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

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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.

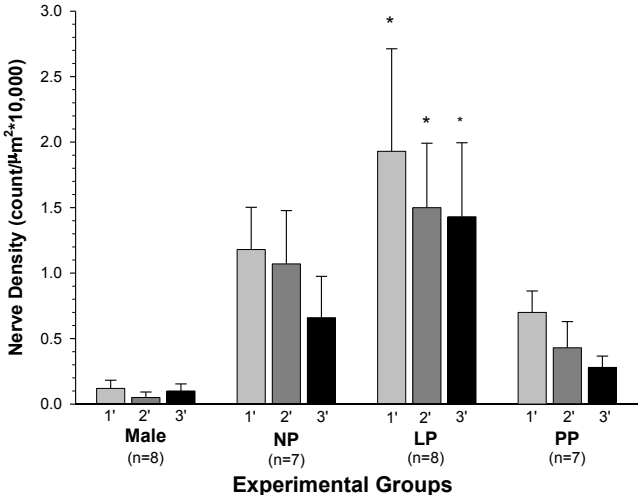

Figure 1 Nerve density of perivascular calcitonin gene-related peptide (CGRP)-containing nerve fibers of posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micron of vascular wall for each arterial segment. * $p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

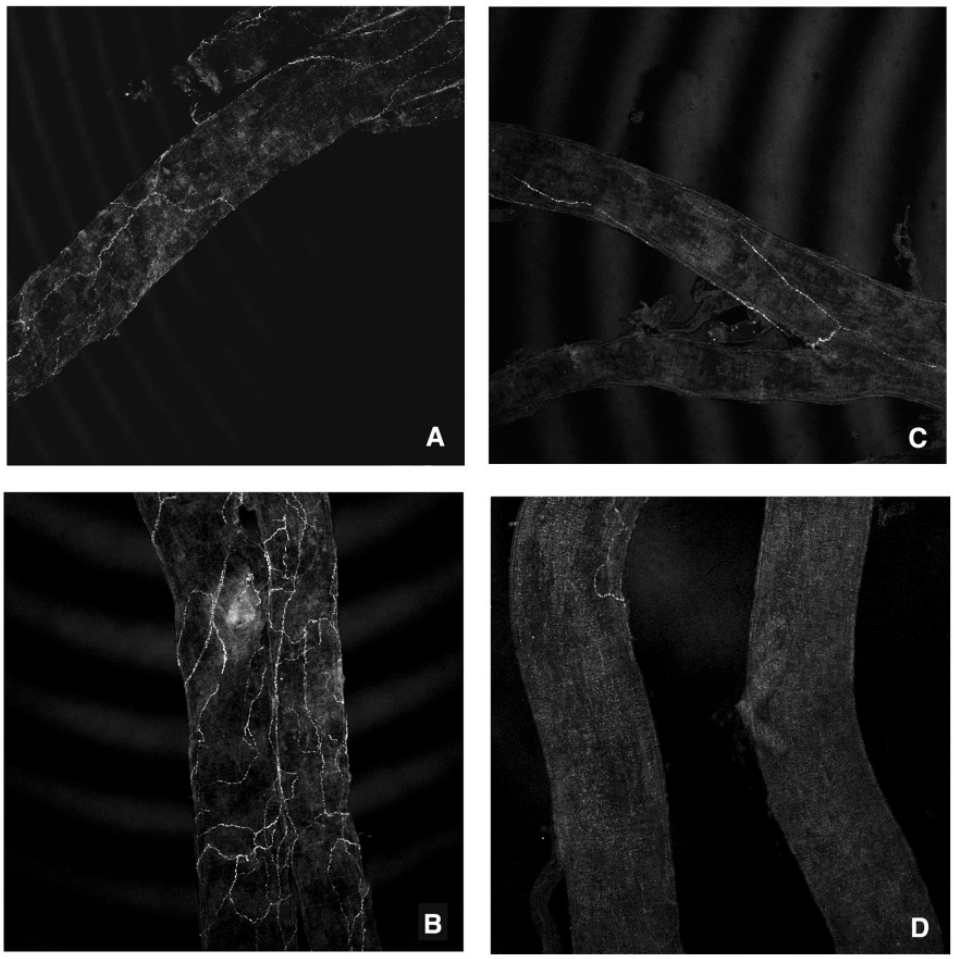

Figure 2 Photomicrographs of posterior cerebral arteries stained for calcitonin gene-related peptide (CGRP)-containing nerve fibers from nonpregnant (**A**), late-pregnant (**B**), postpartum (**C**) and male (**D**) rats. The innervation of CGRP-containing nerve fibers in posterior cerebral arteries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

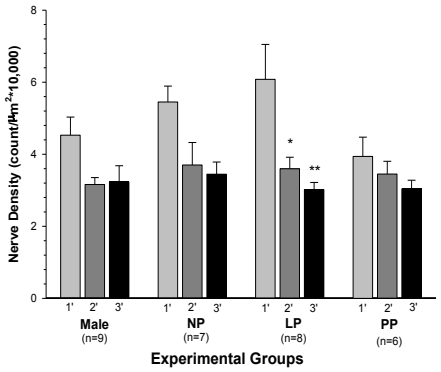

Figure 3 Nerve density of perivascular tyrosine hydroxylase (TH)-containing nerve fibers of posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micrometer of vascular wall for each arterial segment. *p < 0.05 vs.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

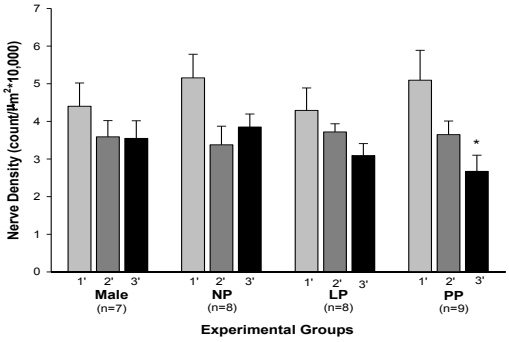

Figure 4 Nerve density of perivascular protein gene product 9.5 (PGP 9.5)-containing nerve fibers of posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is expressed per square micrometer of vascular wall for each arterial segment. *p <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

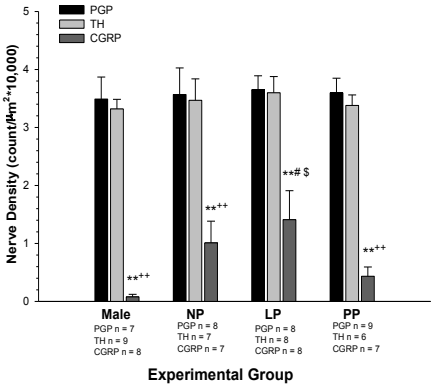

Figure 5 Nerve density of perivascular calcitonin gene-related peptide (CGRP)-, protein gene product 9.5 (PGP 9.5)- and tyrosine hydroxilase (TH)-containing nerves of the entire posterior cerebral artery segment from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. Nerve density is 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.

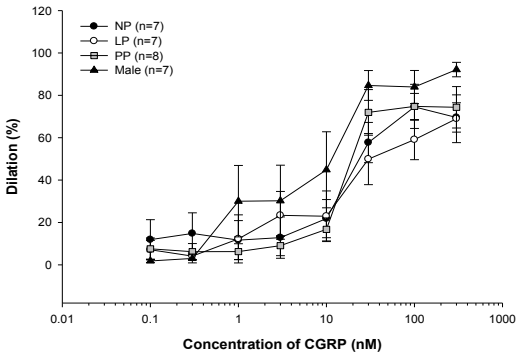

Figure 6 Reactivity curves to α-calcitonin gene-related peptide in posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats. There was no difference in reactivity to CGRP between groups (p > 0.05).

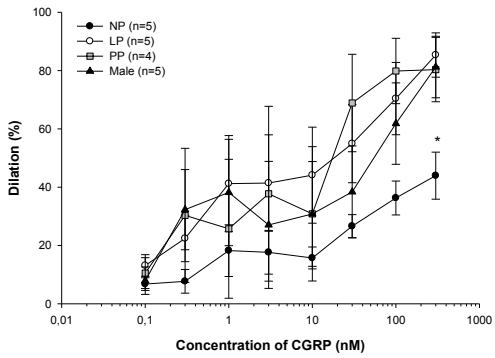

Figure 7 Reactivity curves to α-calcitonin gene-related peptide (CGRP) in posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats in presence of nitro-L-arginine (L-NNA). There was no difference in reactivity to CGRP between groups except for the NP group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

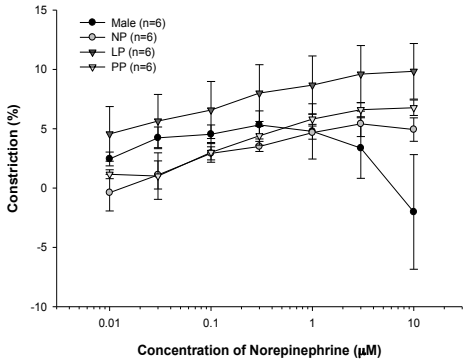

Figure 8 Reactivity curves to norepinephrine (NE) in posterior cerebral arteries from nonpregnant (NP), late-pregnant (LP), postpartum (PP) and male rats in presence of nitro-L-arginine (L-NNA) and indomethacin. Reactivity to NE was considerably less compared with CGRP. There was no difference in reactivity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

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