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

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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 °C. 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 Determination 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 (30 μm^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 $[1-(\Phi_{\text{tone}}/\Phi_{\text{papav}})]\cdot 100\%$, 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 $m = \Delta\Phi/\Delta_{\text{pressure}}$, where $\Delta\Phi$ is the difference in diameter of the myogenic active vessel at the two different pressures and Δ_{pressure} the difference in pressure.

Statistical Analysis

Results are presented as mean \pm 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: $30.2 \pm 6.0\%$ vs. $40.7 \pm 4.6\%$ ($p>0.05$). 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: $36.3 \pm 5.2\%$ vs. $40.0 \pm 5.8\%$ ($p>0.05$).

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

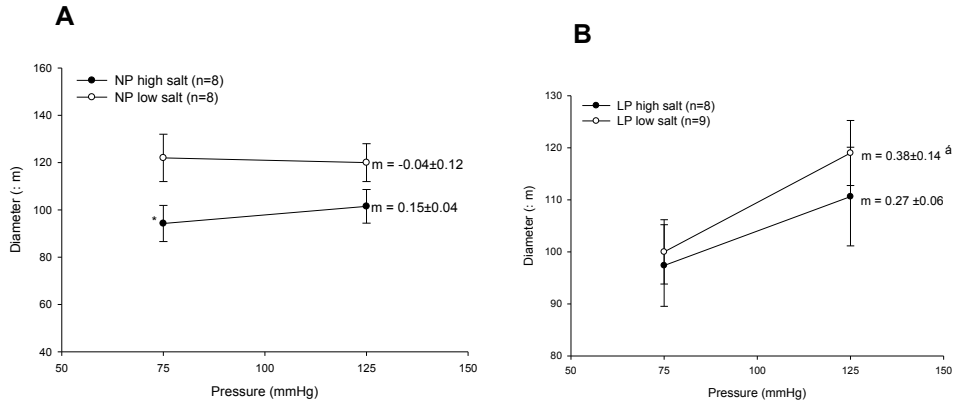


Figure 1 Active pressure vs. diameter curves with slope (m) for posterior cerebral arteries (PCA) from nonpregnant (NP) and late-pregnant (LP) rats. A: both groups of NP animals responded to pressure myogenically, as demonstrated by the small slope. NP hypertensive animals (closed circles) had significantly 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; † p < 0.05 vs. 75mmHg; ‡ p < 0.05 LP vs. NP hypertensive.

Figure 2

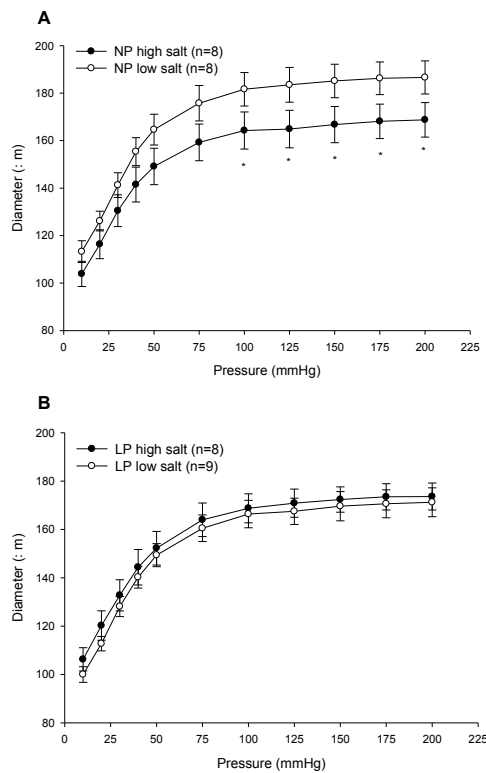


Figure 2 Passive pressure vs. diameter (in μm) curves for PCAs from NP and LP animals. A: the diameters of PCAs in NP hypertensive animals were smaller compared to normotensive control animals. B: the diameters of PCAs in LP hypertensive and normotensive animals were similar. *p = 0.06 vs. NP low salt.

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 varicose 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 \pm 5	142 \pm 5	17.1 \pm 0.8	0.16 \pm 0.01
High salt (n=8)	101 \pm 5	128 \pm 7	13.6 \pm 1.3*	0.14 \pm 0.01
Late-pregnant				
Low salt (n=9)	97 \pm 3	130 \pm 3	15.9 \pm 0.4	0.16 \pm 0.01
High salt (n=8)	98 \pm 6	130 \pm 6	16.0 \pm 1.0	0.17 \pm 0.02

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

Figure 3

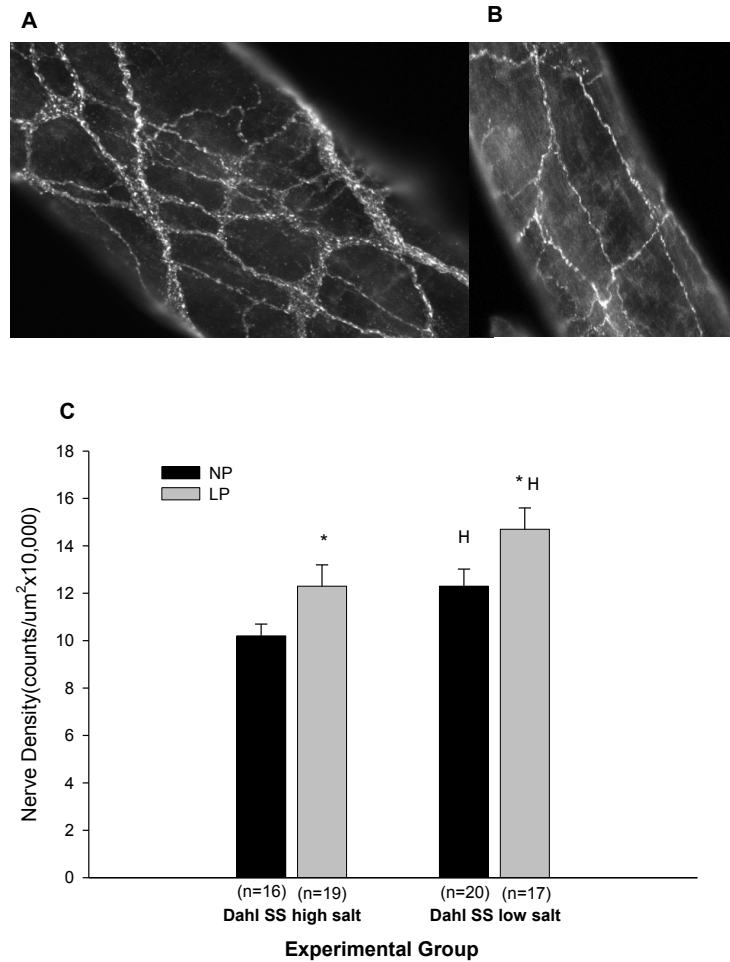


Figure 3 Perivascular nerve fibers and their density in pial PCAs of NP and LP Dahl salt-sensitive rats. A and B: photomicrographs of PCAs stained for perivascular nerves with the pan-neuronal stain, protein gene product 9.5. The arteries were imaged using a fluorescent microscope for Cy3. A: photomicrograph 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; [†] $p < 0.05$ 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 nociceptive 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.

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