

# Pharmacology of Opioid Inhibition to Noxious Uterine Cervical Distension

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**Background:** Reflex abdominal muscle contraction elicited by colorectal distension in male rats is inhibited by  $\mu$ - and  $\kappa$ -opioid receptor agonists and sites of action and receptor subtypes have been probed. The authors examined the pharmacology of opioid agonist inhibition in visceral pain related to the uterine cervix, the source of labor pain.

**Methods:** Ovariectomized female rats were anesthetized with halothane, and metal rods inserted in the uterine cervix through a small midline laparotomy. After a period of stabilization the cervix was distended by manual separation of the rods, using stimuli of 25–100 g, and reflex rectus abdominis electromyographic activity was recorded. After determining the stimulus response relationship, we tested inhibition of reflex activity by (-)U50,488 and morphine and their reversal with norbinaltorphimine, or with naltrexone and methyl-naltrexone, respectively.

**Results:** Cervical distension produced a stimulus-dependent increase in electromyographic activity, with a threshold of 25 g. Morphine and (-)U50,488 produced dose-dependent inhibition of the reflex activity. Log linear regression analysis demonstrated an ID<sub>50</sub> of 0.03 for morphine, and of 0.05 mg/kg for (-)U50,488. These effects were reversed by naltrexone, but not by methyl-naltrexone or norbinaltorphimine.

**Conclusions:** These data suggest that  $\mu$ - and  $\kappa$ -opioid receptor agonists effectively inhibit responses to acute uterine cervical stimulation. Lack of reversal by norbinaltorphimine further supports evidence of a novel  $\kappa$ -opioid receptor by visceral afferents. Lack of morphine reversal by methyl-naltrexone suggests central (spinal or supraspinal) sites of action for inhibition of this visceral noxious stimulus.

VISCERAL pain has only recently received attention. Our knowledge concerning neuroanatomy, neurobiology, and neuropharmacology of nociception derives mainly from research in acute somatic pain and somatic afferents. There is now an emerging literature about visceral pain, with most studies focusing on responses from the colon or the urinary bladder in male animals.<sup>1</sup> However, pain arising from the uterine cervix, which mediates the acute pain of the first stage of labor and from dilatation during gynecologic surgical procedures, or the chronic pain from injury such as cervical carcinoma, has been largely ignored.

We have recently established a model of acute uterine cervical distension (UCD) nociception in the lightly anesthetized rat. Controlled UCD results in a stimulus dependent increase in hypogastric nerve and rectus abdominis muscle activity.<sup>2</sup> Afferents excited by UCD show C fiber conduction velocity, are polymodal in that they respond to mechanical and chemical (bradykinin) stimuli, and are comprised of approximately equal proportions of low and high threshold units. The threshold for single afferents and electromyographic responses to UCD are similar (25 g of UCD), with linear stimulus-response curves, up to a maximum UCD force of 100 g.

Better understanding of the excitatory and inhibitory mechanisms of these visceral afferents at both peripheral and central terminals is necessary to improve pain treatment originating from the uterine cervix. Previously we reported an inhibition of primary afferent and electromyographic responses to acute UCD by three new, highly selective, and peripherally restricted  $\kappa$ -opioid receptor (KOR) agonists,<sup>2</sup> similar to what has been observed in colorectal distension in male rats.<sup>3,4</sup> Estrogen exposure significantly reduced the inhibitory effect of the  $\mu$ -opioid receptor (MOR) agonist, morphine, but not of (-)U50,488, a KOR agonist,<sup>5</sup> suggesting unique pharmacology in this structure in the female reproductive tract compared with the male colon or bladder. The aim of the current study was to further investigate the pharmacology of KOR and MOR agonists, with two primary goals. First, we tested whether MOR inhibition of UCD reflects a central site of action, as it does for colorectal distension.<sup>6</sup> Second, it has been suggested that peripheral  $\kappa$ -opioid receptors responsible for inhibition of colorectal distension are not reversed by norbinaltorphimine (nor-BNI) and DIPPA, two KOR-selective antagonists, and represent a novel, nonclassical KOR.<sup>7</sup> If this were also the case for UCD, development of a peripherally restricted KOR agonist for this novel receptor subtype could represent a new approach to the treatment of labor pain. We therefore examined the ability of nor-BNI to reverse KOR agonist induced inhibition of response to UCD.

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## Materials and Methods

### Animals

The surgical preparations and experimental protocols were approved by the Institutional Animal Care and Use Committee and conformed to the NIH guidelines on the ethical use of animals. Sixty adult female Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 210–305 g at the time of the experiments were studied. Animals were

housed two per cage at 22°C and under a 12 h–12 h light–dark cycle, with free access to food and tap water.

#### *Ovariectomy and Uterine Cervical Distension*

Because estrogen alters responses to opioids in UCD,<sup>5</sup> we controlled estrogen exposure by ovariectomy. Animals were anesthetized with halothane (2% to 3% in 100% O<sub>2</sub>), and ovariectomy performed *via* two small flank incisions. One week later, uterine cervical distension was performed as described previously.<sup>2</sup> Animals were anesthetized with halothane, the carotid artery was cannulated for monitoring of arterial blood pressure and heart rate, and the jugular vein was cannulated for fluid and drug administration. A tracheotomy was performed for mechanical ventilation. Next, halothane was reduced to 0.4–0.8%, a level which allowed detection of electromyographic reflex responses of rectus abdominis muscles to UCD, but prevented purposeful escape behavior. Animals were not restrained in any way, and no neuromuscular blockers were applied. Rectal temperature was monitored continuously and maintained at 37–39°C using a circulating water heating pad and heat lamp. A small midline laparotomy was performed, and fine metal rods were inserted through both uterine cervical ossi *via* a small incision in the uterus. 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 right inguinal region of the rectus abdominis and electromyographic activity was monitored using a window discriminator and spike counter. Average frequencies of electromyographic activity in the first 4 s of a 5 s 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 to avoid tissue injury. For data analysis, the baseline frequency in the absence of stimulation was subtracted from frequencies observed with distension. To quantify cardiovascular response to UCD, mean arterial pressure and heart rate were averaged over the last 3 s before and at the end of the stimulus.

#### *Drug Treatment*

For each individual animal, the UCD force producing approximately 75% maximum response was determined and this distension force was used to examine the effects of opioid agonists and antagonists on UCD. 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 relationship obtained in each animal and approximated in 12.5 g steps. Dose ranges and timing of injection for all used opioid agonists and antagonists were determined in pilot experiments. Drugs were administered intravenously, except nor-BNI, which was

applied subcutaneously. Six animals were tested per group.

The KOR agonist (-)U50,488 (0.01–3 mg/kg) was administered at 5 min intervals in a cumulative manner using half-log increments. The (-) enantiomer was used, because, unlike the (+) enantiomer, it has no MOR activity. To determine the effect of nor-BNI on KOR agonist inhibition to UCD, nor-BNI was administered twice as a 20 mg/kg dose subcutaneously (SC) 48 and 24 h before (-)U50,488 testing.<sup>8</sup> At the end of the cumulative (-)U50,488 dosing experiments, naloxone, 1 mg/kg, was administered intravenously.

The MOR agonist, morphine (0.01–1 mg/kg) was applied in a cumulative manner in half-log increments at 5 min intervals, and the dose which produced 75% maximum inhibition (ID<sub>75</sub>) was determined by linear regression. To test the effects of opioid antagonists, a dose of morphine just above the ID<sub>75</sub> (0.3 mg/kg) was administered, followed either by naltrexone or methyl-naltrexone (0.01–1 mg/kg each), administered in a cumulative fashion in half-log increments at 5 min intervals. As a control, saline was injected at 5 min intervals in 0.3 ml amounts after the 0.3 mg/kg morphine bolus. At the end of the methyl-naltrexone and saline experiments, naloxone, 1 mg/kg, was administered. Finally, all animals were euthanized with an intravenous overdose of pentobarbital.

#### *Drugs*

Drugs used and their sources were halothane (Halocarbon Laboratories, River Edge, NJ); pentobarbital (Nembutal; Abbott Laboratories, North Chicago, IL); morphine sulfate (Astra Pharmaceutical Products, Inc., Westborough, MA), (-) (1S, 2S) U50,488, nor-BNI, and naltrexone (Sigma Chemical Co., St. Louis, MO). Methyl-naltrexone was a generous gift from John F. Foss, M.D., and Chun-Su Yuan, M.D., from the Department of Anesthesiology, University of Chicago, Chicago, IL.

Morphine and methyl-naltrexone were diluted in saline 0.9%. (-)U50,488, nor-BNI, and naltrexone were initially diluted in distilled water, then further to the final concentration with normal saline.

#### *Statistical Analysis*

Comparisons of data were performed by repeated measures of analysis of variance (ANOVA) followed by the Dunnett test. Electromyographic signals in the stimulus-response studies required log transformation for normalization. Electromyographic and cardiovascular data from the morphine antagonism studies were analyzed by a mixed effects repeated measures ANOVA. The inhibition induced by the morphine bolus was considered as baseline and included as a baseline covariate in the statistical model. Corrections were made for multiple comparisons using the Fisher protected LSD method with Bonferroni corrections, when appropriate. Effects of UCD on mean arterial blood pressure and heart rate before and during

UCD were compared by the Mann-Whitney rank sum test on the entire stimulus-response data (3 s before *vs.* 3 s at the end of the UCD stimulus). These cardiovascular data are presented as median and 25<sup>th</sup>-75<sup>th</sup> percentile. All other data are presented as mean  $\pm$  SE. ID<sub>50</sub> was calculated by log linear regression analysis of the entire electromyographic data set. The level of statistical significance was  $P < 0.05$ .

## Results

All animals recovered uneventfully from ovariectomy, and there was a clear shrinkage in the size of uterine horns and the cervix 1 week later, consistent with a reduction in estrogen exposure. Throughout the pharmacologic experiments, a concentration of  $0.6 \pm 0.02$  Vol.% halothane (mean  $\pm$  SEM; range 0.5–0.8 Vol.%) was used. The 75% maximum force was  $83 \pm 1.1$  g (mean  $\pm$  SEM; range 67.5–87.5 g; mode 87.5 g). The baseline frequency in the absence of stimulation was  $0.6 \pm 0.2$  Hz (mean  $\pm$  SEM; range: 0–5.5 Hz). UCD resulted in a stimulus-dependent increase in electromyographic activity in the rectus abdominis muscle with a threshold of 25 g. UCD also evoked a small increase in mean arterial pressure (pre-UCD: 147 [136.5/157] *vs.* UCD: 150 [143/165] mmHg; median [25<sup>th</sup>/75<sup>th</sup> percentile];  $P < 0.01$ ), but not in heart rate (pre-UCD: 390 [360/403] *vs.* UCD: 385 [350/400] beats/min; median [25<sup>th</sup>/75<sup>th</sup> percentile];  $P = 0.7$ ).

Intravenous (-)U50,488 reduced electromyographic response to UCD in a dose-dependent manner, with an ID<sub>50</sub> of 0.052 mg/kg (95% CI: 0.0041, 0.057 mg/kg). Nor-BNI pretreatment failed to prevent inhibition from (-)U50,488 on the electromyographic response, but naloxone did reverse it ( $P = 0.99$ ; Fig. 1). (-)U50,488 after nor-BNI pretreatment decreased pre-UCD mean arterial blood pressure ( $P = 0.02$ ), but not heart rate. (-)U50,488 without nor-BNI pretreatment decreased pre-UCD heart rate, but not blood pressure (table 1). No UCD-evoked blood pressure or heart rate changes were observed in both (-)U50,488 groups ( $P > 0.05$ ; data not shown).

Intravenous morphine produced a dose-dependent reduction in electromyographic response to UCD, with an ID<sub>50</sub> of 0.030 mg/kg (95% CI: 0.007, 0.040 mg/kg; Fig. 2). Similarly, mean arterial pressure and heart rate were decreased dose-dependently (0.1–1 mg/kg;  $P < 0.05$  and 0.03–1 mg/kg;  $P < 0.05$ , respectively). The UCD evoked pressor response ( $11 \pm 4$  mmHg before morphine administration; mean  $\pm$  SEM) changed dose-dependently to a depressor response, with the highest blood pressure decrease at 0.3 mg/kg morphine ( $-10 \pm 4$  mmHg; mean  $\pm$  SEM;  $P < 0.05$ ). The ID<sub>75</sub> for morphine was 0.188 mg/kg (95% CI: 0.117, 2.4980).

Naltrexone antagonized morphine's inhibition of the electromyographic response to UCD with a threshold for

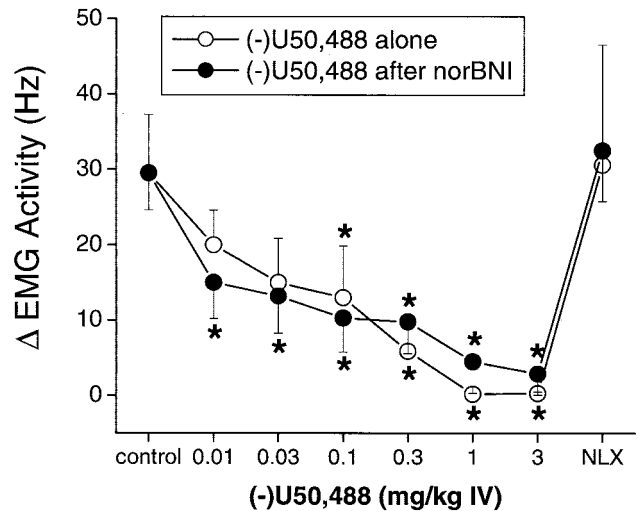


Fig. 1. Change in electromyographic (EMG) activity induced by uterine cervical distension in ovariectomized rats receiving cumulative doses of intravenous (-)U50,488 with (closed circles) or without (open circles) norbinaltorphimine (norBNI) pretreatment. NorBNI, 20 mg/kg, was applied intraperitoneally 48 and 24 h before testing. Each symbol represents the mean  $\pm$  SEM of 6 rats. \* $P < 0.05$  versus control. No differences between groups.

antagonism at 0.03 mg/kg ( $P < 0.05$ ). In contrast, neither saline nor methylnaltrexone reversed morphine-induced inhibition of the electromyographic response to UCD, with no difference between saline and methylnaltrexone. Naltrexone differed from methylnaltrexone and saline with doses starting at 0.03 mg/kg ( $P < 0.05$ ; Fig. 3). Pre-UCD mean arterial pressure and heart rate recovered from the morphine-induced decrease with the first bolus of naltrexone-methylnaltrexone (0.01 mg/kg;  $P < 0.05$ ) similar to saline control except for heart rate, where the time until the second saline bolus was required to recover ( $P < 0.05$ ; table 1). Morphine induced a depressor response during UCD in the saline control group ( $-9 \pm 4$  mmHg; mean  $\pm$  SEM), showing a return to pre-UCD values with the first saline bolus ( $-1 \pm 3$  mmHg; mean  $\pm$  SEM;  $P < 0.05$ ). Although naltrexone or methylnaltrexone group showed no UCD evoked change in blood pressure, no differences between both opioid antagonists and saline were observed ( $P = 0.07$ ). Similar to (-)U50,488, heart rate remained unaffected by the UCD stimulus in the morphine antagonism studies.

## Discussion

The current experiments, using a new model of acute visceral nociception in female rats, support the hypothesis that classic KOR selective antagonists cannot reverse KOR agonist induced antinociception to noxious stimuli to the reproductive tract, providing additional evidence that KOR act on a new peripheral KOR. Our data further support the hypothesis that morphine acts spinally or supraspinally, not peripherally, in inhibition of visceral

**Table 1. Effects of the Different Opioid Agonists and Antagonists on Pre-Uterine Cervical Distension Blood Pressure and Heart Rate**

		Concentrations (mg/kg intravenously)							
		0	Morphine	0.01	0.03	0.1	0.3	1	3
MAP (mmHg)	(-)U50,488	153 ± 7		150 ± 8	155 ± 8	150 ± 6	144 ± 6	147 ± 6	144 ± 5
	Norbinaltorphimine	140 ± 6		142 ± 6	147 ± 7	146 ± 5	147 ± 4	136 ± 5	131 ± 4†
	Naltrexone	155 ± 3*	134 ± 5	145 ± 3*	156 ± 4*	154 ± 4*	151 ± 5*	153 ± 6*	
	Methylnaltrexone	150 ± 6*	129 ± 6	137 ± 3*	141 ± 4*	141 ± 4*	144 ± 5*	143 ± 6*	
	Saline	134 ± 9	122 ± 5	132 ± 3	140 ± 6*	138 ± 7*	139 ± 7*	133 ± 11	
HR (beats/min)	(-)U50,488‡	399 ± 10		404 ± 9	412 ± 7	409 ± 8	407 ± 8	403 ± 10	380 ± 11
	Norbinaltorphimine	383 ± 9		375 ± 15	381 ± 18	388 ± 15	392 ± 11	364 ± 22	365 ± 14
	Naltrexone	377 ± 14*	289 ± 20	352 ± 9*	382 ± 11*	390 ± 13*	385 ± 14*	384 ± 11*	
	Methylnaltrexone	359 ± 13*	287 ± 15	334 ± 10*	365 ± 14*	381 ± 14*	391 ± 14*	404 ± 9*	
	Saline	389 ± 17*	288 ± 8	353 ± 12*	380 ± 16*	406 ± 18*	414 ± 18*	407 ± 23*	

For (-)U50,488 and norbinaltorphimine studies: data represent mean ± SEM, n = 6 per group. For morphine antagonism studies: "morphine" represents data from the 0.3 mg/kg intravenous bolus prior to application of the opioid antagonist or saline; data represent mean ± SEM, n = 6 per group.

\* P < 0.05 versus morphine, 0.3 mg/kg intravenously. † P < 0.05 versus control. ‡ P < 0.05 within group.

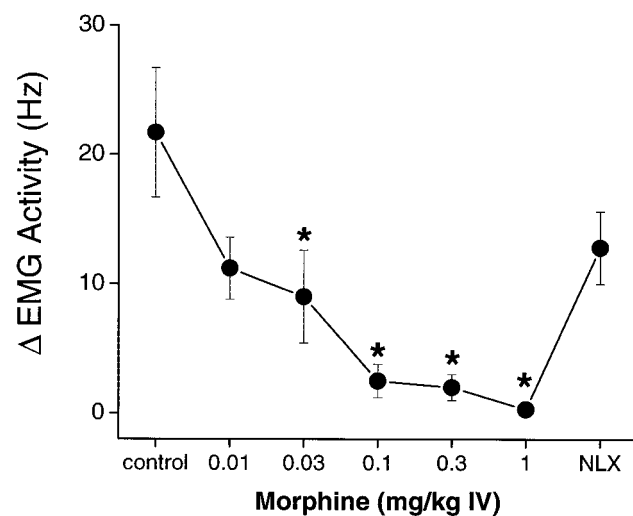
MAP = mean arterial blood pressure; HR = heart rate.

noxious stimuli. These results suggest that observations in colorectal or urinary bladder distension in male rats<sup>3,8,9</sup> likely also apply to the uterine cervix.

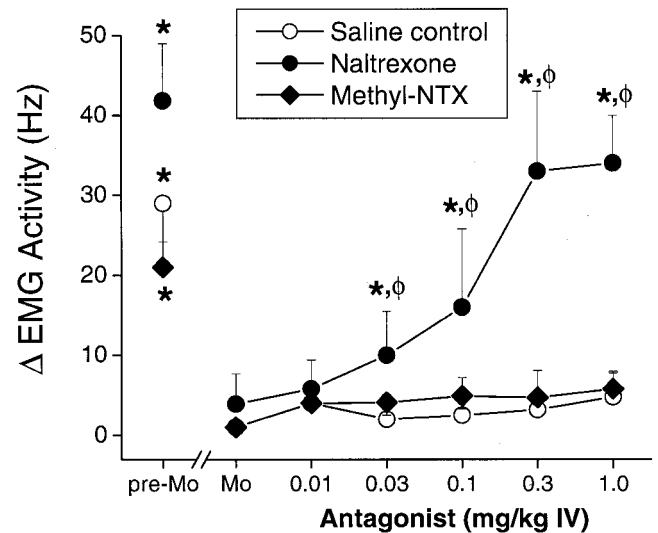
There is increasing interest in the study of visceral nociception, with clear distinctions being made to somatic and neuropathic.<sup>10</sup> Previous studies of the female reproductive tract focus on underlying anatomy<sup>11</sup> or inflammatory changes and sensitization.<sup>12</sup> Acute stimuli previously examined were either distension of the uterine horn,<sup>13</sup> or vagino-cervical probing,<sup>14</sup> which induces immobility and antinociception related to mating behavior in rats. Whether uterine cavity distension is nociceptive in rats is uncertain, and the role of uterine afferents in human pain is not clear.<sup>15</sup> Uterine, but not cervical, afferent and efferent terminals degenerate during preg-

nancy, possibly as protection against local vasoconstriction or myometrial stimulation.<sup>16</sup>

μ-opioid receptor agonists including morphine DAMGO inhibit visceromotor as well as pressor responses to colorectal distension after intravenous or intrathecal application, reversible by naloxone.<sup>17,18</sup> In contrast, morphine did not inhibit responses to colorectal or gastric distension from afferents isolated from the central nervous system.<sup>8,19</sup> These electrophysiologic data suggest a central site of action for MOR agonists to inhibit colorectal nociception in male rats. Our pharma-



**Fig. 2.** Change in electromyographic (EMG) activity induced by uterine cervical distension in ovariectomized rats after inhibition by cumulative doses of morphine. At the end of the experiment, morphine-induced response inhibition was reversed by naloxone (NLX), 1 mg/kg intravenously. Each symbol represents the mean ± SEM of 6 rats. \*P < 0.05 versus control.



**Fig. 3.** Change in electromyographic (EMG) activity induced by uterine cervical distension in ovariectomized rats after inhibition by one morphine (Mo) dose, 0.3 mg/kg intravenous (= Mo). Pre-Mo represents the EMG response to UCD before the morphine application. Reversal of morphine inhibition was tested by applying Naltrexone or methylnaltrexone (methyl-NTX) in a cumulative manner. As a control, intravenous saline was applied cumulatively. Each symbol represents the mean ± SEM of 6 rats. \*P < 0.05 versus Mo. φ P < 0.05 versus saline or methylnaltrexone.

colgic data suggest MOR agonists also act centrally to inhibit uterine cervical nociception. Naltrexone is a non-selective opioid antagonist preferring the MOR, and reaches central sites after systemic administration. Its quarternary derivative methylnaltrexone is impermeable to the blood-brain barrier at doses less than 10 mg/kg, and selectively antagonizes opioid receptor activation in the periphery in this dose range.<sup>20,21</sup> Near complete reversal of morphine-induced inhibition of the electromyographic response to UCD with naltrexone, intravenous 0.3 mg/kg, but lack of effect of over three times this dose with methylnaltrexone supports an exclusive central site of morphine action.

Several studies suggest that a novel  $\kappa$ -like opioid receptor, distinct from the cloned KOR, may be expressed on colorectal afferents. First, differential effects of KOR agonists in behavioral, neurophysiological, and binding studies support the existence of three KORs:  $\kappa_1$ ,  $\kappa_2$ , and  $\kappa_3$ . Benzacetamide KOR agonists like U50,488 or U62,066 prefer binding to  $\kappa_1$ , whereas benzomorphan derivatives like bremazocine prefer  $\kappa_2$  as their binding site. Intravenous U50,488 inhibits response to colorectal or duodenal distension.<sup>22,23</sup> Similarly, we report in the current study a dose-dependent inhibition of intravenous (-)U50,488 of UCD-induced nociception. Second, two KOR-selective antagonists including nor-BNI fail to reverse KOR agonist induced antinociception in several models of visceral pain.<sup>3,4,8,19,24</sup> Although nor-BNI can have a long latency to effect,<sup>25</sup> we used a previously validated 48 h treatment scheme to assure effect.<sup>8</sup> This chronic treatment failed to attenuate the effect of (-)U50,488 in UCD, as in male rats with colorectal distension. (-)U50,488 acted on an opioid receptor, as indicated by complete reversal by naloxone. Third, the structure activity relationship for KOR agonists to inhibit responses to colorectal, gastric, or urinary bladder distension is completely different than that for somatic nociception.<sup>6</sup> (-)U50,488 was more potent ( $ID_{50}$  of 52  $\mu\text{g}/\text{kg}$ ) than in these reports, suggesting that UCD may be more sensitive to KOR agonist inhibition than the male gastrointestinal tract, or that the type of receptor differs in uterine cervical afferents from gastrointestinal afferents. Last, intrathecally applied antisense deoxyribonucleotide against the cloned KOR reverses KOR agonist-induced antinociception to peripherally administered formalin, but not to colorectal distension.<sup>7</sup> The peripheral site of KORs in reducing response to UCD was previously indicated by activity of these agents in recordings from single unit afferents, in which all effects are peripheral, and in activity of KOR agonists with poor penetration into the central nervous system.<sup>2</sup>

Blood pressure and heart rate may increase or decrease in response to painful stimuli, depending on the length, depth, and type of stimulation as well as the anesthetic used.<sup>17,26</sup> UCD evoked an increase in mean arterial blood pressure, but not in heart rate, as observed previously.<sup>2</sup>

This UCD evoked pressor response changed to a depressor response after morphine administration, possibly due to interactions with anesthesia.

This is the first report providing information about reversal of opioid inhibition to noxious stimulation in the female reproductive tract. Reflex electromyographic responses to UCD are inhibited by morphine and (-)U50,488. Most of the pharmacology observed (lack of morphine reversal by a peripherally restricted antagonist and lack of efficacy of a traditional KOR antagonist to inhibit (-)U50,488) are similar to that observed in studies of male rats with gastrointestinal tract distension. The exquisite sensitivity of UCD to inhibition by (-)U50,488 compared with previous studies of males suggests, however, that differences may exist between these visceral structures, and that development of selective KOR agonists may be useful in the treatment of acute and chronic pain originating from the uterine cervix.

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