Estrous cycle variation of TRPV1-mediated cross-organ sensitization between uterus and NMDA-dependent pelvic-urethra reflex activity

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1Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University; 2Department of Physiology, College of Medicine, and 3Department of Obstetrics and Gynecology, Chung-Shan Medical University Hospital, Chung-Shan Medical University, Taichung; 4Department of Obstetrics and Gynecology, Chung-Gung Memorial Hospital, Taoyuan; 5School of Physical Therapy, College of Medicine, China Medical University; 6Division of Urology, Department of Surgery, Taichung Veterans General Hospital, Taichung; 7Department of Medicine, Saint Paul’s Hospital, Taoyuan; and 8Graduate Institute of Biomedical Electronics and Bioinformatics, National Taiwan University, Taipei, Taiwan

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Peng H-Y, Huang P-C, Liao J-M, Tung K-C, Lee S-D, Cheng C-L, Shyu J-C, Lai C-Y, Chen G-D, Lin T-B. Estrous cycle variation of TRPV1-mediated cross-organ sensitization between uterus and NMDA-dependent pelvic-urethra reflex activity. Am J Physiol Endocrinol Metab 295: E559–E568, 2008. First published June 24, 2008; doi:10.1152/ajpendo.90289.2008. —Cross-organ sensitization between the uterus and the lower urinary tract (LUT) underlies the high concurrence of pelvic pain syndrome and LUT dysfunctions, and yet the role of gonadal steroids is still unknown. We tested the hypothesis that cross-organ sensitization on pelvic-urethra reflex activity caused by uterine capsaicin instillation is estrous cycle dependent. When compared with the baseline reflex activity (1.00 ± 0.00 spikes/stimulation), uterine capsaicin instillation significantly increased reflex activity (45.42 ± 9.13 spikes/stimulation, P < 0.01, n = 7) that was corroborated by an increase in phosphorylated NMDA NR2B (P < 0.05, n = 4) but not NR2A subunit (P > 0.05, n = 4) expression. Both intrauterine pretreatment with capsazepine (5.02 ± 2.11 spikes/stimulation, P < 0.01, n = 7) and an intrathecal injection of AP5 (3.21 ± 0.83 spikes/stimulation, P < 0.01, n = 7) abolished the capsaicin-induced cross-organ sensitization and the increment in the phosphorylated NR2B level (P < 0.05, n = 4). The degrees of the cross-organ sensitization increased in a dose-dependent manner with the concentration of instilled capsaicin from 100 to 300 μM in both the proestrus and metestrus stages, whereas they weakened when the concentrations were higher than 1,000 μM. Moreover, the cross-organ sensitization caused by the uterine capsaicin instillation increased significantly in the rats during the proestrus stage when compared with the metestrus stage (P < 0.01, n = 7). These results suggest that estrogen levels might modulate the cross-organ sensitization between the uterus and the urethra and underlie the high concurrence of pelvic pain syndrome and LUT dysfunctions. 

transient receptor potential vanilloid subfamily member 1; N-methyl-D-aspartate; pelvic pain syndrome; central sensitization; spinal reflex potentiation; capsaicin; spinal cord

CENTRAL SENSITIZATION. characterized by a dynamic enhancement of the responsiveness of the capsaicin-sensitive C-fiber to noxious stimulation following injury or inflammation, is a form of reflex plasticity that is presumed to underlie secondary hyperalgesia and tactile allodynia (65, 72). Capsaicin-sensitive afferent fibers arising from the viscera and peripheral tissues express the transient receptor potential vanilloid subfamily member 1 (TRPV1) (8, 9). Although detailed molecular mechanisms have yet to be established, studies investigating TRPV1-dependent nociception or hyperalgesia have demonstrated that the activation of TRPV1 receptors increased the miniature excitatory postsynaptic potentials in a brain slice obtained from the dorsolateral periaqueductal gray neuron through the selective potentiation of glutamatergic N-methyl-D-aspartate (NMDA) neurotransmission (77). When comparing TRPV1 knockout animals with wild-type mice characterized by a postinflammatory hyperalgesia that was reversed by the NMDA antagonist (54), it was found that the TRPV1-dependent modulation on NMDA neurotransmission may underlie the enhancement of pain signaling in the central nervous system and therefore elicits postinjury or postinflammation (12, 21, 45, 62, 63, 74, 75). Recently, phosphorylation of tyrosine residues in the NR2 subunit of NMDA receptors, which defines the properties essential for spinal neural plasticity, has been shown to be an important determinant for the induction of NMDA-dependent postinflammatory hyperalgesia (13, 27).

The uterus is a major source of female pelvic pain (5, 58). Immunohistochemical studies have demonstrated that capsaicin-sensitive primary afferent fibers innervate a rat’s uterine cervix (28, 60, 64), and activation of these fibers caused by injury, infection, or malignancy induces obstetrical/gynecological neuropathic pain and postinflammatory hyperalgesia via central sensitization (6, 29, 40, 51).

Neural-mediated cross-organ interaction in the pelvic cavity, which results from the convergence of sensory pathways within the spinal cord, is necessary for the normal regulation of sexual, bladder, and bowel functions (3, 4). Since the neural substrate for such a cross-organ interaction exists under physiological conditions, alterations in these neural pathways by injury or inflammation may cause the development of overlapping pelvic disorders (43, 52).

The pelvic-urethra reflex, which is the reflexive closure of the urethra caused by bladder distension, is the neural basis for urine continence (15, 16, 17, 22, 35, 36, 48, 49, 50). Neurrogenic hyperactivity and/or dyssynergia are believed to be participants in lower urinary tract dysfunctions, including in-
terstitial cystitis (19, 47). Clinically, lower urinary tract dysfunctions occur almost exclusively in association with pelvic pain syndrome (46, 79). The high concurrence rate of pelvic pain syndrome and lower urinary tract syndrome suggests a possibility of cross-organ interaction between the uterus and the lower urinary tract.

A recent study investigating the pelvic-urethra reflex has demonstrated that the mechanical distension at the ureter induced a cross-organ interaction to modulate pelvic-urethra reflex activity (18). However, so far there has not been an adequate investigation of the hypothesis that an irritation of the uterus may adversely influence the physiological activity of the lower urinary tract. Therefore, the first purpose of this study was to determine whether pharmacological stimulation of the uterus, through the instillation of capsaicin into the uterine horn, might affect the function of the lower urinary tract in a manner of cross-organ interaction.

The level of estrogen dramatically remodels the physiological conditions of the uterus (28, 78). Previous studies demonstrated a large variation in the response of visceral nerve activity to uterine distension across the stages of the estrous cycle (10), with the most dramatic changes occurring between the receptive and the nonreceptive postovulatory stages (6, 10, 55, 56). An interesting point to be explored is whether the activation of the primary afferent fiber arising from the uterus causes a cross-sensitization with the lower urinary tract and whether this cross-organ sensitization changes with exposure to various levels of estrogen. Therefore, the second purpose of this study was to compare and contrast the cross-organ sensitization between the uterus and pelvic-urethra reflex activity at different stages of the estrous cycle.

It is well known that capsaicin activates the nociceptive afferent fibers, causing an acute burning pain, whereas a chronic or high-dose application has been shown to be an analgesic, but such treatments have proven to irreversibly damage the unmyelinated afferent fibers (12, 63). This phenomenon, known as desensitization, has been widely investigated for pain therapeutics. Therefore, we instilled various concentrations of capsaicin into the uterine horn to test first for whether desensitization occurs in the capsaicin-induced cross-organ interaction and second for the possibility of developing pharmacological strategies for pain treatment. Our data confirmed that intrauterine capsaicin instillation induced cross-organ sensitization on the pelvic-urethra reflex activity with a parallel increase in the expression of the phosphorylated NMDA NR2B subunit in the spinal dorsal horn and that the TRPV1 antagonist reversed both. Moreover, treatment with a high dose of capsaicin attenuated the cross-organ sensitization, indicating the possibility of developing a clinical strategy for the treatment of lower urinary tract dysfunction and pelvic pain syndrome.

**MATERIALS AND METHODS**

**Animal preparations.** In this study, we used 73 female Sprague-Dawley rats weighing 200–300 g. They were individually housed in wood chip-lined plastic cages, with free access to water and food, and they were maintained on a 12:12-h light-dark cycle with lights on at 0700. The estrous stage was assessed daily at 9:00 AM by vaginal lavage, using the traditional stage nomenclature (2). Only rats that had two complete regular 4-day estrous cycles before the day of the experiment were used. Measurements were made ~5–8 h after the lights were turned on and when the rats were in either the proestrus (high estradiol and low progesterone levels) or metestrus (low estradiol and high progesterone levels) stage.

Animal care and experimental protocols were done in accordance with the guidelines of the National Science Council of Taiwan, and the experimental protocol was approved by the Committee of Experimental Animal Research at Chung-Shan Medical University.

**Surgical preparations.** Animals were anesthetized with urethane (1.2 g/kg ip). A PE-50 catheter (Portex; Hythe, Kent, UK) was placed in the left femoral vein for administration of anesthetics when needed. A midline abdominal incision was made to expose the pelvic viscera. Both ureters were ligated distally and cut proximally to the sites of ligation. The proximal ends of the ureters drained freely within the abdominal cavity. A bladder cannula was inserted into the lumen of the urinary bladder from a small incision made on the apex of the bladder dome and was secured with cotton thread. The open end of the bladder cannula drained freely throughout the experiment so that reflex activity was not affected by bladder urine distension. Two wide-bore uterine cannulae were inserted into the lumen of the uterine horn through small incisions made on the top and the upper half of the uterine horn and were secured with cotton thread for drug instillation and fluid drainage, respectively (Fig. 1A). The rats were monitored for a corneal reflex and a response to noxious stimulation to the paw throughout the course of the experiment. If responses were present, a supplementary dose of urethane (0.4 g/kg iv) was given through the venous catheter. When the experiments were completed, the animals were euthanized via an intravenous injection of potassium chloride saturation solution.

**Intrathecal catheter.** The occipital crest of the skull was exposed and the atlanto-occipital membrane was incised at the midline with the tip of an 18-gauge needle. A PE-10 catheter was inserted through the slit and passed caudally to the L6-S1 level of the dorsal aspect of the arachnoid space. The volume of fluid within the cannula was kept constant at 10 µl in all experiments. A single 10-µl volume of drug solution was administered, followed by a 10-µl flush of vehicle solution.

**Intraurethral pressure recordings.** To record the intraurethral pressure (IUP) in some experiments, two cotton sutures were placed around the bladder trigone and ligated. A wide-bore intraurethral catheter was inserted into the urethra through the opening of the uterine horn and were secured with cotton thread for drug instillation and fluid drainage, respectively (Fig. 1A). The rats were monitored for a corneal reflex and a response to noxious stimulation to the paw throughout the course of the experiment. If responses were present, a supplementary dose of urethane (0.4 g/kg iv) was given through the venous catheter. When the experiments were completed, the animals were euthanized via an intravenous injection of potassium chloride saturation solution.

**Intraurethral pressure recordings.** Electromyogram electrodes, made of epoxy-coated copper wire (50 µm; M. T. Giken, Tokyo, Japan), were placed intra-abdominally near the external urethra sphincter. The placement of the electrodes was performed using a 30-gauge needle with a hooked electromyogram electrode positioned at the tip (1.0–1.5 mm). The needle was inserted into the sphincter ~1–2 mm lateral to the urethra and then withdrawn, leaving the electromyogram wire embedded in the muscle. The external urethra sphincter electromyogram (EUSE) activities were amplified 20,000-fold by a preamplifier (Grass P511AC, Cleveland, OH) and then displayed continuously on an oscilloscope (Tectronix TDS 3014, Wilsonville, OR) and on a recording system with a sampling rate of 20,000 Hz (MP30). The right pelvic nerve was kept intact, whereas the left pelvic nerve was carefully dissected from the surrounding tissue and transected distally. Then the central stump of the transected nerve was mounted on a pair of stainless steel wire electrodes for stimulation. Single shocks at fixed suprathreshold strengths (5–30 V) were applied from a stimulator (Grass S88) through an isolation unit (Grass SIU 5B) and a constant current unit (Grass CCU1A) with a stimulation of 1–30 Hz for 10 min. This frequency of stimulation was chosen for sampling data because it did not result in response facilitation. The intensity of stimulation was gradually increased from 0 to 30 V, and a stimulus intensity that...
yielded a single spike action potential in reflex activities was usually chosen to standardize the baseline reflex activity.

**Application of drugs.** The drugs used in this study included glutamate (10 μM), NMDA (10 μM), D-2-amino-5-phosphonovalerate (AP5; a glutamatergic NMDA receptor antagonist, 10 μM), 8-methyl-N-vanillyl-trans-6-nonenamide (capsaicin; a natural vanilloid compound, 10, 100, 300, and 1,000 μM), and N-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carbothioamide (capsazepine, a selective TRPV1, 300 μM) (all from Sigma). All drugs were dissolved in artificial cerebrospinal fluid or DMSO and applied in a final DMSO concentration of 1%. A solution of volume identical to the tested agents was dispensed to serve as the vehicle. For the uterine capsaicin instillation, capsaicin was instilled into the uterus 1 min before electric stimulation onset. Pretreated capsazepine and AP5 were injected 1 min before the capsaicin instillation.

**Western blotting.** After the experimental procedures were finished, the rats were decapitated. The dorsal half of the spinal cord segments, from L6-S1 ipsilateral to the stimulation site, was dissected, and the amount of protein was quantitated. Protein samples (20 μg) were separated on SDS-PAGE (12%) and transferred to a nitrocellulose membrane. Membranes were blocked in 5% nonfat milk and probed sequentially with antibodies against phosphorylated NR2A (1:1,000; Toris, Bristol, UK), phosphorylated NR2B (1:1,000; Chemicon, Temecula, CA), and β-actin (1:500; Santa Cruz Biotechnology, Santa Cruz, CA). The blots were incubated with the HRP-conjugated anti-

**Fig. 1.** Cross-organ sensitization on the pelvic-urethra reflex activity caused by uterine capsaicin instillation. A: the experimental arrangements showing the external urethra sphincter electromyogram (EUSE) activity induced by pelvic afferent nerve stimulation recorded under the urinary bladder as it drained freely. The ureter horn was cannulated for capsaicin instillation. B: afferent nerve test simulation (TS; 1–30 Hz) evoked a baseline reflex activity with a single action potential in the EUSE. Intrathecal administration of both glutamate (TS + GLU) and N-methyl-D-aspartate (TS + NMDA) induced a longer-lasting reflex potentiation. Intratheine capsacin instillation produced cross-organ sensitization on the pelvic-urethra reflex activity evoked by the TS (TS + CP). The cross-organ sensitization caused by intratheine capsacin instillation was abolished by both intratheine capsazepine pre-treatment (TS + CPZ + CP) and intrathecal D-2-amino-5-phosphonovalerate (TS + AP5 + CP). C: a single-pulse TS evoked a baseline reflex activity characterized by a single action potential in association with a sharp contraction wave in the intraurethral pressure (IUP). Intratheine capsazine instillation (TS + CP) induced cross-organ sensitization in the reflex activity in association with a parallel elongation in the intraurethral pressure wave. Intratheine capsazepine (TS + CPZ + CP) and intrathecal AP5 (TS + AP5 + CP) pretreatment abolished the cross-organ sensitization in the reflex activity and the elongation in the intraurethral pressure wave. D: summarized data (means ± SE) showing that both intrathecal glutamate (TS + GLU) and NMDA (TS + NMDA) significantly increased the spike number evoked by the TS (**P < 0.01 to TS, n = 7). Intratheine capsacin instillation (TS + CP) significantly increased the spike number evoked by the test stimulation (**P < 0.01 to TS, n = 7) that was abolished by capsazepine (TS + CPZ + CP) and AP5 (TS + AP5 + CP; ##P < 0.01 to TS + CP, n = 7 each).
body (1:2,000) for 1 h at RT and visualized with enhanced chemilumi-
minescence solution (5 min), followed by film exposure (2 min). Densi-
metric analysis of the WB membranes was done with Science Lab 2003 (Fujii). Results were normalized against β-actin and are
presented as means ± SD.

Statistical analysis. All data in the text and figures are means ± SE. Statistical analysis of the data was performed by means of ANOVA. In all cases, a difference of \( P < 0.05 \) was considered as a statistically significant difference.

RESULTS

Baseline pelvic-urethra reflex activity. We first reestablished
a baseline pelvic-urethra reflex activity. As shown in Fig. 1B,
a single pulse pelvic afferent nerve test simulation (TS; 1–30
Hz) evoked a baseline pelvic-urethra reflex activity with a
single action potential. The evoked activity remained rela-
tively constant throughout the stimulation period. The sum-
marized data in Fig. 1D show the mean spike number
counted 10 min following the TS onset (1.00 ± 0.00
spikes/stimulation; \( n = 42 \)).

Glutamatergic agonists induced reflex potentiation. Next,
we examined the role of glutamatergic neurotransmission in
the induction of the pelvic-urethra reflex potentiation. When
compared with the single pulse TS (Fig. 1B), which evoked a
baseline reflex activity with single action potentials, the intra-
theal administration of both glutamate (TS + GLU, 10 μM)
and NMDA (TS + NMDA, 10 μM) induced a longer-lasting
reflex potentiation. The summarized data in Fig. 1D show that
intrathecal glutamate (TS + GLU, 19.06 ± 1.51 spikes/stimu-
lation; \( P < 0.01 \) to TS, \( n = 42 \)) and NMDA (TS +
NMDA, 13.11 ± 0.86 spikes/stimulation; \( P < 0.01 \) to TS, \( n =
42 \)) significantly increased the mean spike number evoked by
the TS when compared with TS alone (TS).

Capsaicin-induced cross-sensitization. After the previous
procedures were finished, we pharmacologically activated cap-
saicin-sensitive afferent fibers arising from the uterus to test the
role of these fibers on the pelvic-urethral reflex activity. As
shown in Fig. 1B, uterine capsaicin instillation produced sen-
sitization in the pelvic-urethra reflex activity (TS + CP) when
compared with the baseline reflex activity with a single action
potential evoked by the TS alone (TS). The reflex sensitization
caused by uterine capsaicin instillation is summarized in Fig.
1D (TS + CP, 45.42 ± 9.13 spikes/stimulation; \( P < 0.01 \)
to TS, \( n = 7 \)). We tested the dose-response relationship using
capsaicin at concentrations of 10, 30, 100, and 300 μM, and the
results showed that the EC50 was 39.81 ± 4.42 μM (\( n = 7 \);
data not shown). Therefore, we instilled the uterus with capsa-
icin at a concentration of 100 μM to induce cross-organ
sensitization in the following experiments, except for the
desensitization experiments, where multiple doses were used.

TRPV1 antagonist. We then tested the participation of
TRPV1 in capsaicin-induced cross-organ sensitization by phar-
macologically blocking the receptor using a TRPV1-selective
antagonist, capsazepine. Intrathecal pretreatment with capsa-
zipine (300 μM) did not affect the baseline reflex activity
evoked by the TS (data not shown), whereas it attenuated the
reflex sensitization caused by uterine capsaicin instillation
(TS + CPZ + CP, 100 μM; Fig. 1B). The summarized data in
Fig. 1D show that the spike number evoked by the TS in
association with uterine capsaicin instillation (TS + CP) de-
creased significantly by the pretreatment with the capsazipine
pretreatment (TS + CPZ + CP, 5.02 ± 2.11 spikes/stimula-
tion; \( P < 0.01 \) to TS + CP, \( n = 7 \)).

NMDA dependence. Since TRPV1-expressing primary affer-
sents are presumed to be glutamatergic, we tested the possibility
that the cross-organ sensitization caused by uterine capsaicin
instillation is mediated by the modulation of spinal gluta-
materic neurotransmission by intrathecally blocking the gluta-
materic NMDA receptor using AP5. As shown in Fig. 1B,
intrathecal injection of AP5 (TS + CP + AP5, 10 μM) also
attenuated the reflex sensitization caused by uterine capsaicin
instillation (100 μM). The summarized data in Fig. 1D show
that AP5 (TS + AP5 + CP) significantly decreased the spike
number evoked by the test stimulation with uterine capsaicin
instillation (TS + CP, 3.21 ± 0.83 spikes/stimulation; \( P <
0.01 \) to TS + CP, \( n = 7 \)).

Secondary responses to cross-sensitization. We then tested
whether the cross-organ sensitization caused by uterine capsa-
icin instillation affects the physiological functions of the ure-
thra. As shown in Fig. 1C, the TS evoked a baseline reflex
activity with a single action potential in accompaniment with a
contraction wave in the IUP. Intrathecal capsaicin instillation
(100 μM) sensitized the evoked activity and elongated the IUP
contraction wave (TS + CP). Both intrathecal capsazepine
(TS + CPZ + CP, 300 μM) and intrathecal AP5 (10 μM, TS +
AP5 + CP) pretreatment abolished the cross-organ sensitiza-
tion caused by the capsaicin instillation as well as the elon-
gation in the IUP contraction wave.

Role of NMDA NR2A and NR2B subunits. Electrophysi-
ological evidence has demonstrated that phosphorylation of ty-
rosine residues in the NR2 subunit is an important determinant
for NMDA-dependent neural plasticity underlying postinflam-
matory hyperalgesia (13, 27). To clarify the role of NR2 sub-
units in the development of cross-organ sensitization caused
by uterine capsaicin instillation, spinal tissues (dorsal half of the L6-S1 level ipsilateral to the stimulated nerve) were
harvested from the rats 10 min following the stimulation onset
of test stimulation in association with uterine saline instillation
(TS + SA) and TS in association with uterine capsaicin
instillation (100 μM) without (TS + CP) or with intrathe-
reterine capsazepine (300 μM) pretreatment (TS + CPZ + CP) or
intrathecal AP5 (TS + CPZ + CP) for Western blot analysis.
As shown in Fig. 2A, when compared with the saline, uterine
capsaicin instillation increased the levels of the phosphorylated
NR2B subunit but did not affect the level of phosphorylated
NR2A. In addition, both intrathecal capsazepine and intrathe-
cal AP5 reversed the increment in phosphorylated NR2B levels
induced by uterine capsaicin instillation (TS + CPZ + CP and
TS + AP5 + CP, respectively). The mean immunoreactivity of
phosphorylated NR2A and NR2B induced by the TS in
association with uterine saline instillation (TS + SA) and
the TS in association with uterine capsaicin instillation without
(TS + CP) or with intrathecal pretreatment with capsazepine
(TS + CPZ + CP) or intrathecal AP5, respec-
tively (TS + CPZ + CP), are summarized in Fig. 2B (\( n = 4 \)).

Stages of the estrous cycle. Since studies have shown that
the TRPV1-mediated painful response in the uterus is highly
dependent upon the gonadal hormonal environment, we rees-
ablished and compared the capsaicin-induced cross-organ sen-
sitization in two major stages of the rat estrous cycle, i.e., the,
proestrus and metestrus stages. The plasma estradiol and pro-
gestosterone concentration of the proestrus (Pro) and metestrus

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(Met) rats are shown in Fig. 2C. Measurements of serum hormone levels confirmed the cycle stage on the basis of a vaginal cytology pattern. TS on the pelvic afferent nerve evoked a baseline reflex activity with a single action potential both in the Pro and Met rats (Fig. 3). Intrauterine capsaicin instillation with a concentration of 100 μM produced reflex sensitization (TS + CP, 100 μM) in both groups. In addition, the reflex sensitization was more elongated in the Pro than in the Met rats. The summarized data in Fig. 3B show that the mean spike number, which was counted 10 min following the onset of TS in both the Pro and Met rats, was significantly increased by uterine capsaicin instillation, with a concentration of 100 μM (100 μM, 40.05 ± 4.23 and 12.89 ± 3.13 spikes/stimulation in Pro and Met, respectively; *P < 0.01 to TS, n = 7). Meanwhile, the increase in the spike number caused by capsaicin instillation was significantly higher in the Pro than in the Met rats (*P < 0.01 to Pro, n = 7).

High-dose capsaicin-induced desensitization. It has been established that a high dose of capsaicin causes irreversible damage to the unmyelinated sensory fibers, which is well known as desensitization. We tested the effect of various concentrations of capsaicin instillation on cross-organ sensitization. In both the Pro and Met rats (Fig. 3A), the pelvic-urethra reflex activity was sensitized by capsaicin instillation. Meanwhile, there was an elongation of the firing duration parallel to the test concentrations from 100 to 300 μM (TS + CP, 100 μM and TS + CP 300 μM, respectively). However, the reflex sensitization weakened when we instilled capsaicin with a concentration of 1,000 μM in both groups of rats (TS + CP 100 μM; Fig. 3, A and B). The summarized data in Fig. 3B show that the mean spike number, counted 10 min following the onset of TS in both the Pro and Met rats, increased in a dose-dependent manner to the concentration of instilled capsaicin at a range from 100 (40.05 ± 4.23 and 12.89 ± 3.13 spikes/stimulation in Pro and Met; n = 7) to 300 μM (65.98 ±
7.15 and 31.03 ± 4.52 spikes/stimulation in Pro and Met; n = 7). The increase in the spike number caused by capsaicin with a concentration of 1,000 μM (31.84 ± 2.21 and 27.13 ± 5.12 spikes/stimulation in Pro and Met; n = 7) was significantly smaller than at 300 μM.

DISCUSSION

In the present study, we instilled capsaicin into the uteruses of female rats and found that this procedure induces cross-organ sensitization on the pelvic-urethra reflex activity as well as the physiological target of this reflex, the contraction wave of the urethra, with a parallel increase in the phosphorylated NMDA NR2B subunit level in the spinal dorsal horn. Moreover, pharmacological blockage of the TRPV1 receptor attenuates the reflex potentiation caused by uterine capsaicin instillation as well as the increment in the phosphorylated NR2B subunit level, indicating that TRPV1 plays a role in the spinal NR2B subunit-dependent cross-organ sensitization induced by uterine capsaicin instillation.

Pelvic pain syndrome, one of the most frequent disorders in women, can lead to years of suffering, disability, and numerous unsuccessful medical treatments. Pelvic pain can arise from several sources, such as the gastrointestinal, urinary, genital, muscular, and skeletal systems. The cross-innervation of visceral organs within the pelvic cavity not only offers a complex sensory pathway in the pelvis that is essential for the dynamic regulation and integration of sexual, bowel, and bladder functions (3, 52, 70) but also underlies the pathophysiological mechanism where injury or inflammation in one pelvic organ may lead to modifications in the function of others (11, 23, 24). For example, patients with irritable bowel syndrome have a significantly higher prevalence of pelvic pain syndrome (52). Moreover, lower urinary tract dysfunction is usually concurrent with pelvic pain syndrome (61, 78). In the present study, we make the first direct demonstration of cross-organ sensitization between the uterus and the urethra caused by capsaicin instillation. We suggest that the cross-organ sensitization presented in this study, at least in part, mimics the pathophysio-
logical condition that occurs during acute uterine inflammation and may provide an animal model for further investigations to explore pelvic pain syndrome and lower urinary tract dysfunction in women.

Although local administration of capsaicin, the natural vanilloid substance, may activate sensory nerves and induce a consecutive neuropeptide release, it mimics the sequel that is triggered during local inflammation (12, 33). Thus, capsaicin is routinely used to identify nociceptive C-fibers and to explore their contribution to physiological and pathological regulatory processes (30, 33). In this study, activation of the capsaicin-sensitive primary afferent fibers arising from the uterus by capsaicin instillation into the uterine horn sensitized the evoked pelvic-urethral reflex activity, which has been suggested to be related to neurogenic urethra hyperactivity (38, 39). Although the detailed mechanisms involved in this cross-organ sensitization require further elucidation, these results imply that urethral instability might be induced because of uterine inflammation via the capsaicin-sensitive, primary afferent, fiber-mediated cross-organ sensitization. We propose that this phenomenon, at least in part, underlies the clinical finding that lower urinary tract dysfunction, characterized by hyperactivity and dyssynergia in the lower urinary tract, often occurs in association with pelvic pain syndrome (53).

In addition, the agonist-induced desensitization of TRPV1 may offer a way to alleviate neuropathic and inflammatory pain (1, 33). In the present study, we instilled increasing concentrations of capsaicin into the uterine horn of rats in both the proestrus and metestrus stages to activate the nociceptive C-fiber, and this procedure induced sensitization in the pelvic-urethra reflex activity in a dose-dependent manner to the concentration of capsaicin from 100 to 300 μM. However, the reflex potentiation weakened after the inducement of capsaicin reached a ceiling of maximal response at a concentration of 300 μM, even when the concentration of capsaicin was increased to 1,000 μM. Our results correlate with previous pharmacological studies (33, 67, 68). Our data demonstrate that desensitization of capsaicin-sensitive primary afferent fibers may attenuate the hyperactivity in the urethra caused by intrauterine irritation. This result implies that vanilloid compound-induced desensitization in the cross-organ interaction between the uterus and the lower urinary tract can be a potential candidate for clinical therapy in secondary neurogenic hyperactivity in the low urinary tract induced by uterine pathology.

TRPV1, expressed by the capsaicin-sensitive nociceptive afferent fibers, is recognized as necessary for visceral nociception (8, 32, 57). In this study, pharmacological blockade of the TRPV1 receptor using the selective antagonist capsazepine abolished the cross-organ sensitization caused by intrauterine capsaicin instillation, indicating that uterine TRPV1 receptors participate in the induction of the cross-organ sensitization between the uterus and the lower urinary tract. Studies investigating the intracellular cascades mediating the agonist-induced desensitization of the nociceptive C-fibers suggest that the activation of the TRPV1 receptor may induce a subsequent entry of extracellular Ca2+ through the channel into the sensory neurons. One of the prominent mechanisms responsible for TRPV1 desensitization is dephosphorylation of the TRPV1 protein by the Ca2+/calmodulin-dependent enzyme phosphatase 2B. Of several agreed-upon phosphorylation sites identified so far, the most notable are the two sites for Ca2+/calmodulin-dependent kinase II, at which the dynamic equilibrium between the phosphorylated and dephosphorylated states presumably regulates agonist binding. However, the detailed mechanism involved in the nociceptive C-fiber desensitization, which has been suggested to alleviate neuropathic and inflammatory pain, still needs further clarification and investigation.

A number of pain syndromes are more prevalent in women than in men, including irritable bowel syndrome, fibromyalgia, and temporomandibular disorders (7, 66). In many women, the severity of symptoms of a pain syndrome fluctuates with the menstrual cycle (20, 25, 26, 34). This phenomenon suggests that gonadal steroid levels may be related to pain severity (31). Estrogen not only affects the urogenital system but is also known to possibly modulate the neural response within the central nervous system (59). We have previously shown that surgical ablation of menses attenuates repetitive stimulation-induced spinal reflex potentiation, and hormone replacement therapy reverses this attenuation caused by the ablation (37). In this study, we further studied the effects of gonadal hormone levels on the cross-sensitization between the uterus and the lower urinary tract. Moreover, the results demonstrate an enhanced cross-organ sensitization in the rats in the receptive stage compared with the rats in the nonreceptive postovulatory stage of the menstrual cycle. This result correlates with the well-established notion that estrogen may facilitate the neural response to noxious stimulation (8, 20, 25, 26, 34, 66).

The precise mechanism involved in estrogen-induced modulation is still under investigation. Estrogen may sensitize afferents by altering the expression or function of mechanosensitive ion channels on nerve endings in the uterus, especially the TRPV1 receptors. Recent studies have shown that the afferent nerve fibers innervating the uterine cervix, which express TRPV1, increase expression with chronic estrogen treatment (55, 56, 61, 78), and capsazepine, a selective TRPV1 receptor antagonist, abolishes estrogen-induced sensitization of uterine afferents. On the other hand, estrogen also modulates glutamatergic NMDA-mediated neurotransmission within the central nervous system. Studies have established that pharmacological or surgical ablation of menses decreases the duration of the NMDA-dependent excitatory postsynaptic potential in the central nervous system (71). Some experiments have confirmed that the NMDA-dependent synaptic plasticity in the CA1 area is modulated by the menstrual cycle (69, 71) or by hormone replacement therapy (76). A more recent study has demonstrated that rats treated with estradiol had an increased level of NMDAR1 mRNA and protein in the central nervous system neurons. Since glutamate is widely utilized by the primary afferent fiber for the neurotransmission on the dorsal horn neuron, including the lumbar sacral level of the spinal cord, the levels controlling the functions of the lower urinary tract (44) and the role of the NMDA receptor in the modulation of cross-organ sensitization caused by uterine capsaicin instillation cannot be excluded.

It is now presumed that peripheral and central mechanisms may underlie cross-organ sensitization. The convergence of sensory fibers from adjacent structures accounts for the peripheral mechanism (42), whereas the central integration of neural activity within the brain and spinal cord is involved in the central mechanisms (14, 43). In this study, cross-organ sensitization is defined by uterine capsaicin instillation sensitizing...
the evoked urethra electromyogram activity; therefore, the involvement of the peripheral convergence of sensory afferent fibers cannot be excluded. On the other hand, the reflex potentiation in this study, similar to central sensitization, is a spinal-mediated neural plasticity manifesting as a potentiation in reflex response to stimuli. In this study, we have shown that the induction and maintenance of reflex potentiation depends on spinal glutamatergic NMDA neurotransmission. Intrathecally blocking the NMDA receptor using AP5 attenuates the capsaicin-induced cross-organ sensitization, indicating that NMDA-dependent spinal central sensitization, at least in this in vivo animal model, may be involved in the cross-organ sensitization between the uterus and the urethra. The results of the induction of cross-organ sensitization are further corroborated by the increase in the level of phosphorylated NMDA NR2B subunit expression at the spinal dorsal horn level, which was reversed by the intrathecal pretreatment with the NMDA antagonist AP5. All of these results imply that an NMDA-dependent central mechanism at the spinal cord level is involved in the capsaicin-induced cross-organ sensitization between the uterus and the urethra.

In summary, these findings not only establish the feasibility of this unique model to study inflammatory disorders of the urethra but may also enable the eventual characterization of the pathophysiological mechanisms involved in the development and overlap of pelvic pain and lower urinary tract dysfunction. These data support the notion that the comorbidity of pelvic pain and urgency syndromes is not coincidental but rather causal in nature. Furthermore, spinal NMDA-dependent reflex plasticity triggered by capsaicin-sensitive primary afferent fibers via the activation of the TRPV1 receptor may set the foundation for the pathophysiological development of cross-sensitization. It may be hypothesized that long-term or ongoing stimulation of these pelvic sensory pathways and reflexes (i.e., pelvic organ cross-talk) may eventually lead to more permanent sensory changes in the nonirritated organ, perhaps leading to neurogenic inflammation and sensitization via the peripheral and central release of neurotrophic factors and other mediators of this phenomenon. Clearly, further research is warranted to expand on the current findings.

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REFERENCES
ESTROUS VARIATION OF CROSS-SENSITIZATION


