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Intrathecal Morphine Reduces the Visceromotor Response to Acute Uterine Cervical Distension in an Estrogen-independent Manner

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Background: **Acute uterine cervical distension (UCD) forms the basis for obstetric and some gynecologic pain. Systemic morphine inhibits the visceromotor response to UCD in rats by an action in the central nervous system, but the effect of morphine is blocked by exposure to estrogen. The purpose of the present study was to determine whether this estrogen blockade of the action of morphine reflects a spinal mechanism.**

Methods: **Virgin Sprague-Dawley rats received estrogen or placebo treatment for 1 week after ovariectomy. Rats were then anesthetized, and the electromyographic response in the rectus abdominis muscle to UCD was recorded in the absence and presence of cumulative dosing with intrathecal morphine.**

Results: **Estrogen treatment did not alter the stimulus–response relationship between UCD and reflex muscle contraction. Intrathecal morphine reduced the visceromotor reflex response to UCD in a dose-dependent manner that was unaffected by estrogen treatment.**

Conclusions: **These data suggest that intrathecal morphine is effective in reducing the visceromotor response to UCD and that the reduction in efficacy of systemic morphine in this model is unlikely to reflect a reduction of the efficacy of morphine at the spinal level. These data agree with clinical studies that indicate that systemic morphine, in doses that reduce acute postoperative pain, have minimal to no effect in women in labor, yet intrathecal injection of opioids provides rapid, complete analgesia.**

SYSTEMIC administration of opioids is the most commonly used treatment for labor pain, yet controlled clinical trials demonstrate that μ-opioid receptor agonists in maximum doses that do not affect fetal heart rate (12 mg morphine, 100 mg meperidine) have no effect on pain intensity during the first stage of labor.[1] Although it is conceivable that this lack of efficacy reflects inadequate doses, hormonal changes during pregnancy that affect μ-opioid receptor agonist efficacy could serve as an alternate explanation. For example, the efficacy and potency of μ-opioid receptor agonists to somatic stimulation decreases with estrogen exposure in female animals, whereas the efficacy and potency of κ-opioid receptor agonists increases.[2,3] Similarly, the potency of μ-opioid receptor agonists is usually greater in male than in female animals,[4] perhaps reflecting greater estrogen exposure in the latter.

In contrast to somatic pain, there has been little investigation of the influence of sex and gonadal hormones on the response to noxious visceral stimuli and their inhibition by opioids. Estrogen alters the threshold for response to uterine[5] or urinary bladder[6] distension, although the relative potencies of μ-opioid receptor agonists in estrogen-exposed and nonexposed groups to these stimuli have not been determined. We recently described the graded visceromotor response to uterine cervical distension (UCD) in anesthetized rats[7] and noted that potency and efficacy of the μ-opioid receptor agonist morphine but not of the κ-opioid receptor agonist U-50,488 were reduced when animals with ovariectomy were treated with estrogen.[8] Opioids act as analgesics at both peripheral and central sites, and the inhibition of UCD-evoked reflexes by morphine reflects a central site of action.[9] The goal of the present study was to determine whether the reduction in morphine efficacy observed with systemic administration reflected a reduction in opioid action in the spinal cord. Because opioids are effective analgesics when administered intrathecally in the first stage of labor,[10] we hypothesized that estrogen would not reduce the efficacy or potency of intrathecal injection of morphine to inhibit the visceromotor response to UCD.

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

Materials and Methods

After approval from the Animal Care and Use Committee, virgin female 10- to 12-week-old Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 200 to 280 g were studied. Rats were housed at 22°C with a 12-h light/dark cycle with free access to food and water.

Ovariectomy and Intrathecal Catheter Insertion

Rats were anesthetized with halothane 1–3% in oxygen, and bilateral ovariectomies were performed *via* laparotomy through flank incisions. An intrathecal catheter was inserted as described previously.[11] Briefly, the atlantooccipital membrane was exposed, and a 30-gauge polyethylene catheter was advanced intrathecally 7.5 cm

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in a caudal direction, such that its tip lay in the lower thoracic/upper lumbar region. Only rats without motor deficits were studied. Catheter tip location was verified at the end of each experiment by complete blockade of the response to UCD by injection of lidocaine through the catheter. After surgery, animals were randomized to receive no treatment or estrogen replacement (n = 6 each) with a 21-day sustained-release 17β-estradiol pellet (Innovative Research of America, Sarasota, FL) implanted subcutaneously in the posterior nuchal region. Animals recovered for 1 week after ovariectomy before study.

Uterine Cervical Distension

Rats were anesthetized with halothane 1–3% in oxygen, with spontaneous ventilation. Mean arterial blood pressure (MAP) was monitored *via* a catheter inserted in the internal carotid artery, and fluid and drugs were administered *via* a catheter inserted in the jugular vein. A tracheostomy was performed for controlled ventilation using a small animal ventilator. The uterus was then exposed through a small low-midline abdominal incision. Two 23-gauge metal rods were inserted into the uterus and through the cervical ossa. One rod was connected to a force transducer for recording distension force, and the other was connected to a silk suture for manual traction. Electromyographic activity of abdominal muscles evoked by cervical distension was recorded by insertion of insulated electrodes into the rectus abdominis muscle. Halothane concentration was adjusted to allow the reflex to occur but no purposeful movement of the limbs.

In each rat, a stimulus–response function was established using UCD distension forces of 25, 50, 75, and 100 *g* force for 10 s. The force producing a 75% maximal response was determined by linear regression, and only this force was used to test the effects of intrathecal morphine. A force of 100 *g* was not exceeded so as to avoid tissue injury of the cervix. All stimuli during the entire experiment, including the definition of the stimulus response, were separated by 5 min. The raw electromyographic data were rectified and integrated, and the difference in integrated electromyographic power between the tenth and the fifth seconds after initiation of UCD was used as the response. In addition, blood pressure response to UCD was calculated as the difference between MAP at the tenth second of stimulation and that just before the beginning of UCD. Rectal temperature was maintained between 38° and 40°C using a warm-water circulating heating pad and heat lamp.

Intrathecal Administration of Morphine

Morphine was diluted in normal saline, and the injected volume was 10 μl. Another 10 μl of saline was injected after each morphine injection to clear the catheter dead space. Morphine was administered in cumulative dose of 0.1, 0.3, 1.0, and 3.0 μg, with doses separated by 10 min. This dose range and timing were chosen on the basis of preliminary studies of single doses. All injections were administered over a period of 2 min to avoid transient electromyographic excitation with rapid injection. After the last injection of intrathecal morphine, 1 mg/kg naloxone was injected intravenously to verify that the decreases of electromyographic activity to intrathecal morphine were opioid receptor–mediated. At the end of the experiments, 10 μl 2% lidocaine was injected to verify that the intrathecal catheter was in the correct position. After intrathecal lidocaine injections, all animals were euthanized with intravenous pentobarbital sodium.

Drugs

Halothane (Halocarbon Laboratories, River Edge, NJ), 17β-estradiol 21-day-release 1.5-mg pellets (Innovative Research of America, Sarasota, FL), morphine sulfate (Astra Pharmaceutical Products, Westborough, MA), naloxone hydrochloride (Sigma Chemical Co., St. Louis, MO), and lidocaine hydrochloride and pentobarbital sodium (Abbott Laboratories, North Chicago, IL) were used. Morphine was diluted in normal saline, and naloxone was initially dissolved in distilled water, then diluted with normal saline.

Statistics

Body weight, threshold for resting electromyography, anesthetic concentration, and 75% maximal stimulus force between groups were compared with a *t* test or Mann–Whitney rank sum test. Stimulus–response relationships for electromyography and MAP were compared using a repeated-measures ANOVA. Within each group, the effect of intrathecal morphine on control values was testing using Friedman's one-way repeated-measures ANOVA on ranks with Duncan's *post hoc* testing. The median effective dose of intrathecal morphine was calculated by log-linear regression analysis of the entire data set of each group. Unless otherwise noted, data are expressed as mean ± SEM, and a value of $P < 0.05$ was considered significant.

Results

All animals recovered without complications after ovariectomy. The ovariectomized group (OVX) was heavier than the estrogen-replaced group (ERT) at the time of the UCD experiments (OVX, 262 ± 5.8 g *vs.* ERT, 220 ± 9.5 g, $P = 0.004$). The groups did not differ at the time of UCD experiments in body temperature (39° ± 0.2°C in each group), halothane concentration (0.92 ± 0.07% in OVX *vs.* 0.87 ± 0.03% in ERT), electromyographic recorder threshold (12% in OVX *vs.* 13% in ERT), or 75% maximum stimulus (87.5 g in each group). The arterial catheter in one animal in the OVX

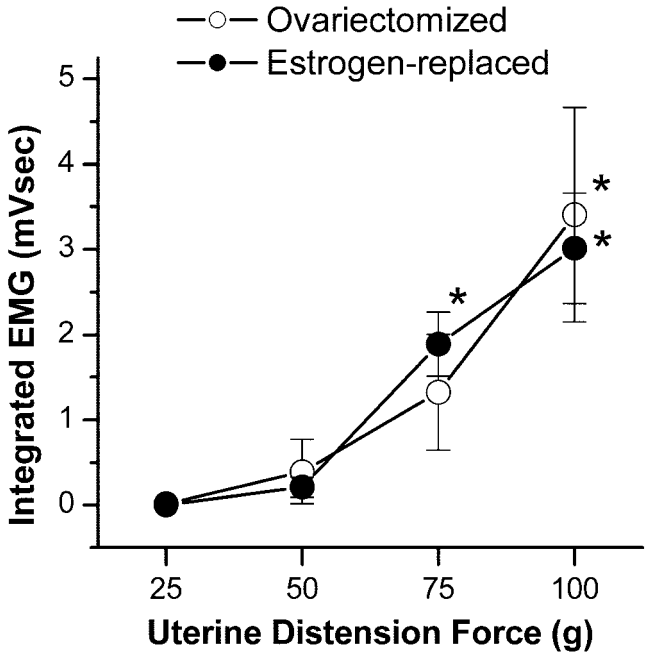

Fig. 1. Electromyographic (EMG) response to UCD in ovariectomized rats with (*solid circles*) or without (*open circles*) estrogen replacement. Each symbol represents the mean ± SEM of six animals. *$P < 0.05$ *versus* 25-g response.

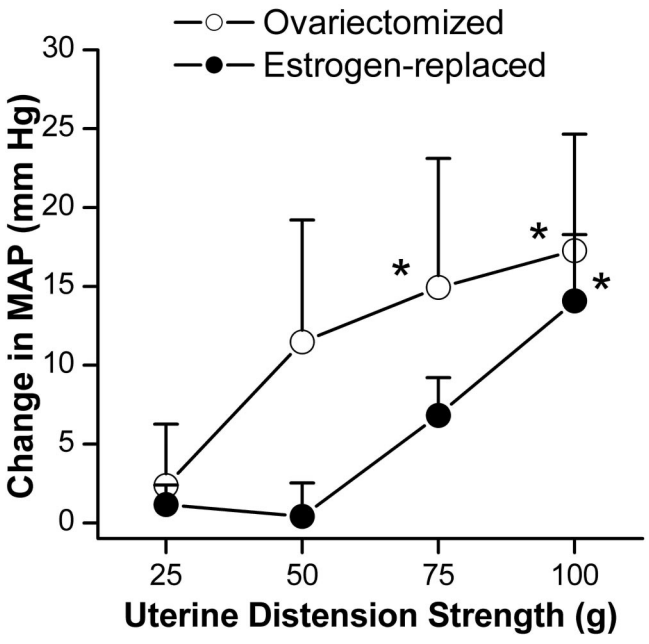

Fig. 2. Change in mean arterial pressure (MAP) with UCD in ovariectomized rats with (*solid circles*) or without (*open circles*) estrogen replacement. Each symbol represents the mean ± SEM of five or six animals. *$P < 0.05$ *versus* 25-g response.

group became occluded shortly after it was inserted; therefore, MAP data from only five animals are reported in this group.

Resting Stimulus–Response Relationships

In the range of UCD force from 25 to 100 *g*, the rectus abdominis muscle electromyographic response increased in a curvilinear fashion similarly in both groups (fig. 1). Repeated-measures ANOVA revealed a stimulus-dependent response with no difference between groups. MAP before each stimulus did not change during the series of stimuli used to determine the stimulus–response relationship (data not shown, $P = 0.288$). There was a stimulus-dependent increase in MAP (determined as the difference in MAP at the end of the 10-s stimulus compared with just at the beginning of each stimulus) in both groups (fig. 2). Although there is an apparent reduction in the MAP response to UCD in the estrogen-replaced animals (fig. 2), this difference was not significant ($P = 0.39$). Further analysis revealed a low power ($\beta = 0.15$ with α set at 0.05) to detect such a difference in MAP.

Intrathecal Morphine Effects

Intrathecal morphine produced a dose-dependent decrease in the electromyographic response to UCD in both groups, with no difference between OVX and ERT (fig. 3). There was a statistically significant, although small, difference in the intrathecal morphine dose to produce a 50% maximal inhibition of the electromyographic response to UCD (0.33 μg/kg; 95% CI, 0.30–0.44 μg/kg) in OVX compared with 0.24 μg/kg; 95% CI, 0.23–0.24 μg/kg, in ERT. MAP before UCD (fig. 4) and the effect of UCD on MAP did not differ between groups (fig. 5).

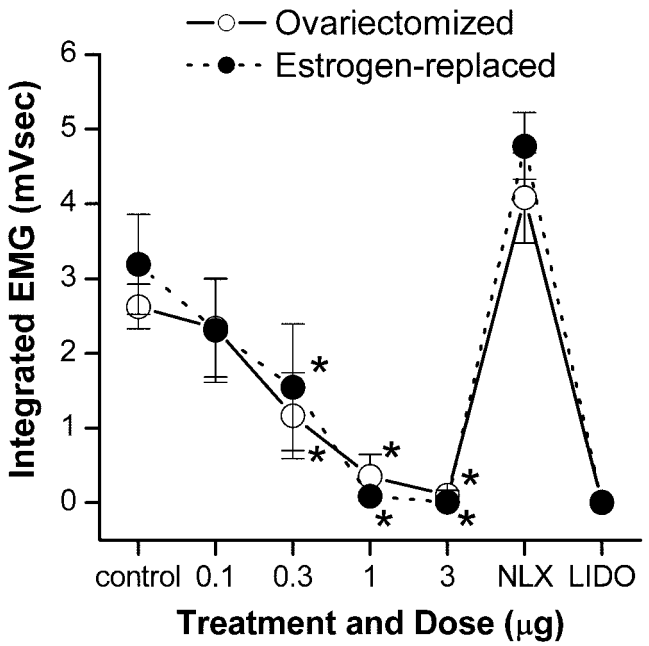

Fig. 3. Electromyographic (EMG) response to UCD in ovariectomized rats with (*solid circles*) or without (*open circles*) estrogen replacement in response to intrathecal morphine followed by intravenous naloxone (NLX, 1 mg/kg) and intrathecal lidocaine (LIDO, 20 μg). Each symbol represents the mean ± SEM of six animals. *$P < 0.05$ *versus* control EMG activity by one-way repeated-measures ANOVA.

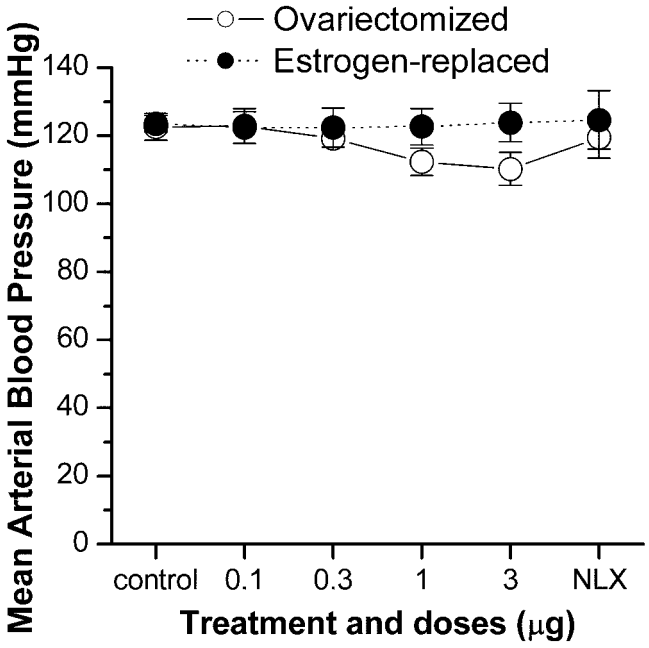

Fig. 4. Mean arterial blood pressure before UCD in ovariectomized rats with (*solid circles*) or without (*open circles*) estrogen replacement in response to intrathecal morphine followed by intravenous naloxone (NLX, 1 mg/kg). Each symbol represents the mean ± SEM of five or six animals. There were no differences in basal mean arterial blood pressure between groups.

Discussion

Reflex response to acute UCD in the anesthetized virgin rat is, of course, not a model of labor pain. It does, however, allow for a systematic study of acute nociception from the uterine cervix, and the present report represents an early step toward the eventual examination of the factors during pregnancy that alter both the neurophysiology of nociception from this structure and its pharmacologic inhibition. The study of UCD will most likely advance our understanding of labor pain more than the study of acute noxious heat stimuli to the skin or noxious distension of other viscera, such as the colon and bladder.

Estrogen Exposure and Response to UCD

As in a previous study,[8] estrogen replacement in ovariectomized animals had no effect on the stimulus response to UCD in the present report. In contrast, there is an effect of stage of estrus cycle on response to noxious visceral stimulation in the rat, with behavioral response to an artificial ureteral stone[12] and escape behavior to vaginal and uterine cavity distension[13] greater during periods of increased circulating estrogen concentrations. This discrepancy may reflect different estrogen-dependent responses in different viscera or differences between phasic and tonic estrogen exposure. For example, estrogen receptor expression in dorsal root ganglion neurons changes in a different manner with estrogen treatment in ovariectomized animals (decrease in estrogen receptor α and increase in estrogen receptor β) than during high estrogen exposure in the intact, cycling animal (both receptor subtypes increase).[14] The dose of estrogen used in the present study results in circulating concentrations near the peak of those observed in normal, cycling animals (50–75 pg/ml)[8] but far less than those observed during pregnancy.

Estrogen Exposure and Response to Opioids

Previous studies have yielded conflicting effects of estrogen on the antinociceptive effects of opioids, even with use of the same stimulus. For example, using a noxious heat stimulus to the skin, the effect of morphine in ovariectomized rats has been reported to be unaffected,[15] decreased,[2] or increased[16] by estrogen replacement. Pregnancy increases threshold to withdrawal from electric shock to the skin in a naloxone-reversible manner, reflecting activation of spinal κ- and δ-opioid receptors.[3,17] However, estrogen administration in ovariectomized animals to levels achieved in pregnancy failed to produce this antinociception.[18]

In contrast to these studies of somatic nociception, there has been little investigation of the modulation by estrogen of the antinociception of morphine to visceral stimuli. We recently reported that the antinociceptive effect of intravenous morphine on the visceromotor response to UCD was lost when estrogen was replaced in ovariectomized rats.[8] Morphine most likely acts to in-

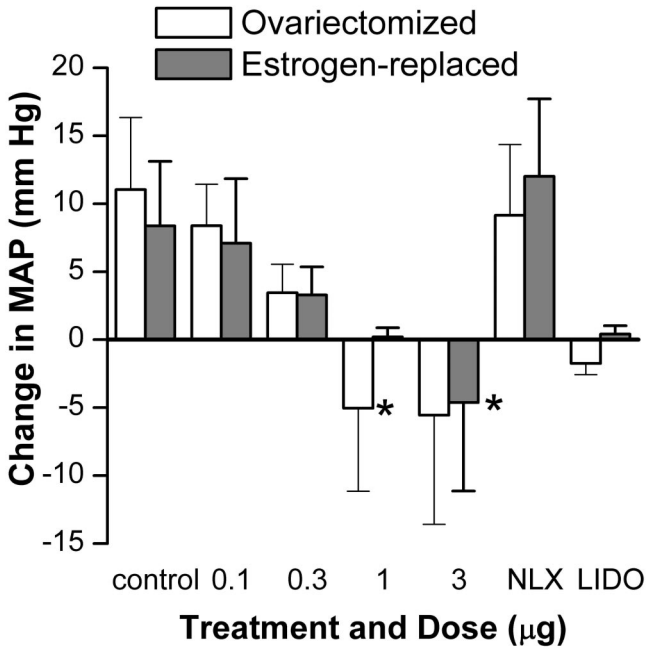

Fig. 5. Change in mean arterial pressure (MAP) with UCD in ovariectomized rats with (*solid bars*) or without (*open bars*) estrogen replacement before and after intrathecal morphine followed by intravenous naloxone (NLX, 1 mg/kg) and intrathecal lidocaine (LIDO, 20 μg). Each symbol represents the mean ± SEM of five or six animals. *$P < 0.05$ *versus* control MAP change before morphine.

hibit the response to noxious visceral stimuli by actions in the central nervous system, because morphine is inactive to reduce afferent responses to distension of the colon,[19] and the inhibition by morphine of response to UCD is antagonized by naltrexone, which enters the central nervous system, but not by methyl-naltrexone, which fails to penetrate into the central nervous system.[9]

The present study observed no difference in the inhibitory response of intrathecal morphine from the visceromotor response elicited by UCD when estrogen was replaced in ovariectomized rats, suggesting that the previously observed effect of estrogen does not reflect a change in responsiveness to μ-opioid receptor stimulation in the spinal cord. Estrogen increases metenkephalin mRNA content in superficial dorsal horn of the spinal cord of ovariectomized rats,[20] although examination of the effects of estrogen on spinal μ-opioid receptor number or response to exogenous ligand has not been studied previously. Intrathecally administered opioids are effective for the treatment of the first stage of labor in doses similar to or lower than those required to treat postoperative pain in nonpregnant women or in men. In addition, to the best of our knowledge, there is no evidence that periods of high estrogen exposure in women before menopause or estrogen replacement therapy after menopause diminishes spinal opioid efficacy. We therefore conclude that the reduction in morphine efficacy in the UCD model with estrogen exposure represents estrogen-induced plasticity supraspinally in the central nervous system.

Increases in MAP and heart rate are sometimes used as measures of nociception, and reduction in these responses by drug treatment is sometimes used as a measure of antinociception. We observed a clear parallel in the dose responses of morphine to reduce the MAP and the electromyographic response to UCD, and estrogen failed to affect either of these dose responses. Whether estrogen reduces the stimulus response to UCD, as suggested by fig. 2, would require study of more animals.

In summary, in an acute UCD model of uterine cervical nociception, intrathecal morphine produces dose-dependent antinociception, and this effect is not altered by estrogen replacement in ovariectomized animals. Intrathecal opioids are effective in the treatment of labor pain, providing a further parallel between labor pain and the UCD model. The reduction in intravenous morphine efficacy previously observed in the UCD model, and perhaps the poor efficacy of intravenous morphine in women in labor, reflects estrogen-induced changes supraspinally in the central nervous system.

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