence the cerebral blood flow and permeability of the blood-brain barrier, especially during acute hypertension, has yet to be discovered.

Future perspectives

1. How pregnancy affects cerebral blood flow, cerebral autoregulation, the blood-brain barrier and cerebral perivascular innervation is currently largely unknown. Studies in animals are needed to create insight in the physiologic adaptations of the cerebral vasculature during pregnancy. In addition, these physiologic adaptations should be compared to the possible maladaptation in animal models of (pre)eclampsia. Furthermore, experiments in higher order animal species should be done to relate more easily to human subjects.

2. How and why the blood-brain barrier disrupts in eclampsia/PRES at relatively low blood pressures, especially at the posterior region of the brain, has yet to be elucidated. The identification of markers for blood-brain barrier disruption in serum or cerebrospinal fluid such as S100B, other neuroinflammatory markers or free hemoglobin deserves further interest. In addition, different levels of the cerebral vasculature should be investigated because only very few data on cerebral veins and penetrating arterioles during pregnancy and concomitant acute hypertension are currently available.

3. The long-term consequences of PRES in non-obstetric patients should be investigated. By including non-obstetric patients, larger cohorts can be studied and more insight can be generated on the pathophysiology of long-term consequences after PRES, both dependent and independent of the underlying disease that causes PRES. In addition, by including non-obstetric PRES patients, more insight can be generated on the underlying pathophysiology of the development of PRES, in other words; what makes the brain vulnerable to blood-brain barrier disruption and edema formation?

4. Objective neurocognitive studies are warranted to better evaluate the cognitive function of formerly eclamptic women. The outcomes of these studies should preferably be linked to neuroimaging findings such as structural MRI, diffusion tensor imaging or fMRI. In addition to neurocognitive testing, other long-term consequences of eclampsia should be ruled out such as visual impairment based on cortical lesions.

5. The cohorts that we studied in this thesis represent a group of young women. Future research should be performed to monitor the development of white matter lesions

during life in this group of patients. Who are the women that develop severe cognitive impairment, dementia and stroke later in life with relation to presence and severity of white matter lesions at a younger age?

6. Since lifestyle intervention has been shown to be effective on several markers for cardiovascular disease shortly after preeclampsia, emphasis should be on prevention of cardiovascular disease with diet, supplements, exercise and treatment of risk factors such as hypertension and diabetes mellitus. Within this group, the women with early-onset preeclampsia should be monitored and coached most intensively considering the high risk they have in comparison to those women who experienced late-onset preeclampsia. Additional research is needed to determine treatment options for women with a history of eclampsia who may complain of suboptimal neurocognitive functioning.

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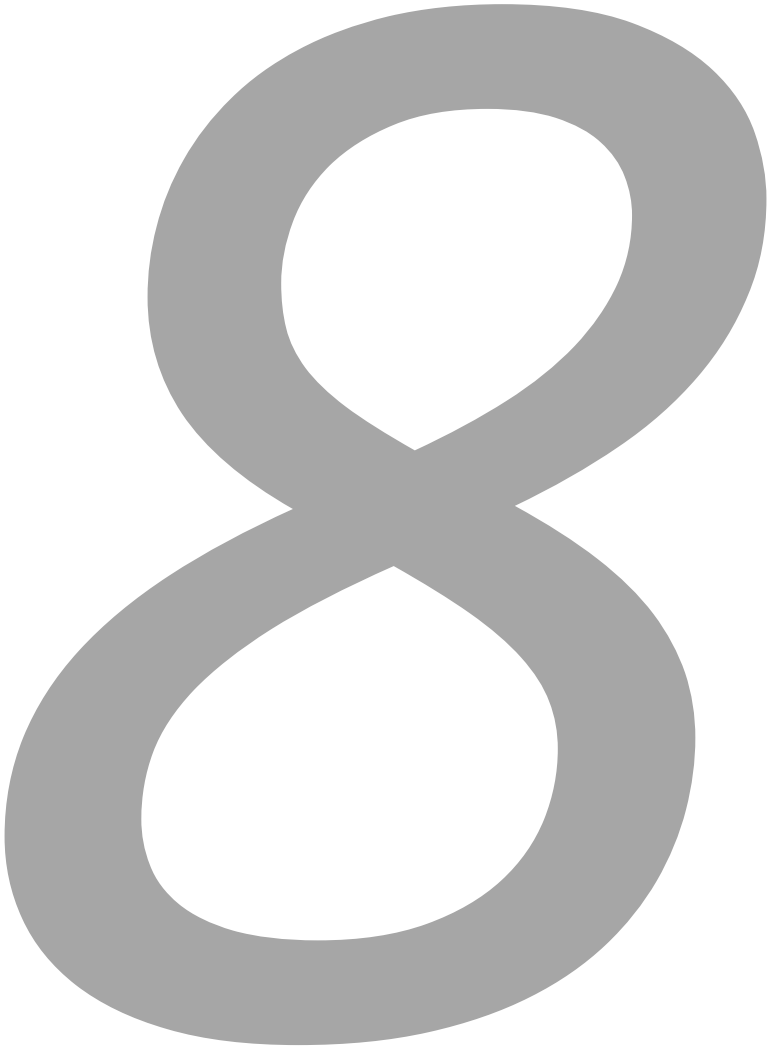
Nederlandse Samenvatting



Pre-eclampsie is een zwangerschapsgeïnduceerde aandoening die voorkomt bij 5-7% van de zwangerschappen. Pre-eclampsie is gedefinieerd als hypertensie en proteïnurie in de tweede helft van de zwangerschap. Het is een systemische aandoening waarbij o.a. de nieren, lever en hersenen aangedaan kunnen zijn. Ten gevolge van gegeneraliseerde endotheeldysfunctie wordt de bloeddoorstroming van deze organen verstoord. Wanneer de hersenen betrokken zijn bij pre-eclampsie, kan eclampsie ontstaan: tonisch-clonische insulten bij een vrouw met pre-eclampsie zonder een andere aanwijsbare oorzaak. De hoge bloeddruk zorgt, in samenhang met endotheeldysfunctie, voor het ontstaan van vasogeen oedeem in het brein wat leidt tot het 'posterior reversible encephalopathy syndrome' (PRES). Wanneer de bloeddruk daalt en de endotheeldysfunctie herstelt, verdwijnt het oedeem en de heersende opvatting is dat de patiënte volledig herstelt. Omdat veel vrouwen na (pre-)eclampsie in meer of mindere mate neurocognitieve klachten blijven houden, zoals klachten met betrekking tot het geheugen en de concentratie, is in **Deel 1** van dit proefschrift gekeken naar de lange termijn gevolgen van (pre-)eclampsie op het maternaal brein. Hypertensieve aandoeningen tijdens de zwangerschap vormen de belangrijkste oorzaken voor maternale sterfte in Nederland en bij het merendeel van deze sterfte is er sprake van cerebrale complicaties. Hoe de hersenen en de cerebrale circulatie reageren op de hemodynamische en hormonale veranderingen tijdens de zwangerschap, is nog vrijwel onbekend en is het belangrijk te onderzoeken waarom de hersenen tijdens de zwangerschap vatbaar zijn voor acute hypertensie en wat de gevolgen daarvan zijn. In **Deel 2** van dit proefschrift wordt in verschillende diermodellen gekeken naar de cererbrale vasculatuur tijdens de zwangerschap met en zonder hypertensie.

In **Hoofdstuk 1** wordt de pathofysiologie van eclampsie besproken en daarbij de verschillende facetten van de cerebrale circulatie. Hieronder vallen de cerebrale autoregulatie, de bloed-hersenbarrière en perivasculaire innervatie van hersenvaten. De cerebrale autoregulatie zorgt ervoor dat ondanks een wisselende perfusiedruk, de cerebrale bloeddoorstroming gelijk blijft, bewerkstelligd door verandering van de vaatdiameter. Wanneer de druk te hoog wordt, wordt de maximale limiet van de cerebrale autoregulatie overschreden. Er ontstaat geforceerde vasodilatatie met hyperperfusie als gevolg. De bloed-hersenbarrière wordt verstoord met als gevolg uittreding van water en plasma in het hersenparenchym. Deze cascade van gebeurtenissen ontstaat bij eclampsie en PRES. De perfusiedruk waarop dit optreedt en de mate van oedeemvorming worden o.a. beïnvloed door de kwetsbaarheid van de bloed-hersen arrière, activatie en dichtheid van de perivasculaire innervatie en veranderingen in de limieten van de cerebrale autoregulatie. De veranderingen die zwangerschap

veroorzaakt in deze facetten van de cerebrale circulatie, worden in dit hoofdstuk besproken.

In **Hoofdstuk 2** werden drie cohorten vrouwen vergeleken: 30 vrouwen met eclampsie in de voorgeschiedenis, 31 vrouwen met pre-eclampsie in de voorgeschiedenis en 30 vrouwen met een normotensieve zwangerschap in de voorgeschiedenis. De vrouwen waren gematched op leeftijd en jaar van zwangerschap. Alle deelneemsters vulden de Cognitive Failures Questionnaire (CFQ) in, een gevalideerde vragenlijst met betrekking op het cognitieve functioneren. De uitkomsten van deze test waren significant slechter in de groep vrouwen met eclampsie in de voorgeschiedenis (43.5 ± 14.6) vergeleken met de controles (36.1 ± 13.9), p = 0.049. Vrouwen met pre-eclampsie in de voorgeschiedenis hadden een vergelijkbare score met de controles (36.9 ± 13.9), echter, dit verschilde niet significant van de eclampsie groep. De mate van cognitieve functiestoornissen nam lineair toe met het aantal insulten dat vrouwen in de eclampsiegroep hadden doorgemaakt. De vrouwen met meer dan twee eclamptische insulten hadden een score van 60.0 ± 14.5 vergeleken met een score van 39.1 ± 10.9 wanneer er één insult was geweest (p = 0.005). De CFQ is een subjectieve test waarbij de deelneemsters vragen kregen over o.a. het geheugen en de concentratie. Deze uitkomsten geven dus enkel een indruk van de subjectieve cognitieve functie in het dagelijks leven van deze vrouwen. Echter, het feit dat vrouwen met meerdere insulten een slechtere cognitieve functie rapporteerden, suggereert dat eclamptische insulten schadelijker zijn dan gedacht en dat er negatieve gevolgen op de lange termijn kunnen bestaan. In **Hoofdstuk 3** werd deze hypothese getest door van 39 vrouwen met eclampsie in de voorgeschiedenis en 29 controles met normotensieve zwangerschappen in de voorgeschiedenis structurele cerebrale MRI scans te maken. Van de vrouwen die, gemiddeld 7 jaar geleden, eclampsie doormaakten, vertoonde 41% cerebrale witte stof afwijkingen. Van de controles was dat, gemiddeld 5 jaar na de zwangerschap, 17%, p = 0.03. Het geschatte volume van de witte stof afwijkingen was 0.041 ml ± 0.016 (± standard error) in de eclampsie groep vergeleken met 0.004 ml ± 0.002 (± standard error) in de controle groep, p = 0.025. Het aantal eclamptische insulten bleek lineair verband te houden met het optreden (p = 0.009) en de ernst (p = 0.042) van witte stof afwijkingen. Deze bevindingen suggereren dat het paradigma dat een vrouw met eclampsie volledig zou herstellen na de zwangerschap, mits er geen cerebrale bloedingen optreden, zou moeten worden herzien. Het gebruik van magnesiumsulfaat ter voorkoming van het ontstaan en opnieuw optreden van insulten zou daarom benadrukt moeten worden. In **Hoofdstuk 4** werd vervolgens van 73 vrouwen met pre-eclampsie in de voorgeschiedenis en 75 controles met een normotensieve zwangerschap de aanwezigheid en het volume van witte stof afwijkingen bepaald op MRI

scans. De gemiddelde leeftijd was 37 jaar en de zwangerschap was gemiddeld 5 jaar geleden. Bij 37% van de vrouwen met pre-eclampsie werden cerebrale witte stof afwijkingen gevonden, terwijl dat in de groep controles 21% was, p = 0.036. Ook het volume van de witte stof afwijkingen was significant groter bij de vrouwen met pre-eclampsie in de voorgeschiedenis (gemiddeld 0.11 ml, mediaan 0.00 ml, range 0.00 – 2.34 ml) vergeleken met de controles (gemiddeld 0.015 ml, mediaan 0.00 ml, range 0.00 – 0.13 ml), p = 0.019. In de subgroepanalyses van de pre-eclampsie groep waren vroege pre-eclampsie (vóór 37 weken amenorroeduur) en huidige hypertensie gerelateerd aan het vóórkomen van witte stof afwijkingen en een groter volume. Er kwam geen relatie naar voren tussen witte stof afwijkingen en neurologische verschijnselen ten tijde van de zwangerschap, aanwezigheid van HELLP-syndroom, toediening van magnesiumsulfaat of een diastolische bloeddruk boven 110 mmHg. De huidige bloeddruk en het huidige lichaamsgewicht waren significant hoger in de pre-eclamptische groep in vergelijking met de controles. Het was helaas retrospectief niet met zekerheid te bepalen welke vrouwen PRES hadden doorgemaakt ten tijde van de zwangerschap met pre-eclampsie, omdat deze vrouwen routinematig geen indicatie hebben voor neurologische beeldvorming. Om die reden kan de hypothese dat cerebraal oedeem een bijdragende factor is geweest voor het ontstaan van witte stof afwijkingen niet verworpen of bevestigd worden, louter gesuggereerd. Een andere, of aanvullende, plausibele verklaring voor de aanwezigheid van witte stof afwijkingen bij vrouwen met pre-eclampsie in de voorgeschiedenis, is het constitutioneel verhoogde risico op hart- en vaatziekten in deze groep. De witte stof afwijkingen die in deze studie gevonden zijn, zouden een uiting van vroege cerebrovasculaire schade kunnen zijn. Vrouwen met vroege pre-eclampsie zijn mogelijk gevoeliger voor deze cerebrovasculaire schade dan vrouwen met à terme pre-eclampsie. Het is daarom belangrijk om in deze groep vrouwen extra aandacht te besteden aan het opsporen en behandelen van risicofactoren voor hart- en vaatziekten.

In **Hoofdstuk 5** werd een ratmodel gebruikt om eclampsie na te bootsen. Twee groepen niet-zwangere (n = 16) en twee groepen zwangere (n = 17) Dahl salt-sensitive ratten werden onderzocht. In beide categorieën werd één groep hypertensief gemaakt door het toedienen van een zoutrijk dieet gedurende twee weken. Hierna werden de arteriae cerebri posterior geïsoleerd om de effecten van zwangerschap en hypertensie te bepalen op de reactiviteit en remodellering van de vaatwand van de cerebrale arteriën. In deze studie werd aangetoond dat de gebruikelijke hypertensieve remodellering wel optreedt bij niet-zwangere dieren maar niet plaatsvond tijdens zwangerschap. In beide groepen zwangere dieren was er een verminderde myogene reactiviteit van de arteriën bij hogere intraluminale drukken. Dit suggereert dat zwangerschap het cerebrale vaatbed gevoeliger

maakt voor verlies van autoregulatie in geval van acute hypertensie. Omdat hetzelfde fenomeen reeds was gevonden in een ander diermodel voor pre-eclampsie kan geconcludeerd worden dat deze bevindingen een effect van de zwangerschap waren en niet van de manier waarop hypertensie werd geïnduceerd. In deze studie werd ook de dichtheid van de totale perivasculaire innervatie in de a. cerebri posterior bepaald, omdat perivasculaire innervatie in pathologische omstandigheden een rol kan spelen in cerebrale autoregulatie. Het bleek dat de dichtheid van perivasculaire innervatie daalde in geval van hypertensie, maar steeg tijdens de zwangerschap ongeacht de aanwezigheid van hypertensie. Welk type neuronen van dichtheid veranderde kon met deze techniek niet bepaald worden en de consequenties van deze verandering zijn nog niet opgehelderd. Daarom werd in **Hoofdstuk 6,** naast de dichtheid van de totale perivasculaire innervatie, ook de dichtheid van de sympatische (tyrosine hydroxilase) en trigeminale (calcitonin gene-related peptide, CGRP) perivasculaire innervatie gemeten in a. cerebri posterior van niet-zwangere (n = 23), zwangere (n = 22), post partum (n = 23) en mannelijke (n = 22) Sprague Dawley ratten. In deze groepen zorgde zwangerschap niet voor een toename van de totale perivasculaire innervatie dichtheid, maar wel van de trigeminale innervatie. De sympatische innervatie bleef gelijk in alle groepen. In deze vaten werd de sensitiviteit voor zowel CGRP als noradrenaline (sympatische neurotransmitter) niet beïnvloed door zwangerschap of sekse. Wat het belang is van de veranderde dichtheid van de trigeminale perivasculaire innervatie tijdens de zwangerschap is onduidelijk. In dit hoofdstuk wordt gespeculeerd over het mogelijke belang hiervan bij hoofdpijn en cerebrale hyperperfusie, met name bij migraine – een aandoening die mogelijk gedeelde eigenschappen met eclampsie heeft.

Een algemene discussie en conclusies worden in **Hoofdstuk 7** gegeven. Er wordt geconcludeerd dat het optreden van eclamptische insulten (PRES) op de lange termijn schadelijker voor het maternale brein is dan tot op heden gedacht werd. Er blijkt een relatie tussen het optreden van witte stof afwijkingen en verminderd subjectief cognitief functioneren waaronder geheugen en concentratie. Verder wordt geconcludeerd dat vroege pre-eclampsie als risicofactor kan worden aangewezen met betrekking tot het optreden van cerebrovasculaire aandoeningen op de lange termijn en reeds binnen enkele jaren na de zwangerschap tot uiting kan komen in de vorm van het vaker voorkomen van cerebrale witte stof laesies. Met betrekking tot de zwangerschapsgerelateerde aanpassingen van de cerebrale circulatie in een proefdiermodel wordt geconcludeerd dat de reactiviteit van cerebrale arteriën verminderd is waardoor de bloed-hersen barrière vatbaarder is voor disruptie en dus oedeemvorming bij het optreden van hoge drukken.

Perivasculaire innervatie lijkt hierin geen rol te spelen, echter verschillende andere oorzaken worden in dit hoofdstuk genoemd zoals remodellering van de vaatwand en een toegenomen capillair dichtheid.

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Annet.

I love my heart and soul
I love all humanity
Join hearts and souls together
Love, peace and harmony
Love, peace and harmony
(Zhi Gang Sha)

Curriculum Vitae NL

Annet Aukes is op 13 maart 1984 geboren te Brederwiede. Annet groeide op in Heerenveen terwijl haar jeugd in het teken stond van theater, muziek en sport. Nadat zij daar het Gymnasium voltooide met het profiel Natuur & Gezondheid, Latijn en wiskunde B_2 is ze in 2002 begonnen met de studie Geneeskunde aan de Rijksuniversiteit Groningen. In haar 3^{e} studiejaar begon zij met het onderzoek naar het brein na eclampsie bij Dr. G.G. Zeeman, wat uiteindelijk resulteerde in promotieonderzoek en dit proefschrift. In 2006 verbleef zij een jaar aan de University of Vermont in Burlington, Vermont, Verenigde Staten. Daar deed ze - aanvankelijk in het kader van haar wetenschappelijke stage - onderzoek naar het brein tijdens de zwangerschap. Later in dat inspirerende jaar werd zij aangenomen voor het MD/PhD programma van de Junior Scientific Masterclass Groningen (promotor Prof. dr. J.G. Aarnoudse) waarna Annet dit onderzoek in het kader van haar promotie voort zette in Vermont bij Prof. dr. M.J. Cipolla. In 2007 ging ze verder met het onderzoek in het UMCG. In 2008 werd haar werk gewaardeerd met de Best Plenary Presentation Award op het International Student Congress of Medical Sciences te Groningen. Haar co-schappen liep zij met veel plezier in het UMCG en de Isala klinieken te Zwolle. Het keuze co-schap Obstetrie & Gynaecologie deed Annet in 2010 in het Kalafong Academic Hospital in Pretoria, Zuid-Afrika waar ze eveneens een onderzoeksproject naar Maternal Near Miss deed bij Prof. dr. R.C. Pattinson. Hierna deed ze nog een extra co-schap Neurologie aan de University of Vermont, College of Medicine. In augustus 2010 behaalde zij haar artsexamen, in december dat jaar rondde ze haar promotieonderzoek af en in 2011 is ze begonnen als ANIOS Obstetrie & Gynaecologie in het Spaarne ziekenhuis te Hoofddorp.

Curriculum Vitae EN

Annet Aukes was born in the Netherlands on March 13, 1984. She started Medical School at the University of Groningen in 2002. In 2004 she started research with Dr. G.G. Zeeman, on patients with a history of eclampsia, what eventually resulted in this thesis. In 2006 Annet spent one year in Dr. M.J. Cipolla's lab, at the University of Vermont, Burlington, VT, USA, investigating the cerebral vasculature in several rodent models of preeclampsia. Based on that work, she was accepted for an M.D./Ph.D. program of the Junior Scientific Masterclass Groningen (promotor Dr. J.G. Aarnoudse). In 2007, she has continued her patient research in Groningen and in 2008 she was awarded the Best Plenary Presentation Award at the International Student Congress of Medical Sciences in Groningen. Annet furthered her medical training by performing her clinical rotations in multiple countries. In the Netherlands she trained at the University Medical Center in Groningen and the Isala clinics in Zwolle. During her elective rotation in Obstetrics & Gynecology in 2010 she trained under Dr. R.C. Pattinson at the Kalafong Academic Hospital, Pretoria, South Africa, which she combined with a research project on maternal morbidity and mortality. She completed her medical training with an additional rotation in Neurology at the University of Vermont, College of Medicine, Burlington, VT, USA. She received her medical degree in August 2010. As of 2011, Annet started her clinical career in Obstetrics & Gynecology at the Spaarne hospital, Hoofddorp, the Netherlands.