Adaptive Plasticity of Vaginal Innervation in Term Pregnant Rats

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Zhaohui Liao, MD^{1,2,3} and Peter G. Smith, PhD^{1,2,3}

Abstract

Changes in reproductive status place varied functional demands on the vagina. These include receptivity to male intromission and sperm transport in estrus, barrier functions during early pregnancy, and providing a conduit for fetal passage at parturition. Peripheral innervation regulates vaginal function, which in turn may be influenced by circulating reproductive hormones. We assessed vaginal innervation in diestrus and estrus (before and after the estrous cycle surge in estrogen), and in the early (low estrogen) and late (high estrogen) stages in pregnancy. In vaginal sections from cycling rats, axons immunoreactive for the pan-neuronal marker protein gene product 9.5 (PGP 9.5) showed a small reduction at estrus relative to diestrus, but this difference did not persist after correcting for changes in target size. No changes were detected in axons immunoreactive for tyrosine hydroxylase (sympathetic), vesicular acetylcholine transporter (parasympathetic), or calcitonin gene-related peptide and transient receptor potential vanilloid type 1 (TRPV-1; sensory nociceptors). In rats at 10 days of pregnancy, innervation was similar to that observed in cycling rats. However, at 21 days of pregnancy, axons immunoreactive for PGP 9.5 and each of the subpopulation-selective markers were significantly reduced both when expressed as percentage of sectional area or after correcting for changes in target size. Because peripheral nerves regulate vaginal smooth muscle tone, blood flow, and pain sensitivity, reductions in innervation may represent important adaptive mechanisms facilitating parturition.

Keywords

autonomic innervation, estrous cycle, parturition, sensory innervation

Introduction

The female reproductive tract undergoes major structural and functional changes associated with hormonal and reproductive status. These include alterations in receptors, endometrial and muscular size, and immune function.¹⁻⁶ Such changes are likely to be important in many aspects of reproduction including ovulation, sperm transport, implantation, gestation, and parturition.

Reproduction-related plasticity is not confined to the target tissues but also occurs in neuronal populations projecting to the reproductive tract. In uterus, it is well established that pregnancy is accompanied by massive degeneration of sympathetic axons.⁷⁻⁹ More recent studies show that changes in reproductive hormonal status also induce reductions in uterine sympathetic nerves.¹⁰⁻¹² Thus, increasing serum estrogen levels deplete uterine sympathetic fibers through a process that appears to involve axonal degeneration,¹ similar to that reported for uterine axons at the termination of pregnancy.^{7,13} While the functional implications of reduced sympathetic innervation are not fully understood, it is thought that reduced excitatory innervation may be important in preventing neurally mediated uterine contractions at stages of the reproductive process where quiescence is required.

Estrogen can also affect innervation in other parts of the reproductive tract. The rodent vagina contains abundant sensory and autonomic innervation, mainly in association with

smooth muscle of the submucosal layer. This innervation is thought to be important in regulating vaginal smooth muscle tone, glandular secretions, blood flow, and sensitivity.¹⁴⁻¹⁷ Hence, changes in vaginal innervation may impact many aspects of reproductive tract physiology that could in turn influence fertilization and delivery.

We previously observed that “surgical menopause” induced by bilateral ovariectomy results in increased densities of vaginal sympathetic, parasympathetic, and sensory axons relative to intact cycling rats. Restoring estrogen levels to those seen in pregnancy reduced innervation densities to those of intact animals.¹⁴ The finding that vaginal target innervation density changes with estrogen levels suggests that menopausal symptoms, such as vaginal dryness, reduced smooth muscle tone,

¹Institute for Neurological Disorders, University of Kansas Medical Center, Kansas City, KS, USA

²Kansas Intellectual and Developmental Disabilities Research Center, University of Kansas Medical Center, Kansas City, KS, USA

³Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA

Corresponding Author:

Peter G. Smith, Hemenway Life Sciences Innovation Center, University of Kansas Medical Center, Mailstop 3051, Kansas City, KS 66160, USA
Email: psmith@kumc.edu

diminished blood flow, and increased sensitivity may be associated with changes in innervation status rather than simply effects on target tissues per se.

These findings raise additional questions. Does vaginal innervation remodeling occur during estrous cycle fluctuations in reproductive hormones, possibly implying a role in receptivity or sperm transport? Are the more sustained and pronounced increases in estrogen in late pregnancy associated with nerve loss, thus facilitating parturition? In the present study, we examined whether normal physiological changes linked to different aspects of reproductive function also are associated with alterations in vaginal innervation.

Materials and Methods

Experimental Preparations

A total of 20 virgin female Sprague Dawley rats (Harlan Breeding Laboratories, Indianapolis, Indiana) at 60 days of age were housed with a 12-hour alternating light–dark cycle and access to food and water ad libitum. Vaginal smears were obtained at 9 to 10 AM daily and estrous cycle stages determined by the established criteria^{10,18}; rats included in these studies showed at least 2 consecutive normal estrous cycles.

To assess vaginal innervation during the estrous cycle, tissues were obtained from 5 rats at estrus (ie, after the surge in serum estrogen that occurs in proestrus) and 5 rats at early diestrus (low estrogen phase of the cycle).¹⁹

For studies on vaginal innervation during pregnancy, tissues were obtained from 10 normally cycling female Sprague Dawley rats mated in proestrus at approximately 60 days of age. Mating was verified by the presence of copulation plugs or spermatozoa in vaginal smears on the following morning (day 1 of pregnancy). Tissue was obtained from one group of rats ($n = 5$) on gestational day 10 (prior to the rise in estrogen) and from a second group ($n = 5$) at gestational day 21 (during sustained estrogen elevation just prior to parturition).²⁰

Virgin rats at estrous and diestrus and pregnant rats at gestational day 10 and day 21 were euthanized with pentobarbital (100 mg/kg intraperitoneal [ip]) followed by thoracotomy, and the vagina was removed through a ventral midline incision in the lower abdomen. The tissue was freed by gently separating it from adherent fascia and the urethra, transecting the cervical junction, cutting through external perigenital skin, and removing the vagina in its entirety. All experimental protocols employed in these studies were in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Kansas Medical Center Animal Care and Use Committee.

Tissue Processing

Vaginal tissue was fixed by immersing in Zamboni's fixative for 24 hours at 4°C, rinsed daily in 0.01 mol/L phosphate-buffered saline ([PBS] pH 7.4) for 7 days, and cryoprotected overnight in 20% sucrose in PBS at 4°C.¹⁴ Tissue was then cut transversely into 4 segments of equal length, embedded in

Tris-buffered saline (TBS) medium (Electron Microscopy Sciences, Hatfield, PA), snap frozen in isopentane precooled with liquid nitrogen, and stored at -80°C until sectioning. Serial cryosections, 10- μm , were cut perpendicular to the longitudinal axis from the 2 central quarters of the vagina. We have shown previously that this region shows the least variability in innervation and therefore provides the best opportunity for relatively uniform sampling.¹⁴ Sections were thaw mounted onto Superfrost/Plus precleaned slides (Fisher Scientific, Pittsburgh, PA) and air-dried.

One set of sections was stained with hematoxylin and eosin and coverslipped with Permount (Fisher Scientific). Additional adjacent sets were rinsed in PBS containing 0.3% Triton X-100 (PBST), blocked for 60 minutes at room temperature in PBST containing 5% normal goat or donkey serum and 1% bovine serum albumin, and incubated overnight in a humidified chamber at room temperature with anti-serum against protein gene product 9.5 ([PGP 9.5] 1:1200; rabbit polyclonal immunoglobulin G [IgG]; AbD Serotec, Raleigh, NC), tyrosine hydroxylase ([TH] 1:200; rabbit polyclonal IgG; Chemicon, Billerica, MA), vesicular acetylcholine transporter ([VACHT] 1:1000; goat polyclonal IgG; Chemicon), calcitonin gene-related peptide ([CGRP] 1:800; rabbit polyclonal IgG; Chemicon), or transient receptor potential vanilloid type 1 ([TRPV1] 1:400; guinea pig polyclonal IgG; Neuromics, Edina, MN). Slides were washed 3 times in PBST and incubated with secondary antisera for 1 hour at room temperature (goat anti-rabbit IgG conjugated to Cy-3, 1:200, for PGP 9.5, CGRP, and TH; donkey anti-goat IgG conjugated to Cy-3, 1:200 for VACHT; Jackson ImmunoResearch, West Grove, PA, or donkey anti-guinea pig IgG conjugated to Cy-3, 1:100 for TRPV-1; Chemicon). Slides were coverslipped with fluoromount G (Southern Biotech, Birmingham, AL). Staining was eliminated by primary antisera omission or preabsorption with the appropriate peptide antigen.

Quantitative Analyses

Digital microscopic images (Nikon Eclipse TE300, Nikon Corp., Tokyo, Japan, with an Optronics Magna Fire camera, Optronics, Goleta, CA) were captured from 2 randomly selected sections from each of the 2 blocks containing the middle quarters of the vagina. In each section, 6 randomly selected fields containing submucosal tissue were captured using illumination for Cy-3 fluorescence. The epithelial layer, which is largely devoid of innervation, was not included. The area occupied by immunoreactive nerves was determined by threshold discrimination (AnalySIS, Soft Imaging System, Lakewood, CO) and divided by the total area of the sampled field to provide an index of innervation density (apparent percentage area).^{10,14} To control for changes in vaginal tissue size which could indirectly influence innervation density, submucosal tissue area was measured planimetrically from adjacent hematoxylin and eosin stained sections. Total nerve area within the submucosal compartment was then calculated

Figure I. Vaginal innervation in the estrous cycle. Immunofluorescence photomicrographs of vaginal innervation were obtained from the central vagina in diestrus (A-E) or estrus (F-J) stage. Sections were immunostained for the pan-neuronal marker PGP 9.5 (A, F) or for

by multiplying the percentage area occupied by immunoreactive nerves by submucosal tissue area.

Statistical Analysis

Values of each rat are expressed as a single average of 24 measurements taken from the 2 central quarters of each vagina. Average values from the 5 rats per group are presented as mean $\pm$ standard error of the mean (SEM). Means for apparent percentage area and total nerve area for each marker were compared between estrus and diestrus and between gestational days 10 and 21 using Student *t* test. In 1 case where the normality assumptions were not fulfilled (CGRP percentage area), statistical significance was confirmed using the Mann-Whitney rank sum test. Differences were considered significant when $P \leq .05$.

Results

Vaginal Innervation in the Cycling Rat

Diestrus. Protein gene product 9.5-immunoreactive (ir) fibers were abundant throughout the connective tissue and muscularis (Figure 1A) but rarely present within the epithelium. Protein gene product 9.5-ir nerve fiber density of rats in diestrus stage was $1.4\% \pm 0.1\%$ (Figure 2A). The total submucosal area was $3.93 \pm 0.25 \text{ mm}^2$ and the corresponding total submucosal nerve area was $0.055 \pm 0.006 \text{ mm}^2$ per section (Figure 2B).

Tyrosine hydroxylase-ir fibers were localized predominantly in blood vessels and muscularis smooth muscle (Figure 1B). Tyrosine hydroxylase-ir fiber density was $0.45\% \pm 0.07\%$ (Figure 2A) and the nerve area was $0.017 \pm 0.003 \text{ mm}^2$ (Figure 2B).

Vesicular acetylcholine transporter-ir fibers were associated mainly with vasculature and nonvascular smooth muscle (Figure 1C). Vesicular acetylcholine transporter-ir nerve density was $0.21\% \pm 0.02\%$ (Figure 2A), corresponding to an area of $0.008 \pm 0.001 \text{ mm}^2$ (Figure 2B).

Calcitonin gene-related peptide-ir fibers were distributed within connective tissue, around blood vessels and within smooth muscle (Figure 1D). Calcitonin gene-related peptide-ir nerve fiber density was $0.47\% \pm 0.03\%$ (Figure 2A) and nerve area was $0.018 \pm 0.001 \text{ mm}^2$ (Figure 2B).

Transient receptor potential vanilloid type 1-ir fibers were also identified mainly within connective tissue, around blood vessels and among muscularis (Figure 1E). Transient receptor potential vanilloid type 1-ir nerve fiber density was $0.21\% \pm 0.01\%$ (Figure 2A) and nerve area $0.008 \pm 0.001 \text{ mm}^2$ (Figure 2B).

Figure I. (continued) tyrosine hydroxylase (TH) as a marker of sympathetic nerves (B, G), vesicular acetylcholine transporter (VACHT) as a marker of cholinergic axons (C, H), and for sensory nerves containing calcitonin gene-related peptide ([CGRP] D, I), and the vanilloid receptor transient receptor potential vanilloid type 1 ([TRPV-1] E, J). Bar in j = 50 μm in all panels.

Figure 2. Quantitative analysis of central vaginal innervation in the estrous cycle. A, Area of sample field occupied by immunoreactive fibers was expressed as the percentage of the total field area (axon density). B, Values were also normalized for changes in target size by multiplying the percentage area by the total area of the submucosal compartment (axon area). Data were obtained during diestrus (black bars) and estrus (open bars) stage. Quantification was conducted in specimens stained for PGP 9.5, tyrosine hydroxylase (TH), vesicular acetylcholine transporter (VACHT), calcitonin gene-related peptide (CGRP), and transient receptor potential vanilloid type 1 (TRPV-1). * $P < .05$ versus diestrus.

Estrus

The overall distribution pattern of PGP 9.5-ir fibers in estrus rats was comparable to that of rats in diestrus (Figure 1F) stage. Protein gene product 9.5-ir nerve fiber density was $1.09\% \pm 0.07\%$ (Figure 2A), which was significantly lower than that in diestrus ($P < .05$) stage. The area of the submucosal tissue compartment in rats at estrus was $4.01 \pm 0.13 \text{ mm}^2$, which is comparable to that in diestrus. Nerve fiber area normalized to tissue volume was $0.044 \pm 0.003 \text{ mm}^2$ (Figure 2B), which was not significantly different from those observed in diestrus stage.

Tyrosine hydroxylase-ir fiber distribution in estrus stage was similar to that of rats in diestrus stage (Figure 1G), and fiber density (Figure 2A) and nerve area (Figure 2B) were both comparable to diestrus and did not show any significant differences ($P > .05$). Vesicular acetylcholine transporter-ir fiber distribution (Figure 1H), nerve density (Figure 2A), and area (Figure 2B) in estrus stage were all similar to those observed in diestrus stage.

The distribution of CGRP-ir fibers at estrus (Figure 1I) was similar to those in diestrus stage. Calcitonin gene-related peptide-ir nerve fiber density (Figure 2A) and nerve area (Figure 2B) were not different from those observed in diestrus stage. Transient receptor potential vanilloid type 1-ir fiber distribution (Figure 1J), density (Figure 2A), and area (Figure 2B) in estrus were all similar to those observed in diestrus stage.

Vaginal Innervation in the Pregnant Rat

Gestational day 10. The distribution patterns for axons immunoreactive for PGP 9.5, TH, VACHT, CGRP, and TRPV-1 at day 10 of pregnancy were all similar to those in the cycling rat vagina (Figure 3A-E). Protein gene product 9.5-ir nerve fiber density was $1.02\% \pm 0.06\%$ (Figure 4A) and submucosal area was $3.751 \pm 0.091 \text{ mm}^2$, with a total nerve area of $0.038 \pm 0.002 \text{ mm}^2$ (Figure 4B). Tyrosine hydroxylase-ir nerve fiber density was $0.47\% \pm 0.04\%$ (Figure 4A), corresponding to an area of $0.018 \pm 0.001 \text{ mm}^2$ (Figure 4B). Vesicular acetylcholine transporter-ir nerve fiber density was $0.23\% \pm 0.02\%$ (Figure 4A), and the nerve area was $0.009 \pm 0.001 \text{ mm}^2$ (Figure 4B). Calcitonin gene-related peptide-ir nerve fiber density was $0.46\% \pm 0.03\%$ (Figure 4A), with a nerve area of $0.017 \pm 0.001 \text{ mm}^2$ (Figure 4B). Transient receptor potential vanilloid type 1-ir nerve fiber density was $0.21\% \pm 0.02\%$ (Figure 4A) and nerve area was $0.008 \pm 0.001 \text{ mm}^2$ (Figure 4B).

Gestational day 21. Fiber distribution patterns for PGP 9.5-ir, TH-ir, VACHT-ir, CGRP-ir, or TRPV-1-ir in rats at day 21 of pregnancy were similar to those in cycling rats and rats at day 10 of pregnancy but appeared to be less abundant (Figures 3F-4J). Submucosal area was $4.60 \pm 0.08 \text{ mm}^2$, which was greater than that at day 10 of pregnancy ($P < .01$).

In rats at day 21 of pregnancy, PGP 9.5-ir nerve fiber density was reduced by 40% as compared to rats in early pregnancy ($P < .01$; Figure 4A). When expressed as total area, PGP 9.5-ir innervation was 27% less ($P < .01$; Figure 4B).

Tyrosine hydroxylase-ir nerve fiber density at gestational day 21 was decreased by 41% ($P < .01$; Figure 4A), and TH nerve area was similarly decreased by 28% ($P < .05$; Figure 4B). Vesicular acetylcholine transporter-ir fiber density in late pregnancy was reduced by 45% ($P < .01$; Figure 4A), and VACHT nerve area was decreased by 33% ($P < .05$; Figure 4B).

Calcitonin gene-related peptide-ir nerve fiber density was decreased by 35% ($P < .01$; Figure 4A) and nerve area by 20% ($P < .05$; Figure 4B). Transient receptor potential vanilloid type 1-ir nerve fiber density was 41% less than that observed in early pregnancy ($P < .01$; Figure 4A), and nerve area was reduced by 28% ($P < .05$; Figure 4B).

Discussion

Vaginal Innervation During the Estrous Cycle

The female reproductive tract undergoes substantial remodeling to meet reproductive needs regarding fertility and

Figure 3. Vaginal innervation in pregnancy. Immunofluorescence photomicrographs of vaginal innervation were obtained from the central vagina at day 10 post-coitus (A-E) or day 21 (F-J). Sections were immunostained for the pan-neuronal marker PGP 9.5 (A, F) or for

Figure 4. Quantitative analysis of central vaginal innervation in pregnancy. A, Area of sample field occupied by immunoreactive fibers was expressed as the percentage of the total field area (axon density). B, Values were also normalized for changes in target size by multiplying the percentage area by the total area of the submucosal compartment (axon area). Data were obtained at day 10 of pregnancy (black bars) and at day 21, just before parturition (open bars). Quantification was conducted in specimens stained for PGP 9.5, tyrosine hydroxylase (TH), vesicular acetylcholine transporter (VACHT), calcitonin gene-related peptide (CGRP), and transient receptor potential vanilloid type I (TRPV-1). * $P < .05$ versus day 10; ** $P < .01$ versus day 10.

pregnancy. Both uterus and vagina express the estrogen receptors alpha and beta, which vary during the course of the estrous cycle.²¹ There were similarities between these 2 closely related tissues in their responses to circulating hormones. Thus, uterine epithelium shows marked morphological changes in concert with the estrous cycle,²² and similar changes in vaginal epithelial composition have also been described.²³ However, there also appears to be some notable differences. Uterine myometrial smooth muscle mass varies across the estrous cycle stages, with smooth muscle size increasing at proestrus and estrus relative to diestrus.^{1,10,24} In contrast, our current morphometric studies did not reveal significant changes in vaginal

Figure 3. (continued) tyrosine hydroxylase (TH) as a marker of sympathetic nerves (B, G), vesicular acetylcholine transporter (VACHT) as a marker of cholinergic axons (C, H), and for sensory nerves containing calcitonin gene-related peptide ([CGRP] D, I), and the vanilloid receptor transient receptor potential vanilloid type I ([TRPV-1] E, J). Bar in J = 50 μ m in all panels.

submucosal/muscularis size between estrus and diestrus. This suggests that differences exist in tissue responsiveness to hormones and perhaps other factors with respect to morphological plasticity.

Tissue-specific differences in hormonal responsiveness apply to changes in innervation as well. Uterine innervation in rodents undergoes marked variation during the estrous cycle, with large numbers of sympathetic axons degenerated during the high-estrogen phase of the estrous cycle.^{1,10,25} In contrast, while a modest reduction in the density of total innervation was detected between estrus and diestrus, no significant change could be demonstrated in the overall axonal area or in any subpopulation. This suggests that variation in hormonal status during the estrous cycle is insufficient to affect vaginal axon density, while adequate to elicit uterine changes. Nonetheless, it should be noted that estrous-related changes are reported to occur in some indices of vaginal neural function, including in nociceptive threshold²⁶ and neuropeptide receptor expression.²⁷ Thus, while significant changes do occur in some vaginal neural properties in the course of the estrous cycle, these are not accompanied by major axonal remodeling.

Vaginal Plasticity in Pregnancy

Remodeling of the vaginal canal is important for accommodating passage of the fetus during delivery. Studies on the pregnant rat vagina have revealed dramatic differences in cellular composition beginning early in pregnancy. These include increased surface area, reductions in collagen-packing density, and a shift in smooth muscle cell phenotype from contractile to secretory.²⁸ In the present study, we noted a significant increase in the overall area of the submucosal layer of the vagina between early and late pregnancy. As this was prior to parturition, it is unlikely that changes in vaginal wall size are due to mechanical distension²⁹ but rather are attributable to other pregnancy-related changes. Because estrogen increases the thickness of the vaginal muscularis,³⁰ this hormone is likely to be a factor in promoting the increased size of the muscular/submucosal layer in the latter stages of pregnancy. Indeed, we have shown previously that estrogen administration reverses muscular atrophy associated with ovariectomy. However, because chronic estrogen administration alone did not fully restore the muscularis size to that seen in the intact rat, other factors apparently are also responsible for maintaining vaginal smooth muscle.¹⁴

In contrast to the relatively constant levels of vaginal innervation throughout the estrous cycle, immunostaining for the pan-neuronal marker PGP 9.5 showed a substantial reduction in innervation density in late as compared to early pregnancy. This decrease persisted even after correcting for differences in target tissue size, indicating that is not simply due to the “dilution” of constant numbers of nerves in a larger target volume. The finding that vaginal innervation varies during pregnancy but not the estrous cycle further supports the notion that the vaginal and uterine innervations respond differently to hormonal changes. Thus, while uterine sympathetic nerves are

depleted by the peak estrogen levels achieved during the estrous cycle (ie, ~40 ng/ml¹⁹), data obtained in the present study and others³⁰ indicate that vaginal innervation is not. Similarly, a single injection of 10 µg/kg 17β-estradiol elicits dramatic uterine sympathetic axon depletion³¹ but does not alter vaginal innervation (unpublished data). In contrast, a sustained elevation of estrogen to the levels similar to that of late pregnancy (~80 ng/ml³²) causes a reduction in vaginal innervation concordant with that seen in late pregnancy.¹⁴ Thus, depletion of vaginal innervation appears to require relatively high physiological serum estrogen levels that are sustained for a period of time.

Physiological Significance of Reduced Vaginal Innervation at Term Pregnancy

The importance of vaginal remodeling to accommodate parturition is well appreciated, and prior studies have shown dramatic changes in vaginal cellular phenotype and extracellular matrix composition.^{28,33} Our findings suggest that vaginal adaptation in preparation for parturition also extends to neural remodeling. There are several implications regarding the observed reduction in the density of autonomic and sensory axons at the end of pregnancy.

Vaginal smooth muscle is richly endowed with TH-ir noradrenergic sympathetic axons derived predominantly from the lower lumbar and sacral ganglia and traveling in the hypogastric nerve.^{14,15,17,34} These axons are excitatory to the smooth muscle cells, inducing smooth muscle contraction,^{35,36} which is believed to contribute to a relatively high level of resting tone. At the end of pregnancy, there appears to be a clear change in vaginal smooth muscle cell function, shifting away from a contractile phenotype toward one more, consistent with secretory function.²⁸ Changes in TH-ir sympathetic innervation would seem consistent with this altered status. Reductions in the number of excitatory sympathetic nerves would most likely result in decreased excitatory neuroeffector input, thus resulting in reduced smooth muscle tone. This would have substantial relevance to child birth; reduced excitatory innervation in conjunction with diminished contractile capacity is expected to facilitate passage of the fetus through the vaginal canal at childbirth. The intriguing question of whether the smooth muscle phenotypic shift is accompanied by altered sympathotrophic properties leading to axon loss, or whether diminished innervation contributes to phenotypic changes in smooth muscle, awaits further investigation.

Sympathetic nerves are believed to play a major role in regulating vaginal blood flow by mediating noradrenergic vasoconstriction.⁶ Thus, reductions in TH-ir sympathetic innervation would likely increase vaginal blood flow, and it has been reported that vaginal blood flow is in fact greater in ovariectomized rats receiving estrogen supplementation.³⁷ Such an increase in flow at term pregnancy could be important in facilitating the production of glandular secretions and may also be protective of vaginal tissues during ischemic episodes associated with vascular compression associated with parturition. If

so, then reduced sympathetic vasoconstrictor input could also prove to be an important adaptive mechanism in minimizing tissue damage and in promoting tissue repair.

Parasympathetic postganglionic axons originating in the paracervical ganglia also play a central role in regulating vaginal physiology. In the present study, we identified these parasympathetic axons using the marker, VACHT, which is selective for axons that synthesize and store acetylcholine. However, previous studies have shown that these neurons co-express nitric oxide synthase, consistent with the synthesis of nitric oxide.³⁸ Accordingly, these axons may be capable of mediating both cholinergic and nitrergic neurotransmission. Our findings of decreased number of VACHT-ir parasympathetic axons at term pregnancy is consistent with an earlier report that elevated serum estrogen is associated with diminutions in both vaginal nitric oxide synthase activity and nerve density.³⁹ There is evidence that release of nitric oxide can act on vaginal tissues to relax vaginal smooth muscle and to increase vaginal blood flow.^{15,40} However, it has also been shown that muscarinic receptors are present on vaginal tissues and their activation results in vaginal smooth muscle contraction.⁴¹ Thus, reductions in parasympathetic innervation could have mixed effects on vaginal physiology, at least some of which would diminish the contractile state of vaginal smooth muscles and thus be presumably adaptive in facilitating childbirth.

Sensory axons originating in lumbar dorsal root ganglia and traveling in the hypogastric nerve represent a substantial subpopulation within the vagina.^{14,15,42} These CGRP-ir nerves are believed to be important in several aspects of vaginal physiology. Calcitonin gene-related peptide-ir fibers innervate the vaginal musculature in the rat, where they are reported to elicit a mild relaxation.^{43,44} Whether the loss of this modest inhibitory input through sensory axon pruning at term pregnancy is significant in the face of diminutions in more potent sympathetic and parasympathetic excitatory input is unclear. Calcitonin gene-related peptide-ir nerves also innervate the vasculature⁴⁰ where their antidromic release of sensory neuropeptides results in vasodilation and increased capillary permeability associated with neurogenic inflammation.⁴⁵ It again seems likely that the ultimate physiological outcome will represent the sum of diminished sensory and nitrergic dilator input in the face of reduced sympathetic constrictor innervation.

While the effects of sensory nerve pruning at late pregnancy on vascular and nonvascular smooth muscle tones are unclear, it does seem that loss of sensory innervation could serve a major adaptive role with regard to pain sensation during birth. Our findings of reduced CGRP-ir nerves in the vagina at term extend those of a previous study showing significant reductions in CGRP content of the rat cervix at parturition.⁴⁶ Because vaginal peptide levels were reduced despite maintained content in parent ganglia, these earlier findings are consistent with the terminal axon loss that we observed in the present study.⁴⁶

While CGRP-ir recognizes many sensory nociceptor neurons and their projections, this peptide can also be expressed by other types of neurons.⁴⁷ A more selective marker, at least

for some nociceptors, may be the vanilloid receptor TRPV-1, which identifies capsaicin-sensitive axons mediating heat sensitivity and burning pain.⁴⁸ Immunostaining for TRPV-1 revealed a reduction in axon numbers similar to that seen with CGRP-ir, confirming that populations of nociceptor axons are reduced within vaginal tissue prior to parturition. This reduction in pain-sensing axons may represent an important adaptive mechanism, as it may alleviate some of the discomfort and pain associated with natural childbirth.

Collectively, these findings support the idea that term pregnancy, at least in rodents, is characterized by significant reductions in innervation of the vaginal canal. Loss of autonomic and sensory axons is likely to contribute to adaptive mechanisms associated with parturition including relaxation of vaginal smooth muscle tone, altered blood flow, and diminished pain sensitivity.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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