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Estrogen Reduces Efficacy of μ- but Not κ-Opioid Agonist Inhibition in Response to Uterine Cervical Distension

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Background: **Although the uterine cervix is a common source of acute and chronic visceral pain in women, there is practically no neurobiological investigation of nociception from this visceral organ. With use of a novel model of uterine cervical distension nociception in rats, the estrogen dependency of opioid agonist–induced inhibition was investigated.**

Methods: **Sprague Dawley rats were anesthetized with halothane and bilateral ovariectomy was performed, after which placebo or estrogen treatment was administered for 1 week. Animals were reanesthetized and fine metal rods were inserted into the uterine cervix for manual distension. Reflex contraction of the rectus abdominis in response to distension was recorded before and after cumulative dosing with the μ-opioid agonist morphine and the κ-opioid agonist (−)U50488.**

Results: **Uterine cervical distension increased reflex abdominal muscle contraction with a threshold of 75 g, regardless of estrogen treatment. Morphine and (−)U50488 reduced the reflex response to cervical distension in a dose-dependent manner. Estrogen reduced the inhibitory effect of morphine but not that of (−)U50488.**

Conclusions: **It has been suggested that μ-opioid agonists are less potent in females than males, whereas κ-opioid agonists are more potent in females than males. These data suggest that estrogen may influence the action of opioids, at least against visceral pain, which may explain this sex difference. In addition, these data suggest that κ-opioid agonists may be effective in the treatment of pain originating from the uterine cervix, regardless of estrogen status.**

PAIN research, especially regarding acute nociception, has focused on somatic stimuli, and most of the known neuroanatomic and neurobiological characteristics of nociception relate to somatic afferents, which comprise the majority of sensory afferents. Although there has been recent interest in visceral nociception, most studies of this topic examine responses to stimulation of colon or urinary bladder.[1] Pain arising from the uterine cervix occurs commonly in acute settings, such as obstetric labor pain or uterine cervical dilatation in gynecologic surgery, and in chronic settings, such as with cervical carcinoma, yet there have been few studies of the neurophysiologic aspects of uterine cervical afferents and none with the natural stimulus of cervical distension. We recently established a model of acute uterine cervical distension (UCD) nociception in the lightly anesthetized rat, wherein controlled distension results in stimulus-dependent increases in activity of single afferents in the hypogastric nerve.[2] All afferents recorded that responded to UCD had C fiber conduction velocities. Approximately half are low threshold, responding to UCD of 6.6 ± 2.7 g, and half are high threshold, responding to UCD of 55 ± 8.8 g.[2] All afferents recorded were polymodal, in that they also responded to topical bradykinin. As with colorectal distension, this UCD results in reflex contraction of the rectus abdominis musculature. The threshold to reflex electromyographic response (25 g UCD) is similar to that of single-unit afferent responses, and both responses increase monotonically, with similar slopes up to 100 g UCD force.[2]

Better treatment of pain originating from the uterine cervix requires a better understanding of excitatory and inhibitory influences on these visceral afferents and their integrated responses in the central nervous system. We have previously shown that neurophysiologic and reflex responses from acute UCD, like colorectal distension, can be inhibited by peripherally restricted κ-opioid agonists.[2] The purpose of the current study was to investigate whether peripherally and centrally active μ- and κ-opioid agonists inhibit response to acute UCD, since both types of agonists are in clinical use. In addition, several study reports have suggested a sex dependency and estrogen dependency to opioid potency for analgesia and have demonstrated that this dependency differs between opioid receptor subtypes.[3] Specifically, it has been suggested that estrogen decreases inhibition of μ-opioid agonists and increases inhibition of κ-opioid agonists in response to noxious somatic stimuli.[4,5] Therefore, another purpose of the current study was to examine whether estrogen reduces responses to these opioid agonists from stimulation of a visceral organ of the female reproductive tract, the uterine cervix.

Methods

Animals

After approval was obtained from the Animal Care and Use Committee of Wake Forest University School of Medicine, 40 female Sprague-Dawley rats weighing 205–305 g at the time of surgery were studied. Animals were housed at 22°C and under a 12 h/12 h light/dark cycle, with free access to food and water.

Ovariectomy

Animals were anesthetized with halothane (2–3% in 100% oxygen), and ovariectomy was performed *via* two

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small laparotomies at the flanks. We then implanted subcutaneously either 17β-estradiol time-released pellets or sham pellets. These pellets have been extensively used in rats to provide a stable, 21-day continuous release of estradiol, resulting in circulating concentrations of 200–250 pg/ml, approximately the peak levels occurring during the normal estrous cycle of this species, compared with 20–35 pg/ml before and after the cycle peak.[6]

Uterine Cervical Distension

One week after ovariectomy, animals were reanesthetized with halothane, the carotid artery was catheterized for monitoring of arterial blood pressure and heart rate with a computerized data-acquisition system, the jugular vein was cannulated for fluid and drug administration, and a tracheotomy was performed for mechanical ventilation. Halothane was then reduced to 0.5–0.7%, a level which allowed for reflex response of rectus abdominis muscles to UCD but prevented purposeful escape behavior. Animals were not restrained in any way, and no neuromuscular blockers were used. Rectal temperature was monitored continuously and maintained at 37–39°C by means of a circulating-water heating pad and heat lamp. A small midline laparotomy was performed, and fine metal rods were inserted through both uterine cervical ossa. Manual distraction of one of the rods resulted in distension of the uterine cervix, quantified by a force transducer attached to the other metal rod *via* a silk suture.

To quantify reflex responses to UCD, uninsulated needle electrodes were inserted in the rectus abdominis and electromyographic activity was monitored with a computerized window discriminator and a spike counter. Average frequencies of electromyographic activity in the first 4 sec of a 5-sec distension to 25, 50, 75, and 100 g were recorded, with stimuli separated by 3-min intervals. A distension force of 100 g was not exceeded, in order to avoid tissue injury. For data analysis, the baseline frequency in the absence of stimulation was subtracted from frequencies observed with distension.

Drug Treatment

For each individual animal, the UCD force producing approximately 75% maximum response was determined, and this distension force was used to examine inhibition from opioids. The maximum response was always obtained at 100 g UCD force. The force needed to produce a 75% maximum response was calculated by linear regression from the stimulus–response relation obtained for each animal. This 75% maximum force was 80 ± 1.5 g (range, 67.5–87.5 g).

Animals received intravenously either the μ-opioid agonist, morphine (0.01–1 mg/kg), or the κ-opioid agonist, (−)U50488 (0.01–3 mg/kg) in a cumulative manner, with doses separated by 5 min and increasing in half-log amounts. Dose ranges and timing of injection were determined in pilot experiments to reflect the active therapeutic range and time of peak drug effect. At the end of the pharmacologic experiments, naloxone, 1 mg/kg, was administered intravenously, before the animals were euthanized with an overdose of intravenous pentobarbital.

Drugs

Drugs used and their sources were halothane (Halocarbon Laboratories, River Edge, NJ); pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL); 17β-estradiol 21-day–release 1.5-mg pellets and placebo pellets (Innovative Research of America, Sarasota, FL); morphine sulfate (Astra Pharmaceutical Products, Westborough, MA); and (−)(1S, 2S)U50,488 (Sigma Chemical, St. Louis, MO). Morphine was diluted in saline 0.9%. (−)U50,488 was diluted in distilled water initially to 10 mg/kg and then further to the final concentration with saline 0.9%.

Statistical Analysis

Repeated-measures ANOVA was used to test for the effects of estrogen-replacement therapy and placebo on electromyographic findings, mean arterial pressure, and heart rate in the morphine and (−)U50,488 groups. Electromyographic analyses required log transformation to normalize the data and included baseline and halothane as covariates. Mean arterial pressure and heart rate were analyzed as percentage of baseline for the repeated-measures analyses. *Post hoc* comparisons were corrected for multiple comparisons with use of the Fisher protected least-significant difference test with Bonferroni corrections. Data are presented as mean ± SE. The median infective dose was calculated by log linear regression analysis of the entire data set. Cardiovascular effects were compared by one-way repeated-measures ANOVA, followed by the Dunnett test for *post hoc* comparison against baseline. To evaluate differences due to anesthesia, halothane, mean arterial blood pressure, and heart rate were compared by Student *t* test. Effects of UCD on mean arterial blood pressure and heart rate were compared by the Mann–Whitney rank sum test on the entire stimulus-response data. The level of statistical significance was $P < 0.05$.

Results

All animals recovered uneventfully from ovariectomy, and there was a clear difference in size of uterine horns and the cervix 1 week later between groups, with growth in the estrogen-treated animals and shrinkage in the placebo pellet–treated animals. The baseline frequency in the absence of stimulation was 0.67 ± 0.08 (mean ± SEM; range: 0–10.5). UCD resulted in a stimu-

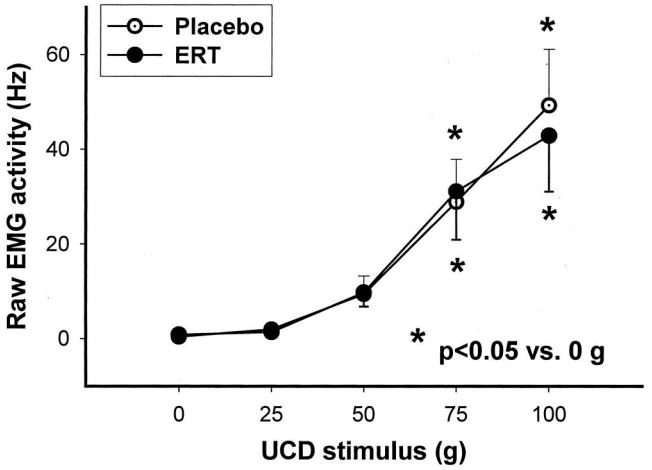

Fig. 1. Electromyographic activity (measured as frequency) as a function of uterine cervical distension force before opioid drug administration in ovariectomized rats and administration of estrogen treatment (closed circles) or placebo (open circles). Each symbol represents the mean ± SEM for six rats. * $P < 0.05$, in comparison with no stimulus. No differences between groups.

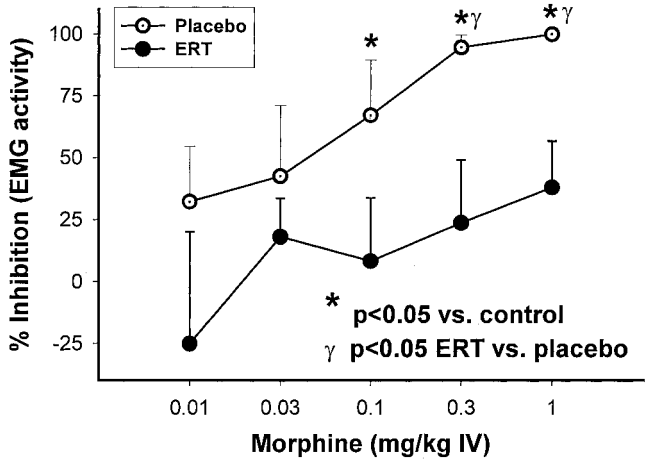

Fig. 2. Percent inhibition of electromyographic activity induced by uterine cervical distension in ovariectomized rats with estrogen (estrogen-replacement therapy [ERT]; closed circles) or placebo (open circles), receiving cumulative doses of intravenous morphine. Each symbol represents the mean ± SEM for six rats. * $P < 0.05$, in comparison with control. γ $P < 0.05$, in comparison with estrogen-replacement therapy.

lus-dependent increase in electromyographic activity in the rectus abdominis muscle, with a threshold of 75 g, and no difference between estrogen-treated and placebo pellet–treated animals (fig. 1). In contrast to our previous study with use of pentobarbital and α-chloralose for anesthesia, UCD (80 ± 1.5 g force) exerted a significant increase in blood pressure (157 ± 145/167 *vs.* 164 ± 153/174 mm Hg; median ± 25th and 75th percentile; $P = 0.037$) in the halothane0anesthetized animal but not in heart rate (data not shown).

Intravenous morphine produced a dose-dependent reduction in response to UCD, with a median infective dose of 0.034 mg/kg (95% confidence interval, 0.013–0.042 mg/kg) in the absence of estrogen. In contrast, estrogen treatment after ovariectomy resulted in a complete blockade of inhibitory effect of morphine ($P = 0.005$; fig. 2). Morphine's inhibition of the electromyographic response to UCD in placebo pellet–treated animals was completely antagonized by naloxone, 1 mg/kg (fig. 2). Morphine also reduced blood pressure and heart rate before each UCD, with no differences between estrogen-treated and placebo pellet–treated animals (table 1).

Intravenous (−)U50,488 also reduced electromyographic response to UCD in a dose-dependent manner, and with potency similar to that of morphine (median infective dose, 0.053 mg/kg; 95% confidence interval, 0.0045–0.057 mg/kg) in the placebo pellet–treated animals). In contrast to morphine, however, the effect of (−)U50,488 was not reduced by estrogen treatment (median infective dose, 0.052 mg/kg; 95% confidence interval, 0.0041–0.057 mg/kg; fig. 3). (−)U50,488 reduced blood pressure and heart rate before each UCD, with no differences between estrogen-treated and placebo pellet–treated animals (table 1).

The concentration of halothane used was 0.4–0.8 vol% but was higher in the estrogen-treated group than in the sham group: 0.600 ± 0.036 *versus* 0.502 ± 0.024 vol% (mean ± SEM; $P = 0.046$). However, including halo-

Table 1. Effects of Morphine and (−)U50,488 on Blood Pressure and Heart Rate

			Concentrations (mg/kg IV)						
			0	0.01	0.03	0.1	0.3	1	3
Morphine	Sham	MAP (mmHg)	166 ± 4	162 ± 3	163 ± 2	152 ± 4*	146 ± 3*	137 ± 4*	
	ERT	MAP (mmHg)	157 ± 10	159 ± 10	160 ± 9	154 ± 10	149 ± 10	144 ± 9*	
Morphine	Sham	HR (beats/min)	396 ± 15	395 ± 16	384 ± 15	338 ± 19*	307 ± 13*	285 ± 13*	
	ERT	HR (beats/min)	323 ± 17	314 ± 15	320 ± 17	291 ± 11*	262 ± 6*	257 ± 5*	
(−)U50,488	Sham	MAP (mmHg)	158 ± 6	154 ± 6	154 ± 6	147 ± 6*	145 ± 6*	141 ± 6*	138 ± 9*
	ERT	MAP (mmHg)	151 ± 4	150 ± 5	148 ± 6	141 ± 7*	133 ± 6*	126 ± 7*	121 ± 7*
(−)U50,488	Sham	HR (beats/min)	396 ± 23	393 ± 26	387 ± 30	386 ± 29	366 ± 32	353 ± 27	346 ± 27*
	ERT	HR (beats/min)	327 ± 25	339 ± 26	336 ± 28	325 ± 28	328 ± 25	321 ± 29	322 ± 28

Sham represents placebo, estrogen replacement therapy (ERT) represents 17β-estradiol treated animals. Data are mean ± SEM; n = 6 per group.

* Significantly ($P = 0.05$) different from baseline (= 0 mg/kg IV).

MAP = mean arterial blood pressure; HR = heart rate; IV = intravenously.

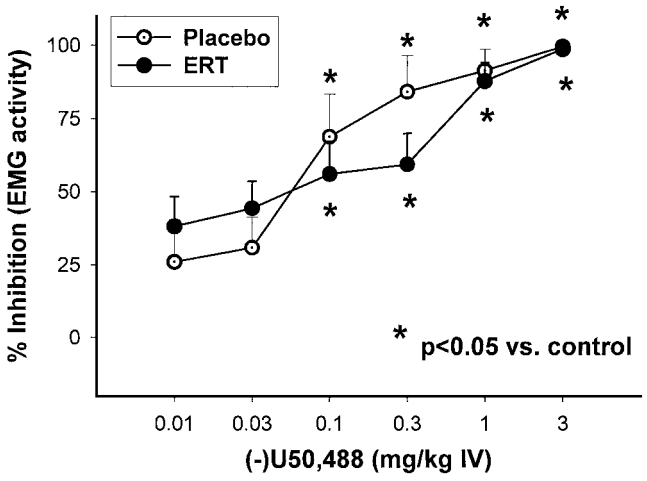

Fig. 3. Percent inhibition of electromyographic activity induced by uterine cervical distension in ovariectomized rats with estrogen (estrogen-replacement therapy [ERT]; closed circles) or placebo (open circles), receiving cumulative doses of intravenous (−)U50,488. Each symbol represents the mean ± SEM for six rats. * $P < 0.05$, in comparison with control.

thane as a covariate in our repeated-measures analyses of electromyographic features did not alter significance of the *P* values in comparison with the analyses without halothane as a covariate.

Discussion

The current study used a new model of acute visceral pain to examine hormonal influences on opioid analgesic responsiveness. We chose as a first step in this investigation to study a simple stimulus (acute UCD) and a simple hormonal status (no estrogen *vs.* chronically high estrogen). Thus, caution should be exercised in extrapolating these results to more complex conditions, such as repeated cervical distension (such as occurs during labor), presence of chronic uterine cervical distension, pregnancy, or the combined effects of progesterone and estrogen. Nonetheless, estrogen alone has powerful biologic effects that could dominate the response in many of these conditions, and we feel that the current study is an important first step in examination of determinants of analgesic efficacy against pain of the reproductive tract in females.

Visceral pain exhibits clear distinctions from somatic pain in clinical characteristics, neurophysiologic basis, and pharmacologic inhibition. The current study provides novel data about visceral pain originating from the uterine cervix. Previous studies of pain in the female reproductive tract have focused on the uterus and vagina[7,8] and have investigated the role of chronic disease, especially chronic inflammation, on sensitization and altered neurophysiology.[9] However, whether uterine cavity distension is nociceptive in rats is uncertain, and the role of uterine afferents in human pain is not clear.[10] For example, uterine efferent and afferent terminals degenerate during pregnancy,[11] a phenomenon thought to be protective against neurally induced local vasoconstriction and myometrial stimulation, and "vigorous palpation" of the uterine body during cesarean section with local anesthesia is reported to not be painful.[12]

In contrast, uterine cervical afferents do not degenerate during pregnancy,[11] UCD is clearly painful, and manual dilation of the cervix in women during cesarean section under local anesthesia is reported to reproduce the pain sensation of labor.[12] What little we know about uterine cervical nociception dates back several decades and involves descriptions of hypogastric afferent activity elicited by mechanical probing of the vaginal or external surface of the cervix.[13,14] As with other visceral organs, mechanical stimulation of the cervix results in increased firing frequency of these afferents. Our recently described UCD model produces selective distension internally from the cervical ossa and results in afferent firing at similar distension forces that result in reflex constriction of abdominal muscles.[2] This is similar to colorectal or bladder distension, which results in similar stimulus–response relations for afferent activity, reflex abdominal muscle constriction, and escape behavior in the awake animal.[15–18] For technical reasons, we have not been able to induce selective UCD in awake animals; thus, we do not know if we would also observe escape behavior at distension forces resulting in reflex abdominal muscle contraction in the lightly anesthetized animal. Although this weakens the interpretation of the responses observed in the current study as being nociceptive, the above observations with colorectal and bladder distension and the known noxious character of UCD in women suggest that we are indeed investigating a nociceptive reflex.

Blood pressure and heart rate typically increase to noxious stimuli in the lightly anesthetized animal, although, depending on anesthesia type as well as length, depth, and type of stimulation, depressor responses may be observed.[14,19] This is particularly true of visceral stimulation, which can result in reflex activation of the vagus and decreases in heart rate, with occasionally observed decreases in blood pressure.[16] Thus, it is not surprising that, at the level of halothane anesthesia used in the current study, UCD increased blood pressure but failed to alter heart rate. Another possible explanation for this observation is our relatively short stimulation time of 5 sec, compared with, *e.g.*, 20 sec, as it is used in colorectal or bladder distension.[14,15]

The threshold for electromyographic response to UCD in the current study in ovariectomized rats, 75 g, is considerably greater than the threshold we previously observed in intact female rats (25 g). Similar increases in response threshold have been observed in recordings from gracile nucleus with uterine distension[20] and in behavior and electromyographic responses to bladder

distension.[16] Although estrogen had the expected trophic influence on the uterine body and cervix, we were unable to determine whether the different thresholds observed in the ovariectomized animals in the current study, in comparison with intact animals previously described,[2] are due to a generalized change in visceral responsiveness related to estrogen following ovariectomy or to the variability within the experimental settings. We chose the 1-week period of estrogen exposure on the basis of previous electrophysiologic studies of uterine body afferent responses[20] and on behavioral responses to spinal cholinergic analgesics.[21] It is conceivable that a shorter period of estrogen exposure could have also affected opioid response without the confounding factors of trophic or atrophic responses from 1 week after ovariectomy.

Morphine inhibited the electromyographic response to UCD in ovariectomized animals without estrogen replacement in the current study, similar to observations concerning colorectal distension in male rats.[22] The reduction in morphine effect after estrogen treatment is in accordance with the reduction by estrogen in the potency of morphine antinociception against heat stimuli to the skin observed by others.[4] The cause of this reduction and its relevance to human pain are uncertain. Many studies of rodents have demonstrated greater potency of μ-opioid agonists in males than in females, not related to pharmacokinetic differences.[3] This could reflect a greater estrogen exposure in the females, although most of these studies did not examine estrous stage of the rats at the time of investigation. Studies of humans, in contrast, have mostly failed to observe meaningful differences in morphine potency between the sexes.[3] A recent well-controlled trial involving volunteers demonstrated greater potency of morphine in women than in men, but estrogen status at the time of experimentation in these cycling women was not determined.[23] Estrogen exposure *in vitro* reduced μ-opioid receptor expression and responses to morphine in a neuronal cell culture.[24] Changes in opioid efficacy during human pregnancy have not been systematically examined. Morphine and meperidine, in doses typically producing analgesia in nonpregnant patients (10 and 100 mg, respectively), failed to reduce pain intensity ratings of uterine contractions during first stage of labor.[25] Similarly, morphine use in the first 24 h after cesarean section averages 50–70 mg in published studies,[26,27] greater than typically observed after other gynecologic laparotomy procedures.[28] Our data lead us to speculate that this lack of efficacy at small doses and the need for greater doses of μ-opioid agonists in the peripartum period reflect 8–25 times higher circulating estrogen concentrations.[29]

The selective κ-opioid agonist (−)U50,488 reduced electromyographic response to UCD, consistent with similar observations with colorectal or bladder distension.[17,30] Activity of κ-opioid agonists in afferent recordings and with peripherally restricted agents suggests that a major site of action of these agents to UCD is on afferent terminals.[2] This is similar to colorectal distension, wherein κ-opioid but not μ-opioid agonists inhibit evoked afferent activity.[30] A major difference in somatic and visceral afferents is their expression of κ-opioid but not μ-opioid receptors, as further confirmed by blockade of opioid responses to noxious visceral stimuli in mice lacking the κ-opioid receptor gene but not in those lacking the μ-opioid receptor gene.[31,32]

We failed to observe an effect of estrogen on inhibition induced by (−)U50,488, suggesting that this agent may retain potency in settings of high estrogen exposure. There is a small variability in κ-opioid receptor immunostaining in the spinal cord of female rats during the estrous cycle,[33] although whether these receptors are on central afferent terminals or other sites is not known. It is unlikely that the differences we observed in the current study between μ-opioid and κ-opioid agonists reflected differences in affecting depth of anesthesia. Thus, resting blood pressure and heart rate, as indirect measures of such depth of anesthesia, were reduced by both morphine and (−)U50,488 in an estrogen-independent manner. Although pregnancy decreases halothane MAC in ewes and rats, this reduction in rats is not correlated to progesterone levels.[34] In our study, however, a slightly greater concentration of halothane in the estrogen-treated than the placebo group was necessary to achieve the same initial electromyographic response, blood pressure, and heart rate.

The clinical relevance of the use of κ-opioid agonists in treatment of acute or chronic visceral pain is difficult to determine for existing drugs, butorphanol and nalbuphine, that have limited agonist efficacy and are limited by their central side effects. Preliminary analysis of a clinical trial concerning chronic visceral pain suggests that newer, peripherally restricted, full κ-opioid agonists demonstrate remarkable efficacy without central side effects (unpublished observations, J.C.E., July 2001).

In summary, reflex electromyographic response to UCD is inhibited in ovariectomized rats by both morphine and (−)U50,488. The κ-opioid agonist but not morphine retains efficacy during estrogen replacement. These data are consistent with findings in studies of other visceral organs and support the development of κ-opioid agonists for treatment of acute and chronic pain originating from the uterine cervix, including labor pain.

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