Role of Capsaicin in a Murine Model of Labor and Delivery

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ABSTRACT

Background: The objectives of this study were to develop a murine model of labor and delivery and to use this model to examine whether capsaicin diminishes labor pain and expedites delivery.

Methods: To develop a murine model of labor pain, the authors identified and compared the incidence of four proposed pain behaviors in 46 mice: (1) No analgesia in labor and the postpartum period, and (2) increasing doses of an analgesic, morphine. The model was then used to examine the impact of topical cervical capsaicin on: (1) labor pain behaviors and (2) labor progress by examining its impact on the time from treatment to delivery of the first pup and on the duration of delivery per pup. The treatment was randomly allocated and the behavioral observation was blinded.

Results: In the absence of analgesia, there was a statistically significant decrease in all four proposed pain behaviors in the postpartum period compared with labor (cumulative 55.0 ± 16.1/h vs. 16.1 ± 8.7/h; P < 0.0001). Additionally, morphine reduced their incidence during labor in a dose-dependent manner (cumulative 55.0 ± 16.1/h control, 46.4 ± 15.8 morphine 0.1 mg/kg/h, 34.6 ± 5.6/h, morphine 0.5 mg/kg/h; P = 0.1988, 0.0014). In addition, the incidence of identified pain behaviors was reduced by pericervical capsaicin (cumulative 55.0 ± 16.1/h control, 38.9 ± 15.4 capsaicin, P = 0.02).

Conclusions: In this pilot study, the authors developed a novel mouse model of labor and delivery. Pericervical capsaicin applied days before delivery reduces labor pain behaviors.

ABOR is associated with significant pain in most women.1–3 Currently available labor pain treatments are highly resource intensive, and they are not uniformly available or without side effects. A new, inexpensive alternative method for labor induction that also noninvasively reduces labor pain would be desirable.

Sensory nerves play a major role in most visceral organs and this is attributed to the release of neuropeptides from the stimulated nerve terminals.4–7 In addition to the nociceptive functions of these nerves, it has been proposed that enhancement of cervical ripening occurs due to the release of neuropeptides that, in turn, orchestrate a local inflammatory response.8 This regulatory function is a characteristic trait ofafferent neurons that express the TRPV1 (transient receptor potential cation channel subfamily V member 1) receptor and that are sensitive to capsaicin (8-methyl-N-vannillyl-6-nonenamide). Pregnancy is associated with an ingrowth and enhanced sensitivity of c-fibers into the uterine cervix.9–11 Prolonged desensitization of TRPV1 receptors after cervical application of capsaicin may reduce pain transmission in labor. Another proposed role for TRPV1 and capsaicin in parturition relates to cervical ripening where capsaicin facilitates the release of neuropeptides important in cervical ripening. Thus, we hypothesized that the topical use of capsaicin will enhance labor induction and reduce labor pain.

Animal models for labor pain, which would allow the testing of capsaicin and other agents that have lagged. Recently,
Tong et al. identified phasic stretching behaviors in the rat, which were validated as labor pain behaviors. These behaviors were associated with the onset of labor, were greatly diminished after delivery, and were reduced in a specific and dose-dependent manner by morphine. In our study, we developed an analogous model in mice, which is valuable for genetic and proteomic studies. We have extended the Tong model to mice and have examined the impact of capsaicin on labor pain and progress.

**Materials and Methods**

**Animals and Surgical Procedures**

The study protocol was approved by the Institutional Animal Care and Use Committee at Columbia University Medical Center. A total of 46 time-mated C57/B6 mice (Jackson Laboratories, Bar Harbor, ME) were included in this study. The study animals were housed individually with a 12:12 h light–dark cycle and with free access to food and water. On gestational day 15, animals were randomly assigned to one of four groups: (1) saline-lidocaine (S-L) a control group where the animal received systemic saline via a microosmotic pump and lidocaine 4% (100 µl, Ferndale Laboratories, Ferndale, MI) intravaginally, (2) M1-L, where the animal received systemic morphine (0.1 mg/kg/h) via pump and lidocaine intravaginally, (3) M5-L, where the animal received systemic morphine (0.5 mg/kg/h) via pump and lidocaine intravaginally, and (4) saline-capsaicin (S-C), where the animal received systemic saline via pump and lidocaine followed by capsaicin 0.05% (100 µl, Chattem, Inc., Chattanooga, TN) intravaginally.

For surgical preparation, animals were anesthetized using isoflurane (2–5%) in oxygen with spontaneous ventilation. Adequate anesthesia was assured by paw pinch before any surgical intervention. A microosmotic pump (Alzet, Durect Corp., Cupertino, CA), prefilled with morphine or saline, was inserted into a pocket beneath the skin behind the animal’s neck (subcutaneously) and was sutured in place. This pump was used to systemically administer either saline or morphine. One half of a milliliter of 0.25% of bupivacaine was injected around the incision for additional reduction of discomfort. A fire-polished pipette was subsequently inserted into the mouse’s vagina to administer lidocaine followed, in some cases, by capsaicin, if indicated by group assignment. All animals emerged from the surgical procedure rapidly without evidence of pain behaviors including vocalization, freezing, abnormal gait, or rubbing the surgical site. The surgical technician created a code to identify animals and was not involved in behavioral scoring. The investigator involved in behavioral scoring (A.F.) was blinded to the treatment group.

Systemic saline was used as a placebo for the systemic infusion of morphine dissolved in isotonic saline. Intravaginal lidocaine, in turn, was used as a control for vaginal manipulation in animals as well as to limit any acute discomfort that might still be present secondary to capsaicin treatment (in the S-C group) when the animals emerged from general anesthesia. Of note, topical lidocaine would be expected to last for minutes to hours although these animals delivered approximately 3 days after application of the study drug. As such, lidocaine was used as a control in all study animals.

**Behavioral and Labor Progress Assessment**

For the purpose of behavioral observation, each animal was subsequently placed in an individual chamber with clear plastic walls in an isolated study room. Each of the dams was videotaped with a continuous loop digital video camera for 72 h, which included delivery of all pups. The placement of a mirror within the chamber facing the camera allowed for optimal monitoring of the animals. At the end of the experiment, video recording was stopped. All videotaped births were reviewed by a single observer (A.F.), who was blinded to treatment group assignment.

Proposed phasic stretching behaviors that were recorded included squashing, lateral contraction, elongation, and arching. These were selected in part based on the description of Tong et al. with the addition of a fourth behavior (arching), which was identified by our team in preliminary work. These four behaviors were quantified for each mouse in all study groups during a period that started 2 h before the time of delivery of the first pup and lasted until 30 min after the delivery of the last pup. The incidence of each of the four behaviors was determined for each mouse.

Each behavior incidence was compared between labor and postpartum periods in mice with saline infusions, in the absence of an analgesic, as well as with increasing morphine doses to demonstrate that these phasic stretching behaviors were pain related. The incidence of each of the four behaviors was examined in the capsaicin-treated animals to determine whether the intravaginal application of capsaicin had any analgesic efficacy. In turn, the potential effect of capsaicin on murine labor progress was examined using two approaches. The time from treatment to delivery of the first pup and the duration of delivery per pup were determined for each dam. The means of these variables were compared between the saline only (S-L) and the capsaicin (S-C) groups.

**Offspring Assessment**

The number of identified pups as well as their viability and birth weights was recorded for each mouse. The weight of the pups at 3 weeks of age was also determined. It has been reported that perinatal injection of high-dose capsaicin has been associated with corneal ulcers and an abnormal response to heat stimuli. To investigate potential adverse effects to the pups of maternal treatment with capsaicin, hind paw withdrawal latency to a heat stimulus was assessed in pups born to control dams (S-L) and pups born to dams treated with capsaicin (S-C), as previously described. In addition, microscopic examination of the eyes of these pups...
was pursued and the results were again compared between the two groups.

**Statistical Analysis**
The data are displayed as mean ± SD. The effect of morphine on phasic stretching behaviors was assessed with ANOVA for repeated measures with morphine dose as the factor and Tukey–Kramer Multiple Comparison’s test using InStat3 (Graphpad Software, La Jolla, CA). The effect of capsaicin is tested with a one-way ANOVA. All hypothesis testing was two-tailed. The effect of capsaicin was analyzed with a two-tailed t test. A P value of less than 0.05 was considered statistically significant.

**Results**
The four groups studied are described in a flowchart along with depictions of the four putative pain behaviors (fig. 1). No mice were lost to follow-up. The incidence of each of the four phasic stretching behaviors was compared between the labor and postpartum periods in mice saline infusion. As shown in figure 2, all four phasic stretching behaviors were greatly reduced after birth. Furthermore, morphine reduced each phasic stretching behavior in a dose-dependent manner. The cumulative incidence of all four behaviors was also diminished in a dose-dependent manner with morphine, as illustrated by figure 3. In order to be clear that morphine did not reduce pain behavior due to generalized sedation, other behaviors not thought to be pain related, such as eating, rearing, nest building, and grooming were scored and found not to be reduced by morphine treatment in labor (data not shown). Figure 3 also demonstrates the impact of capsaicin on the incidence of each of the four phasic stretching behaviors, in relation to saline and the different doses of morphine. Cervical application of capsaicin, 3 days before delivery reduced the cumulative incidence of the proposed labor pain behaviors. Additionally, the median time from treatment to delivery and the duration of delivery per pup were both diminished with the use of capsaicin (fig. 4).

There was no difference in weight gain between pups exposed to capsaicin and those that were not; the mean weight at 3 weeks was 8.48 grams ± 0.9 in the capsaicin-exposed pups (n = 69) and 8.44 grams ± 1.2 in the unexposed pups (n = 48), with P > 0.05. There was also no difference in heat pain sensitivity between pups exposed to capsaicin compared with those that were not; hind paw withdrawal latency to a heat stimulus was 8.00 s ± 1.94 and 8.03 s ± 1.56 in the capsaicin-exposed and unexposed pups, respectively at 3 weeks of age. Furthermore, no corneal lesions were identified in the pups exposed to capsaicin during birth with slit lamp examination.

**Discussion**
Induction of labor is one of the most commonly performed obstetrical procedures, and, in fact, rates of labor induction in the United States have increased over the past two decades.1,2
Despite the frequency of labor induction, the timing of delivery and the treatment of its associated pain cannot always be ideally managed with the currently available techniques. The number of existing agents for labor induction remains limited, and the process itself can be associated with a number of adverse pregnancy outcomes. Moreover, labor, particularly when induced, is associated with substantial pain in most women. As such, it is estimated that nearly 85% of women in the United States choose to have pain reduction during labor. Yet, currently available labor pain treatments are highly resource intensive and are not without side effects. Of further concern, access to labor pain treatments represents an important healthcare disparity not only internationally but also in many parts of the United States. Hence, women living in medically underserved communities remain at risk for undertreatment of labor pain. A new and inexpensive alternative method for labor induction, which also reduces labor pain would be desirable.

The ethical and regulatory requirements for studying new diagnostic and treatment modalities in humans are conservative in pregnancy, hence the need for animal models. Unfortunately, animal models for labor pain, which would allow the testing of capsaicin and other agents, have lagged behind those for the study of other types of pain such as postoperative, neuropathic, and cancer pain. This is in part because of the disagreement on whether labor and delivery is painful in smaller mammals, the most common subjects of animal research. However, pioneering work in rats by Eisenach et al. has clearly established that mechanical cervical dilation and labor activates the same neural pain pathways and that pain is manifested by reflex phasic muscular contractions. In fact, Tong et al. recently identified well-characterized labor pain behaviors in rats. The current study allowed us to develop an analogous murine model of labor pain. For this purpose, we characterized and examined four phasic stretching behaviors, namely squashing, elongation, arching, and lateral contraction. We argue that these specific behaviors represent labor pain because they did not occur

**Fig. 3.** Phasic stretching behaviors were reduced with increasing concentrations of morphine. The incidence of all four phasic stretching behaviors diminished with increasing concentration of morphine. With systemic morphine 0.5 mg/kg/h, squashing was reduced ($P < 0.01$), elongation was reduced ($P < 0.05$), arching was reduced ($P < 0.05$), and lateral contraction was reduced ($P < 0.001$). Capsaicin treatment reduces the incidence of phasic stretching behaviors with respect to control. The incidence of elongations are reduced ($P < 0.01$) and the incidence of arching behaviors are reduced ($P < 0.01$).
before visible abdominal contractions began and we showed that, in the absence of analgesia, they were much more frequent in labor compared with the postpartum period. To further support this observation, we demonstrated that each of the four behaviors was reduced with morphine, a known analgesic, in a dose-dependent manner.

After model development, attention was turned to investigation of a potential role for capsaicin, an agent that activates and then desensitizes the TrPV1 receptor that is highly expressed in the uterus and cervix in pregnancy. The mechanisms that instigate parturition in humans have been remarkably elusive. Sensory nerves play a major role in most visceral organs and this has been attributed to the effects of neuropeptides released from stimulated nerve terminals. This local regulatory function is a characteristic trait of primary afferent neurons, which express the TrPV1 receptor. These neurons are specifically sensitive to activation and desensitization by capsaicin, a natural TRPV1 ligand found in all mammals except for the naked mole rat. Although the analgesic actions of capsaicin through desensitization of the TRPV1 receptors are well recognized in peripheral nociception, little is known about their role in parturition. Capsaicin-sensitive afferent nerve fibers expressing TRPV1 receptors have been shown to be abundant in the uterus and cervix of several species, including humans and rodents. During pregnancy and labor, almost complete disappearance of TRPV1 positive nerve fibers in the corpus has been demonstrated, although expression within the cervix appears to be upregulated.

Activation of the TRPV1 receptor by capsaicin results in an influx of Ca2+ and Na ions, membrane depolarization, and release of neuropeptides causing a transient burning sensation. This initial phase is followed by dose-dependent, prolonged desensitization. Once the TRPV1 receptor is desensitized, transmission via C-type primary afferent receptors, the predominant nociceptors in the uterine cervix at term, is reduced, leading to prolonged pain relief that is dose dependent. Hence, capsaicin acts as an irritant in the uterine cervix, causing a burning sensation upon initial contact; this phase, however, is transient and is followed by reduced pain transmission for a prolonged period. It is postulated that in addition to its analgesic effects, activation of the TRPV1 receptor by capsaicin may lead to enhancement of cervical ripening through the release of neuropeptides that orchestrate a series of local inflammatory responses. As such, capsaicin may play a dual role in facilitating parturition and in providing prolonged analgesia.

Capsaicin, an active extract of the chili pepper, has a wide array of medical and nonmedical applications. In the current study, we demonstrated that a single intravaginal application of capsaicin, 3 days before parturition is associated with a statistically significant reduction in labor pain behaviors in mice. The optimal time of application or dose of capsaicin for this indication remains unknown and will be determined in a larger dose-finding, timing trial in the future. Although proper toxicology would be required before trial in humans with a viable pregnancy, there was no evidence of failure to thrive, changes in pain sensitivity, or corneal damage in any of the pups delivered during the course of this study.

In conclusion, we developed a novel mouse model that can be used to examine the effects of different treatment modalities on labor progress and pain. Using this model, we demonstrated that capsaicin, which possibly has ripening properties, reduces labor pain behaviors. The combination of analgesic and ripening properties of capsaicin

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Fig. 4. Effect of capsaicin on: (A) Time from treatment to delivery and (B) Duration of delivery per pup. Capsaicin-exposed dams had a lower time from treatment to delivery (hours) and duration of delivery per pup (minutes) compared to controls with only saline infusion.
is appealing to obstetric healthcare providers. Capsaicin may emerge as a new, minimally invasive, natural adjunct to medical induction of labor, one of the most commonly performed obstetrical procedures in the United States. In addition, there is a potential global role for capsaicin, particularly in regions with limited access to analgesia and labor-induction agents.

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References