Chronic Estrogen Sensitizes a Subset of Mechanosensitive Afferents Innervating the Uterine Cervix

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Liu, Baogang, James C. Eisenach, and Chuanyao Tong. Chronic estrogen sensitizes a subset of mechanosensitive afferents innervating the uterine cervix. J Neurophysiol 93: 2167–2173, 2005; doi:10.1152/jn.01012.2004. Estrogen increases reflex nocifensive responses to distension of the uterus and the urinary bladder, but estrogen’s effects on afferent response to distension of the uterine cervix, the site of obstetric and some gynecologic pain, has not been studied. Here, single fiber recording of hypogastric nerve responses to uterine cervical distension were obtained from ovariectomized (OVX) rats and OVX rats treated with estrogen (ES). Spontaneous activity was greater in the ES group (13 of 24 units; 54%) than in the OVX group (6 of 27 units; 22%). ES differentially altered the response of low- and high-threshold units to distension. For high-threshold units, firing frequency was increased two- to fourfold with 60–100 gm distension in ES compared with OVX groups (P < 0.05). In contrast, the response of low-threshold units to distension was not altered by ES. About one-half of units tested in each group responded to a temperature increase from 35 to 49°C. A greater proportion of thermosensitive units were also mechanosensitive in the ES group (7 of 8 afferents, 88%) than in the OVX group (5 of 11 afferents, 45%). Acute application of ES in OVX rats failed to evoke or increase distension-induced responses. These data show the polymodal nature of afferent fibers innervating the uterine cervix. Increased spontaneous activity with ES may play a part in remodeling of the cervical tissue, whereas selective sensitization of high-threshold units by ES might underlie increased pain responses to cervical distension. Failure of acute ES treatment to mimic this suggests a genomic effect.

INTRODUCTION

Labor pain is a very common and severe form of acute visceral pain but has received little research attention in comparison with somatic pain. The uterus and endocervix are innervated mainly by afferent fibers in the hypogastric nerve through which sensory information, including obstetric or gynecologic pain from distension, infection, or malignancy, is transmitted to the CNS (Berkley et al. 1990; Peters et al. 1987). Behavioral studies and clinical experience with paracervical blockade to provide pain relief in parturients and nonpregnant patients undergoing dilatation of the cervix indicates the hypogastric nerve is relevant to pain from this structure (Bonica 1994).

Previous studies have focused on inflammation or distension of the uterus or vagina to understand mechanisms of chronic pain from these structures, or pressure applied to the vaginal surface of the uterine cervix, which induces antinociception and mating related behaviors in rodents (Berkley et al. 1988; Komisaruk and Larsson 1971; Peters et al. 1987; Wessellmann et al. 1998). Because these are not necessarily relevant to pain produced by distension of the lower uterine segment and endocervix, we developed a model of uterine cervical distension. Validation studies indicate that uterine cervical distension results in stimulus-dependent increases in hypogastric afferent nerve activity, reflex contraction of the abdominal musculature, and c-Fos expression of dorsal horn neurons in the upper lumbar spinal cord dorsal horn (Sandner-Kiesling et al. 2002; Tong et al. 2003), consistent with a noxious visceral stimulus.

It has been reported that afferents innervating some viscera are polymodal. For example, cardiac afferents are excited both by low pH and by bradykinin (Pan et al. 1999). Colorectal afferents are excited by both distension and noxious heat (Su et al. 2000). Our previous study indicated that uterine cervical afferents are stimulated by bradykinin and distension and are primarily or exclusively C-fibers (Sandner-Kiesling et al. 2002). One purpose of this study was to extend these preliminary observations to determine whether uterine cervical afferents respond to noxious heat and whether there was a relationship between their response to mechanical and thermal stimuli.

The uterine cervix is dramatically remodeled by estrogen (ES) and progesterone, yet whether uterine cervical afferent responses change with exposure to these hormones is unknown. Previous studies indicate a large variation in sensitivity and response of hypogastric nerve activity to uterine cavity distension across the estrous cycle, with the most dramatic changes occurring between the receptive stage and the nonreceptive, postovulatory stages of the cycle (Berkley et al. 1990; Bradshaw et al. 1999; Robbins et al. 1990, 1992). The cervix plays a key role in pregnancy maintenance and labor initiation, but, to date, there are no reports on the effects of ES on sensitivity of hypogastric afferents innervating the cervix. A second purpose of this study was to examine the tonic effects of ES on the hypogastric nerve response to uterine cervical distension.

Classically, ES is considered to act via cytosolic receptors to modulate gene expression, with an obligatory delay for changes in expression to be manifest. Additionally, ES can also cause rapid effects (Kelly et al. 1999; Wong et al. 1996) through either its receptor in the cytoplasm (Wong et al. 1996) or through novel, membrane-associated estrogen receptors (Kelly et al. 1999; Toran-Allerand et al. 2002). Behavioral studies show that peripheral application of estradiol rapidly
modulates behavioral responsiveness to painful stimuli at the spinal level (Evrard and Balthazart 2004). A final purpose of this study was to determine whether acute application of ES, in a time frame excluding genomic effects, could mimic the action of tonic ES exposure on uterine cervical afferent activity and its response to uterine cervical distension (UCD).

METHODS

Animals

Twenty-seven adult virgin female Sprague-Dawley rats (Harlan, Indianapolis, IN) were used, weighing 200–250 gm on the day of the experiments. The experimental protocol was approved by our institutional Animal Care and Use Committee. Rats were housed two per cage on 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.

Ovariectomy and ES/placebo pellet implantation

Rats were anesthetized with halothane (2–5% in oxygen) with spontaneous ventilation. A 1-cm subcostal incision was made in the right flank below the right costal margin, and both ovaries and the surrounding tissues were identified and resected. A pellet (1.5 mg) containing either 17β-estradiol (ES group; Innovative Research of America, Sarasota, FL) or placebo [ovariectomy (OVX) group] was implanted subcutaneously ipsilateral to the incision. Rats were allowed a 7-day recovery period before the nerve recording experiment. Animals had free access to water and food postoperatively. Animals showing signs of abnormal recovery, such as significant weight decrease, inability access to water and food, or wound infection were excluded and killed with intraperitoneal pentobarbital.

Surgical preparation for hypogastric nerve recording

The hypogastric nerve was prepared for single unit recording as previously described (Sandner-Kiesling et al. 2002). In brief, on the day of experiment, the rat was anesthetized with inhalation of halothane (2–5% in oxygen) with spontaneous ventilation. The right jugular vein and carotid artery were catheterized for fluid administration and continuous monitoring of arterial blood pressure and heart rate (Dash Su, Astromed, West Warwick, RI). A tracheotomy was performed for mechanical ventilation. A lower abdominal laparotomy was performed via a midline incision to expose the uterus and cervix. After acute preparation, the halothane concentration was reduced and maintained at 1% throughout the experiment. Body temperature was maintained at 38°C by a heating pad (Harvard Apparatus). Pancuronium, 0.6 mg/kg initially, and 0.1 mg/kg every 45–60 min, was given intravenously for muscle relaxation. At the end of experiment, the rat was anesthetized with inhalation of halothane (2–5% in oxygen) for mechanical ventilation. A lower abdominal laparotomy was performed for mechanical ventilation. A lower abdominal laparotomy was performed via a midline incision to expose the uterus and cervix. After acute preparation, the halothane concentration was reduced and maintained at 1% throughout the experiment. Body temperature was maintained at 38°C by a heating pad (Harvard Apparatus). Pancuronium, 0.6 mg/kg initially, and 0.1 mg/kg every 45–60 min, was given intravenously for muscle relaxation. At the end of experiment, the rat was killed with intravenous sodium pentobarbital.

The abdominal wall was retracted laterally, the intestines were retracted rostrally with gauze, and the right ureter body was displaced toward the left side to expose the retroperitoneal space. PE50 tubing was inserted into the urinary bladder for continuous drainage. The right hypogastric nerve was identified behind the bifurcation of aorta and vena cava, crossing the media aspect of the iliacus psoas major muscle, and trifurcating into a posterior branch innervating the descending colon and rectum, an anterior branch innervating the urinary bladder, and a large central branch innervating the uterus and cervix. At the level of the lower uterine segment, the hypogastric nerve divides into two branches: an ascending branch, which innervates the uterine horn, and a descending branch, which innervates the lower uterine segment and cervix. Once the nerve innervating the uterine cervix was identified and dissected, its distal end was cut at the aortic bifurcation level and draped on a platform covered with warm mineral oil. Under the dissecting microscope, the nerve sheath was carefully removed. The nerve filaments were dissected gradually until single unit activity was obtained.

UCD

Under direct vision, two sterilized hollow metal rods were inserted through the cervical osses as previously described (Sandner-Kiesling et al. 2002). One end was attached to a metal stand for manual distension (20, 40, 60, 80, and 100 gm), and the other end was connected to a force transducer (FT 03, Grass Instruments, Quincy, MA). Distension was applied for 10 s, with a 3-min interval between distensions.

Thermal stimulation

To administer a heat stimulus, one of the hollow metal rods was connected to a three-way stop cock, allowing perfusion with heated saline solution, controlled by a regulated heater pump. A 25-G needle tipped applicator (PGC Scientific, Frederick, MD) was inserted on the surface of the tissue close to the UCD rod for temperature monitoring. The heater pump was set to such a degree that the maximum temperature at the site of cervix reached to 45°C. Typically, uterine cervical tissue temperature increased from 35 to 45°C with heated saline perfusion over a 40-s period.

Hypogastric nerve recording

Single unit activity was recorded with a unipolar platinum electrode. The action potential of the afferent was amplified and processed through an audio amplifier (model AM8, Grass Instruments) and an oscilloscope (model 450, Gould, Cleveland, OH). The single unit was identified initially by examining the waveform and the spike amplitude using a window discriminator (Sciworks 3.0, Dataview Technology) 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 Pentium computer through an A/D 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).

Several search methods were applied to verify the recorded single unit received input from uterine cervix. Units were included if they increased activity in response to gentle stroking of the surface of cervix with a glass rod, distending the cervix, or topical application of bradykinin (10 mg/ml) to the receptive field of afferents by using a cotton-tipped application for 3 min (Sandner-Kiesling et al. 2002) and did not respond to mechanical stimulation of the bladder or surrounding tissues. Units were classified as low threshold (LT) if they responded to distension of 20 gm, high-threshold (HT) if they either responded to distension ≥40 gm, or mecano-insensitive if they did not respond to distension of 100 gm but were excited by bradykinin. The spike number during a 10-s distension was determined, and the absolute response spikes to distension was calculated by deducting the basal spikes from the distension-induced frequency. To determine conduction velocity, a pair of bipolar electrodes were placed in the uterine cervix, an electric stimulation was applied with a Grass Stimulator (Grass S43, Grass Instruments), and the evoked electrical signal was recorded on an oscilloscope.

Acute ES treatment

To test whether acute administration of ES might alter afferent responses to UCD, 17β-estradiol was applied topically using a cotton-tipped applicator for 30–60 min in a concentration of 1 μM on the
surface of cervix in eight rats. In four other rats, a PE50 catheter was inserted through the femoral artery and advanced such that its tip was at the take off of the uterine artery from the iliaca artery, and 17β-estradiol (1 μM) was infused intra-arterially at 20 μl/min through this catheter. At the end of some experiments, bradykinin (10 mg/ml) was applied either topically or intra-arterially. A total of 19 units were studied in this section.

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); mineral oil (Fisher Scientific, Pittsburgh, PA); 17β-estradiol (Innovative Research of American, Sarasota, FL); and β-estradiol (Sigma Chemical).

Data acquisition and analyses

With the employment of the DataWave data-acquisition system, 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/s 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. Data are expressed as mean ± SE and were analyzed by repeated measures one- and two-way ANOVA and by Student’s two-tailed t-test. χ² analysis was used to compare afferent unit populations across groups. The criterion for significance was P < 0.05.

Results

General properties of afferents

A total of 70 units from 27 animals was recorded: 51 for mechanical stimulus response studies and 19 to test the effect of acute estrogen treatment. All units tested exhibited conduc-

Mechanosensitivity

In both groups, there was a mixture of LT, HT, and mecano-insensitive units. The proportion of mecano-insensitive units was lower in the ES group (4 of 24 units, 17%) than in the OVX group (10 of 27 units; 37%, P < 0.05), whereas these groups did not differ in the proportion of HT or LT units. However, the response to UCD was greater in the ES than the OVX group in the overall population because of a selective effect on HT units.

HT UNITS. HT units comprised 40% (8 of 20) of mecano-sensitive units in the ES group and 58% (10 of 17) of mecano-sensitive units in the OVX group. A typical UCD stimulus response to an HT unit is shown in Fig. 1A. Spontaneous activity of HT units in the ES group (2.01 ± 0.65 spikes/s) was significantly greater than in HT units in the OVX group (0.32 ± 0.17 spikes/s; Fig. 1B, columns). Additionally, UCD-induced response in HT units of the ES group was significantly greater than in HT units of the OVX group (Fig. 1B). The ratio of response between groups ranged from 2.2- to 3.1-fold depending on UCD force.

LT UNITS. LT units comprised 60% (8 of 20) of mecano-sensitive units in the ES group and 41% of mecano-sensitive units in the OVX group (χ² = 3.02, P > 0.05). A typical UCD stimulus response to a LT unit is shown in Fig. 2A. Spontaneous activity of LT units in the ES group (3.51 ± 1.08 spikes/s) was significantly greater than in LT units in the OVX group (1.42 ± 0.60 spikes/s; Fig. 2B, columns). In contrast to HT units, however, ES did not increase the response to UCD in LT units (Fig. 2B). The ratio of response at the 100 gm UCD force of ES to OVX groups was 0.83, which did not differ significantly from 1.0.

Thermosensitivity

Forty of the 51 units examined were tested for thermosensitivity. Eight of 16 fibers in the ES group (50%) responded to
heat. Among the eight units sensitive to heat, seven (87.5%) responded to distension, and most of these were HT units (5 HT and 2 LT). Eleven of 24 fibers in the OVX group (48%) responded to heat. Among the 11 units sensitive to heat, 5 (46%) responded to distension (3 HT and 2 LT), which is significantly lower than (87.5%) in the ES group. Groups did not differ in proportion of units that were temperature sensitive ($P > 0.05$). Most units began responding at a tissue temperature of 42–43°C (Fig. 3, A and B), although a few fibers responded at 39°C.

Acute application of ES

Neither topical application nor intra-artery infusion of 17β-estradiol increased UCD-induced hypogastric nerve activity. Taken together, the response to a 60 gm UCD stimulus in the ES group before and after application of 1 μM 17β-estradiol for 30 min was 0.96 ± 0.21 and 1.11 ± 0.22 spikes/s, respectively ($n = 8$, $P > 0.05$). In the OVX group, the response to a 60g UCD stimulus was 0.72 ± 0.39 and 0.77 ± 0.43 spikes/s before and after 17β-estradiol, respectively ($n = 5$, $P > 0.05$; Fig. 4). Additionally, acute 17β-estradiol administration did not alter the number of units with spontaneous activity or the rate of spontaneous firing in units which were spontaneously active.

**DISCUSSION**

This study provides strong evidence that uterine cervical afferents are polymodal, responding to mechanical, thermal, and chemical stimuli. Tonic ES exposure affects these afferents in multiple and inhomogeneous ways. ES increases the proportion of heat-sensitive afferents that respond to mechanical distension. In HT afferents, which are good candidates for nociceptors, ES increases both spontaneous activity and the mechanical distension stimulus response relationship. In LT afferents, ES only increases spontaneous activity without affect on the mechanical distension stimulus response relationship. These effects of ES likely reflect genomic rather than membrane actions.

**Relevance of hypogastric afferents to pain**

This study focuses on hypogastric afferents innervating the cervix and lower uterine segments, because several lines of evidence supports their relevance to pain. This area receives dual afferent innervation—the hypogastric nerve, with afferent cell bodies in T11–L2 dorsal root ganglia in the rat and T10–L1 in the human, and the pelvic (rat) or pudendal (human) nerve, with afferent cell bodies in L6–S3 dorsal root ganglia of the rat and S1–S3 of the human. Local anesthetic blockade of hypogastric afferents by paracervical injection or low thoracic epidural blockade relieves the pain of the first stage of labor and cervical dilatation in nonpregnant women, whereas epidural blockade of sacral segments does not (Brown et al. 1989). In contrast, mechanical pressure on the vaginal surface of the cervix, which stimulates primarily pelvic or pudendal nerve afferents, results in antinociception and mating behavior in rodents (Komisaruk and Wallman 1977) and heterotopic analgesia in humans (Whipple and Komisaruk 1985). Previous neurophysiologic and behavioral studies have focused on the uterine body and have largely concluded that, in the absence of
for response in the current study (afferents innervating the colon (73%), although the threshold by heat. This is somewhat less than that observed in sacral nerves (Riedel 1976; Su and Gebhart 1998). We found that, bers have been found in the pelvic, vagus, and splanchnic this component of inflammation. Thermosensitive afferent fi-

showing stimulation and sensitization of visceral C-fibers to in other viscera (Lombardi et al. 1981; Su and Gebhart 1998), consistent with previous study of hypogastric afferents (Sand-

ner-Kiesling et al. 2002). These data are consistent with studies spinal cord dorsal horn (Sandner-Kiesling et al. 2002; Tong et al. 2003), consistent with a noxious visceral stimulus.

Polymodal responses

All units tested in this study responded to bradykinin, consistent with previous study of hypogastric afferents (Sandner-Kiesling et al. 2002). These data are consistent with studies in other viscera (Lombardi et al. 1981; Su and Gebhart 1998), showing stimulation and sensitization of visceral C-fibers to this component of inflammation. Thermosensitive afferent fi-

bers have been found in the pelvic, vagus, and splanchnic nerves (Riedel 1976; Su and Gebhart 1998). We found that, among total 40 fibers in both groups, 19 (48%) were activated by heat. This is somewhat less than that observed in sacral afferents innervating the colon (73%), although the threshold for response in the current study (>42°C) is similar to previous reports (El Ouazzani and Mei 1982; Su and Gebhart 1998).

Afferent sensitivity to heat in uterine cervical afferents implies the presence of transient receptor potential V1 (TRPV1) and/or other heat-transducing ion channels (Nagy and Rang 1999; Savidge et al. 2001). Although we did not test for capsaicin sensitivity in this study, others have observed TRPV1 receptors in urinary bladder epithelium (Birder et al. 2001), capsaicin-induced afferent excitation in the urinary bladder and gut of rats (Su et al. 1999), and pain from capsaicin application to the intestinal mucosa of humans (Drewes et al. 2003). TRPV1 ion channels are important to sensitization processes, and afferent sensitivity to heat in this study suggests that tissue inflammation and lowered pH, known to sensitize afferent terminals, in part via action at this channel, may occur in uterine cervical afferents.

Effects of ES

Some studies in humans and rodents indicate that tonic ES exposure reduces pain threshold to somatic stimuli and decreases efficacy of μ-opioid receptor agonists (Fillingim et al. 1999; Ratka and Simpkins 1991). We previously showed that tonic ES exposure reduced the potency and efficacy of the μ-opioid receptor agonist, morphine, to block the UCD-evoked visceromotor response (Sandner-Kiesling and Eisenach 2002). This study extends these observations by examining the time-dependent effects of ES on characterized visceral afferents. Although we did not measure plasma ES concentrations in this study, our previous use of these pellets resulted in a steady-state plasma ES concentration of 50–75 pg/ml (Sandner-Kiesling et al. 2002), similar to high proestrous levels, and all animals with ES treatment in this study exhibited uterine and cervical hypertrophy, consistent with an ES effect. The increased compliance of the cervix could have artifactually altered the mechanosensitivity of afferents if they respond to change in length rather than force. This is unlikely, since ES affected the two subclasses of afferents completely differently.

Tonic ES exposure produced three effects in this study. First, although ES did not affect the heat stimulus response relationship (Fig. 3B) or the proportion of uterine cervical afferents that responded to heat, the proportion of heat-sensitive afferents that also responded to distension was greater (7 of 8 afferents, 88%) in the presence of ES than in its absence (5 of 11 afferents, 45%). This suggests that ES exposure does not alter overall expression of TRPV1 or other heat-sensitive channels, but increases the likelihood that mechanosensitive afferents could be stimulated and sensitized by such channels.

Second, ES increased spontaneous activity of both LT and HT units and decreased the proportion of mechano-insensitive units in this study. This is similar to the effect of ES on spontaneous activity in hypothalamic and midbrain neurons after tonic exposure (Haskins and Moss 1983) and on hippocampal neurons during high ES exposure during proestrus (Scharman et al. 2003). Whether this effect in the cervix is due to a direct action of ES on cognate receptors on afferents themselves or reflects sensitization from the effects of ES in other elements of cervical tissue is not clear. Pelvic nerve afferents that innervate the cervix express ES receptors (Papka et al. 1998), and increased ES receptor signaling commences labor by inducing structure changes (ripening) of the cervix (Winkler et al. 1999). Spontaneous activity could reflect spontaneous pain or, alternatively, release of calcitonin gene related peptide and substance P from these afferents (Mowa et al. 2003a,b) into the cervical tissue to participate in the inflammatory response that is cervical ripening.

Third, ES selectively enhanced mechanosensitivity in HT afferents in this study. HT and LT units have been previously noted in the colon (Sengupta and Gebhart 1994a,b) and the uterine cervix (Sandner-Kiesling et al. 2002). HT mechanosensitive afferent fibers encode through the noxious range only, whereas LT mechanosensitive afferent fibers encode throughout the entire physiological and noxious range. Consistent with this study, others have noted an increase in mechanosensitivity of spinal neurons and hypogastric and pelvic afferents by ES or during proestrus (Berkley et al. 1990; Ji et al. 2003). Curiously, we did not observe an increase in the visceromotor response to UCD by tonic ES exposure (Sandner-Kiesling and Eisenach 2002;
Shin and Eisenach 2003), although this has been observed with colorectal distension (Ji et al. 2003).

In addition to the classical genomic pathway involving a cytosolic receptor, ES can directly produce rapid actions by interaction with membrane-bound receptors (Kelly and Levin 2001; Wong and Moss 1991). Since many of these nongenomic effects are present within seconds, and all are present within minutes of ES exposure, the lack of efficacy of acute ES treatment for ≦60 min in this study argues against such a mechanism and for a genomic ES receptor mechanism for the effects observed on uterine cervical afferents. Our studies of acute ES treatment examined only one stimulus strength (60%; ~70% maximum effect in the stimulus-response studies), and it is conceivable that a subtle effect of ES might have been observed at differing stimulus strengths.

Summary

In summary, many uterine cervical afferents traveling in the hypogastric nerve are polymodal, responding to chemical, mechanical, and thermal stimuli. ES treatment increases the proportion of heat-sensitive units that are also mechanosensitive, suggesting an up-regulation of TRPV1 or other heat-sensing ion channels. In addition, ES increases spontaneous activity of all mechanosensitive afferents, although whether this indicates pain or part of the cervical remodeling process is not answered by these experiments. Finally, ES selectively increases mechanosensitivity in HT afferents, consistent with increased sensitivity to pain from UCD, although whether this is due to an action of ES on afferents themselves or is a sensitization process reflecting ES-induced changes in cervical stromal cells is not known.

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