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Pregnancy Increases Excitability of Mechanosensitive Afferents Innervating the Uterine Cervix

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Background: **Labor pain derives primarily from stimulation of afferents innervating the uterine cervix and lower uterine segment. The authors have previously shown that the excitability of these afferents is regulated by sex hormones and test in this study whether pregnancy also alters their excitability.**

Methods: **After animal care committee approval, Sprague-Dawley rats (nonpregnant, pregnant days 17 and 21) were anesthetized, and two metal rods were placed through the cervix for distension. The right hypogastric nerve was dissected and carefully teased until recording from a single unit was obtained. Spontaneous activity and the response to a graded distension (20–80 g) were recorded for off-line analysis.**

Results: **A total of 151 fiber units were recorded. Pregnancy was associated with an increase in spontaneous nerve activity in the absence of a mechanical stimulus (median of 0.98 and 1.56 Hz from pregnant days 17 and 21, respectively, compared with 0.45 Hz in nonpregnant; $P < 0.01$). The proportion of fibers responding to the weakest stimulus (20 g) was significantly greater in pregnant than in nonpregnant animals. The response to graded distension differed significantly among groups, with day 21 > day 17 > nonpregnant.**

Conclusions: **Afferents that innervate the uterine cervix sprout into this tissue during late pregnancy, and estrogen increases excitability of these mechanosensitive afferents. Here, the authors show that excitability also increases during pregnancy. These data suggest that, close to the onset of labor, there is an increased input to the spinal cord from cervical distension and an increased depolarization of afferent terminals in the cervix, effects that could influence pain and the progress of labor.**

PAIN during the first stage of labor reflects activity in the hypogastric nerve, as evidenced by its relief by paracervical injection of local anesthetic,[1] and reflects primarily activation of nerves innervating the lower uterine segment and cervix, as evidenced by reproduction of labor pain by manual distension of these structures, but not the uterine fundus.[2] In rats, distension of the lower uterine segment and cervix increases firing of afferents in the hypogastric nerve,[3] and activates neurons in the thoracolumbar spinal cord dorsal horn[4] with a pattern similar to that observed with labor and delivery in this species.[5] These data suggest that the study of hypogastric afferents in the rat could improve our understanding of the neurophysiology of labor pain. Over the past few years, we have demonstrated that afferents in the rat hypogastric nerve that innervate the lower uterine segment and endocervix are primarily or exclusively C fibers[6]; respond in a polymodal fashion to mechanical distension, noxious heat, and chemical stimuli such as bradykinin[7]; and become more excitable in the presence of tonic estrogen.[7]

The effects of pregnancy on properties of nerves innervating the uterus and cervix are unknown, yet several observations suggest changes may occur during pregnancy. The cervical ripening process that immediately precedes labor involves release of proinflammatory substances, including prostaglandins, cytokines, and nitric oxide, into the cervical tissue,[8] and these inflammatory substances are known to sensitize nociceptors.[9] In addition, nerve density in the cervix increases in late pregnancy both in mice and in humans,[10,11] which could further increase afferent activity from mechanical or chemical stimulation during uterine contractions. The primary purpose of this study was to explore the neurophysiologic properties of uterine cervical afferents in the hypogastric nerve and to compare their responses to mechanical stimuli during late pregnancy.

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

Materials and Methods

Animals

After approval by the Animal Care and Use Committee at Wake Forest University School of Medicine, Winston-Salem, North Carolina, three groups of Sprague-Dawley rats (Taconic, Germantown, NY) weighing 200–300 g on the day of experiment were studied: nonpregnant (n = 25), pregnant day 17 (n = 23), and pregnant day 21 (n = 26). The number of rats studied reflected the plan to examine approximately 50 afferents in each group. Nonpregnant rats were housed two per cage, and pregnant rats were housed singly, with a 12–12 h light–dark cycle. The ambient temperature was kept at 22°C, and rats had free access to standard food and tap water.

Surgical Preparation for Hypogastric Nerve Recording

The hypogastric nerve preparation for single-unit recording and controlled uterine cervical distension were performed as previously described.[6,7] In brief, on the day of experiment, the rat was anesthetized with inhalation of halothane (2% in oxygen), and the right jugular vein and carotid artery were catheterized for fluid administration and continuous monitoring of arterial blood

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pressure and heart rate. A tracheotomy was performed for mechanical ventilation at 60 breaths/min. A lower abdominal laparotomy was performed *via* a midline incision to expose the uterus and cervix. The abdominal wall was retracted laterally, and the intestines and uterine body were retracted rostrally and laterally with gauze soaked with mineral oil. Polyethylene tubing was inserted into the urinary bladder for continuous drainage. Under direct vision, two sterilized hollow metal rods were inserted through the cervical ossa. One end was attached to a metal stand for manual distension (20, 40, 60, 80 g), and the other end was connected to a force transducer. Distension was applied for 10 s, with a 3-min interval between distensions. The right hypogastric nerve was identified, and the branch innervating the uterine cervix was carefully separated. Its proximal end was cut at the aortic bifurcation level and draped on a 5 × 10-mm epoxy-covered metal platform covered with warm mineral oil. Under the dissecting microscope, the nerve sheath was carefully removed. The nerve filaments were dissected gradually using titanium tweezers (model 555227F; World Precision Instruments, Sarasota, FL) until single-unit activity was obtained.

Hypogastric Nerve Recording

Single-unit activity was recorded with a unipolar platinum electrode. The activity of the afferent was amplified and processed through an audio amplifier. The single unit was identified initially by examining the waveform and the spike amplitude using a window discriminator at a rapid sweep speed as well as by checking the recorded sound frequency related to each spike activity. Furthermore, the signals were digitized at a sampling rate of 20 kHz and recorded on a computer through an analog–digital interface card for subsequent off-line analysis. An amplitude threshold was set for the recorded action potential of nerve fibers. Single-unit recording was ensured by checking the constancy of the shape and polarity of the displayed spike waveform. Discharge frequency was quantified by using the data acquisition and analysis software and window discriminator (Sciworks 3.0; Datawave Technology, Berthoud, CO).

Units were included, using criteria previously used in the study of these afferents,[6,7] if they increased activity in response to gentle stroking of the surface of cervix with a glass rod, distending the cervix, or, at the end of the experiment, topical application of bradykinin (10 mg/ml) to the receptive field of afferents by using a cotton-tipped applicator for 3 min, and did not respond to mechanical stimulation of the bladder or surrounding tissues. Spontaneous unit activity was defined as any nerve firing in the absence of stimulation for 1 min.

With window discrimination, we could distinguish up to three fibers with different amplitudes during each trial. The basal (control) discharge rate was computed as the mean number of spikes per second for 1 min before manipulation. For each fiber, the average effect of distension on the fiber's activity was defined as the discharge rate in the 10-s interval of active distension. The absolute spike response to distension was calculated by subtracting the frequency in the absence of distension to that during distention. Units were classified as previously described[6] as low threshold if they responded to distension of 20 g and high threshold if they did not respond to distension of 20 g.

During fiber recording, halothane concentration was maintained at 1% for stable fiber recording. Body temperature was maintained at 38°C by a heating pad. Pancuronium, 0.6 mg/kg initially and 0.1 mg/kg every 45–60 min, was administered intravenously for muscle relaxation to facilitate nerve exposure and dissection. At the end of experiment, the rat was killed with intravenous sodium pentobarbital.

Drugs

Drugs used and their sources were halothane (Halocarbon Laboratories, River Edge, NJ), pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL), bradykinin (Sigma Chemical, St. Louis, MO), and mineral oil (Fisher Scientific, Pittsburgh, PA).

Statistical Analyses

Spontaneous activity data were not normally distributed, are presented as median [25th, 75th percentiles], and were analyzed by one-way analysis of variance by ranks. Elicited activity data were normally distributed, are expressed as mean ± SE, and were analyzed by repeated-measures two-way analysis of variance. Chi-square analysis was used to compare afferent unit proportions across groups. The criterion for significance was $P < 0.05$.

Results

General Properties of Afferents

A total of 151 units from 74 animals were recorded for spontaneous activity and response to uterine cervical distension: 51 units from nonpregnant rats, 49 from pregnant day 17, and 51 from pregnant day 21. There were no differences among experimental groups in rectal temperature, blood pressure, or halothane concentration during recording (data not shown).

Spontaneous Activity

Spontaneous unit activity was determined in all recorded fibers from the three groups. The proportion of units with spontaneous activity by this definition did not differ between nonpregnant (84% of units) and pregnant (88% of units on day 17 and 84% of units on day 21) animals. Pregnancy did have an effect, however, on the magnitude of spontaneous activity. In an analysis of the

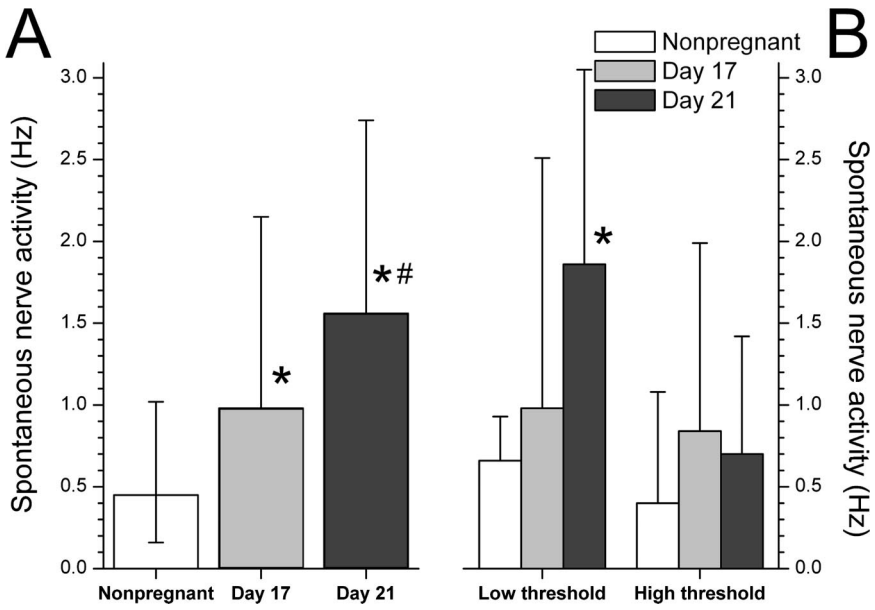

Fig. 1. Spontaneous activity of afferents increases during pregnancy (*A*), and this is due primarily to an increase in spontaneous activity of low-threshold afferents (*B*). Values are median ± 25th and 75th percentiles (*A*) or + 75th percentile (*B*) of 51 nonpregnant, 49 pregnant day 17, and 51 pregnant day 21 units. * $P < 0.05$ compared with nonpregnant. # $P < 0.05$ compared with day 17 pregnant.

total population of fibers, including those with no spontaneous activity, there was a progressive increase in median frequency of spontaneous activity during pregnancy (fig. 1A). This was primarily due to an increase in spontaneous activity in low-threshold afferents (fig. 1B). Similar results were obtained with separate analysis of only units with spontaneous activity, with all groups differing significantly from each other (nonpregnant, 0.67 [0.28, 1.44] Hz; day 17, 1.07 [0.59, 2.41] Hz; day 21, 1.90 [1.03, 3.04] Hz).

Threshold for Elicited Response

Pregnancy was associated with a change in threshold, using the previously defined dichotomy of low- and high-threshold fibers[6] (fig. 2A). We also examined in a secondary analysis the thresholds for the entire population of fibers in each group. The median threshold for elicited neural activity differed among groups by analysis of variance on ranks ($P = 0.03$) with *post hoc* testing using the Dunn test compared with nonpregnant control values yielded differences of borderline significance ($P = 0.08$ for day 17 *vs.* nonpregnant and $P = 0.05$ for day 21 *vs.* nonpregnant; fig. 2B).

Mechanosensitivity

Fibers typically responded to uterine cervical distension in a monotonically increasing fashion with stimulus intensity (fig. 3A). There was a progressive increase in the stimulus response of all fibers studied to uterine cervical distension during pregnancy, with all groups differing in the order nonpregnant < day 17 pregnant < day 21 pregnant (fig. 3B). This reflected an increase in

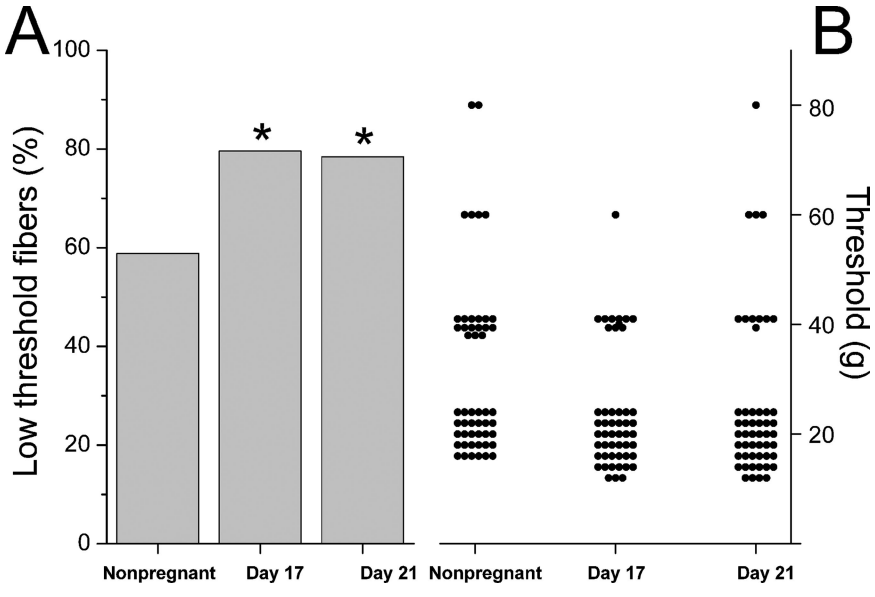

Fig. 2. Effect of pregnancy on threshold to uterine cervical distension. (*A*) The proportion of low-threshold fibers (defined as those responding to 20 g distension) increases during pregnancy. (*B*) Thresholds, with each fiber's threshold indicated as a *solid circle*. * $P < 0.05$ compared with nonpregnant.

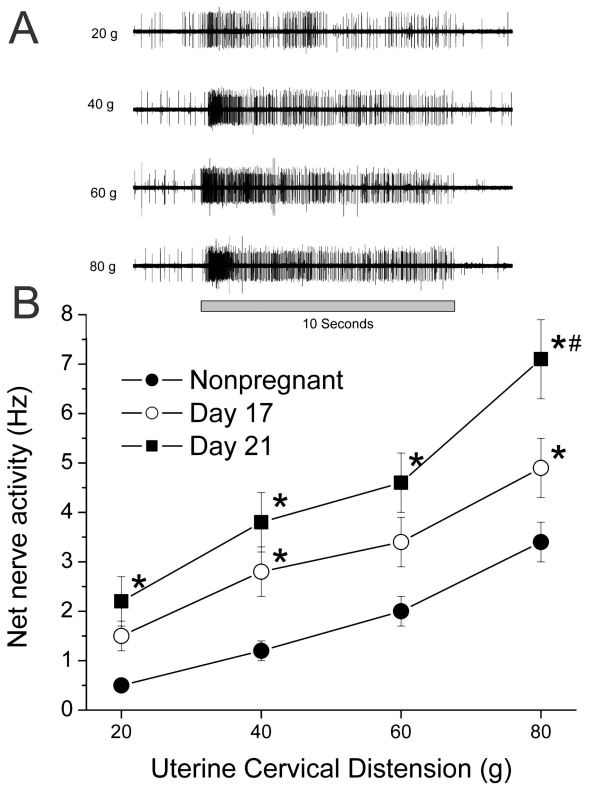

Fig. 3. (*A*) A typical response to uterine cervical distension in a single fiber from a 21-day-pregnant rat. (*B*) Net nerve activity (elicited activity during distension minus activity in the absence of stimulation) during uterine cervical distension increases progressively during pregnancy. Values are mean ± SE of 51 nonpregnant, 49 pregnant day 17, and 51 pregnant day 21 units. * *P* < 0.05 compared with nonpregnant. # *P* < 0.05 compared with day 17 pregnant.

the stimulus response in low-threshold fibers (fig. 4A) but not in high-threshold fibers (fig. 4B).

Illustration

To illustrate the effects of pregnancy on phasic uterine contractions, we used the linear regression of the stimulus response function from nonpregnant and day 21 pregnant animals, adding the median spontaneous activity in each group to the calculated y-intercept. This yielded a calculated nerve firing (in Hz) as a function of distension pressure (in g) of nerve firing = 0.07 + 0.0426 * distension pressure for the nonpregnant animal and nerve firing = 2.24 + 2.9051 * distension pressure for the day 21 pregnant animal. With these formulas, we calculated the expected firing of afferents from nonpregnant and pregnant animals using an input function of a sine wave of distension pressure with a baseline of 3 mmHg, peak of 15 mmHg, and frequency of 1/min to simulate intrauterine pressures generated during labor in the rat.[8] Figure 5 depicts this input function of pressure and the calculated nerve firing. In addition, we integrated the nerve firing functions to yield the cumulative number of action potentials generated by this pattern of

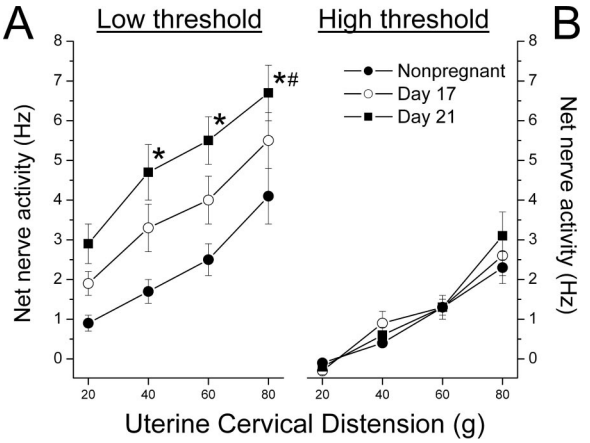

Fig. 4. Pregnancy increases the net nerve activity (elicited activity during distension minus activity in the absence of stimulation) during uterine cervical distension in low-threshold fibers (*A*) but not in high-threshold fibers (*B*). Values are mean ± SE of 30 nonpregnant, 39 pregnant day 17, and 40 pregnant day 21 low-threshold fibers and 21 nonpregnant, 10 pregnant day 17, and 11 pregnant day 21 high-threshold fibers. * *P* < 0.05 compared with nonpregnant. # *P* < 0.05 compared with day 17 pregnant.

uterine activity in the nonpregnant and pregnant state (fig. 5).

Discussion

Pregnancy exerts a profound effect on the afferents innervating the lower uterine segment and cervix of the rat. Spontaneous and distension-induced activity increases more than threefold from the nonpregnant to the term pregnant animal, and cumulative nerve firing in our simulation based on these observations shows more than a sixfold increased total activity in only 15 min, with continued divergence over time. These fundamental observations generate several hypotheses regarding the role of uterocervical afferents in pain of labor and obstetric outcome.

The current results complement anatomical studies that suggest that physiologic changes in late pregnancy would enhance pain during labor. Although there is a relative denervation of the uterine fundus during pregnancy, afferents to the lower uterine segment and cervix branch dramatically in late pregnancy in rodents and humans.[10,11] Assuming that generator potentials would summate at regions of convergence of such branching, this phenomenon could lead to increased sensitivity to peripheral stimulation, including mechanical distension, and could partly underlie the increased response to distension observed in the current study.

Regardless of the cause, the current neurophysiologic data would predict that nociception from uterine cervical distension would increase in late pregnancy compared with the nonpregnant condition, in agreement with clinical and laboratory observations. Therefore, the threshold for behavioral response to uterine distension

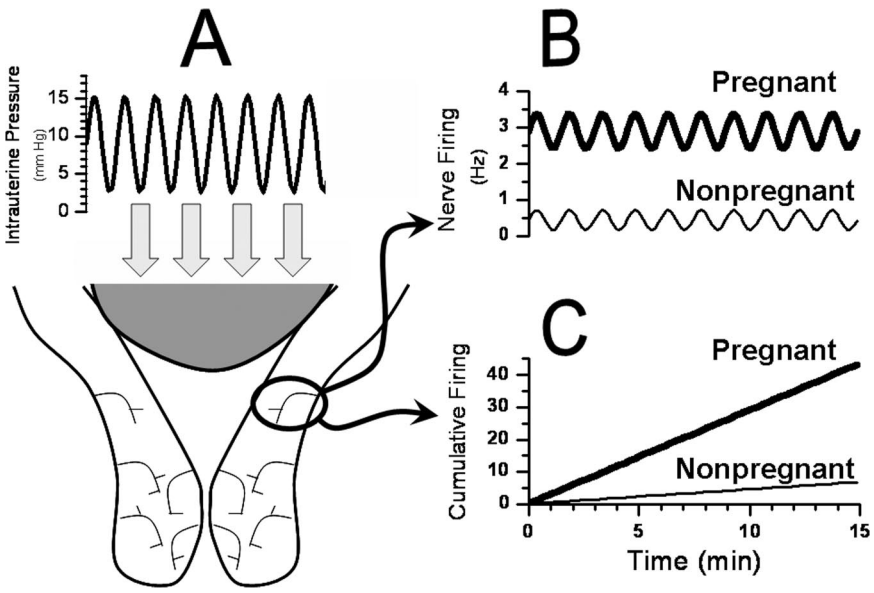

Fig. 5. Illustration of the relation between uterine cervical distension force and hypogastric nerve firing. (*A*) Intrauterine pressure increases in a cyclical fashion with a frequency of approximately 1/min in the rat, resulting in peak pressures of approximately 15 mmHg. Based on the spontaneous activity and stimulus–response relations observed in the current study, this increase in uterine cervical distension pressure results in nerve activity that is greater in the 21-day-pregnant rat (*B*, *thick line*) than in the nonpregnant rat (*B*, *thin line*). The cumulative firing of the hypothetical afferent over the 15-min period diverges over time, with greater activity in the pregnant than in the nonpregnant animal (*C*).

in the nonpregnant rat is greater than 40 g,[12,13] yet nocifensive behaviors similar to those observed with experimental renal calculi[14] occur during labor in this species, with intrauterine pressures exceeding 20 mmHg only during active pushing.[8] Similarly, uterine contractions of similar intensity to those in early labor occur in many women throughout pregnancy, but these contractions before late pregnancy are usually painless.[15]

Increased innervation density could, by summation of generator potentials, decrease the threshold for afferent firing, and this might explain the decreased threshold observed in the current study. Alternatively, threshold could be decreased and stimulus response could be increased by peripheral sensitization induced by a change in the environment surrounding the afferent terminals in the lower uterine segment and cervix. Cervical ripening in late pregnancy is thought to be controlled and modulated by a variety of factors, including increased local release of cytokines, prostaglandins, and nitric oxide.[8] Many of these products are released by macrophages, which infiltrate the cervix just before the onset of labor and play a key role in cervical ripening.[16] Cytokines are known to increase spontaneous activity of somatic nociceptive afferents[17] and sensitize them, leading to reduced threshold to mechanical stimuli.[18,19] Prostaglandins regulate the excitability of somatic nociceptive afferents[20] and primary sensory neurons.[21] Nitric oxide may also act in concert with prostaglandins to induce peripheral sensitization.[22] Taken together, these studies suggest that the increased spontaneous activity and higher response to distension in uterine cervical afferents in late pregnancy in the current study may reflect peripheral sensitization of afferent terminals from substances released during the cervical ripening process.

Hormonal changes in late pregnancy could also trigger increased excitability of uterine cervical afferents. There is a functional withdrawal of progesterone just before the onset of labor, leading to unopposed estrogen signaling.[23] Lumbosacral afferent neurons which innervate the uterus express estrogen receptors,[24] and estrogen increases their synthesis of substance P.[25] We previously showed that tonic estrogen exposure increases excitability of hypogastric afferents and their response to uterine cervical distension in nonpregnant female rats.[7] Curiously, in that study, estrogen selectively increased excitability of high- but not low-threshold afferents, whereas pregnancy in the current study increased excitability primarily of low-threshold afferents. Whether this indicates that estrogen signaling in afferent neurons is not responsible for the change in excitability during late pregnancy or that there is a difference between tonic or phasic stimulation of estrogen or the interaction between estrogen and progesterone is unclear.

We speculate that increased afferent traffic in late pregnancy could contribute to the labor process in addition to increasing pain perception. Substance P and calcitonin gene–related peptide concentrations increase in uterocervical afferents and substance P concentration increases in cervical stromal tissue just before the onset of labor,[25,26] suggesting that afferent released neuropeptides could play a role in cervical ripening by enhancing plasma extravasation leading to edema, increasing leukocyte migration, and enhancing cytokine production from leukocytes into the cervical tissue.[27] Indeed, transection of sensory nerves to the uterine cervix results in obstructed labor in rodents,[28] although it is unclear whether this is due to an effect on cervical ripening or on expulsive efforts in late labor.[8] Nonetheless, the potentially huge increase in neuropeptide release from uterocervical afferents in late pregnancy predicted by the current study (fig. 5) suggests that afferents could play an important modulatory role on the progress of

labor and that their manipulation should be examined in regulation of cervical ripening.

There are several limitations to the current study in addition to the species difference between rats and humans. All experiments occurred during general anesthesia and acute injury to the hypogastric nerve, the peritoneal cavity, and the lower uterine segment and cervix, and these conditions could alter the functional properties of the recorded afferents. In addition, we used a mechanical stimulus search paradigm, and it is not clear that the sample of afferents we obtained reflects the population of afferents, which innervate these structures. Finally, we measured neither nociception from the stimulus applied nor the release of neuropeptides into the lower uterine segment or cervix from the stimulus, so we can for the moment only speculate on the functional consequences of the changes in nerve activity we observed.

In summary, pregnancy increases spontaneous activity as well as the response to uterine cervical distension in afferents that innervate the lower uterine segment and cervix. Some aspects of this increased excitability occur in a progressive manner from day 17 to day 21 of pregnancy in the rat, and some are restricted to high-threshold afferents. The cause and functional consequences of this increased excitability are under study, but these results suggest that there is a large effect of late pregnancy on uterine cervical afferent excitability, which could carry important implications regarding pain during labor and the role of these afferents in the cervical ripening, and hence labor, process.

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