Acute Uterine Irritation Provokes Colonic Motility *via* Transient Receptor Potential A_1-dependent Spinal NR2B Phosphorylation in Rats

Hsien-Yu Peng, Ph.D., Chou-Ming Yeh, M.D., M.Sc., Jen-Kun Cheng, M.D., Ph.D., Yat-Pang Chau, Ph.D., Ting Ruan, Ph.D., Gin-Den Chen, M.D., Ming-Chun Hsieh, M.Sc., Cheng-Yuan Lai, B.S., Tzer-Bin Lin, Ph.D.

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ABSTRACT

Background: Patients with inflammatory gynecological/obstetrical problems often complain of irritable bowel syndrome. The authors examined whether acute uterus irritation reflexively provokes colonic motility in rat preparations.

Methods: A modified colon manometry and striated abdominal muscle electromyogram activity in response to mustard oil (MO) instillation into the uterine horn were continuously recorded in anesthetized rats. The lumbosacral (L6-S1) dorsal horn was dissected to assess the level and the cellular location of phosphorylated NR2B subunit using Western blotting and immunofluorescence analysis, respectively. Finally, the uterine transient receptor potential A_1 or spinal NR2B subunit was pharmacologically blocked to elucidate its roles.

Results: MO (0.1%, 0.2 ml) injected into the lower uterine horn dramatically provoked colonic hypermotility characterized by rhythmic colonic contractions (about 3–4 contractions per 10 min, n = 7) accompanied by synchronized electromyogram firing in the abdominal muscle (about 4–5 folds of control, n = 7). In addition to provoking colonic hypermotility, MO administration also up-regulated phosphorylated (about 2–3 folds of control, n = 7), but not total, NR2B expression in the dorsal horn neurons. Both intrathecal Ro 25–6981 (a selective NR2B subunit antagonist; 10 μM, 10 μl) and intrauterine HC-030031 (a selective transient receptor potential A_1 receptor antagonist; 30 mg/kg, 0.2 ml) injected before the MO instillation attenuated the MO-induced colonic hypermotility and spinal NR2B phosphorylation.

Conclusion: The comorbidity of gynecological/obstetrical and gastrointestinal problems is not coincidental but rather causal in nature, and clinicians should investigate for gynecological/urological diseases in the setting of bowel problems with no known pathological etiology. **(ANESTHESIOLOGY 2014; 120:436-46)**

ACUTE irritation of pelvic organs was shown to provoke phosphorylation of the NR2B subunit of glutamatergic *N*-methyl-D-aspartate receptors (NMDARs) in the lumbosacral dorsal horn.[1–4] Both focal knockdown of spinal NR2B expression[3] and spinal administration of reagents selectively antagonizing NR2B phosphorylation[1–3] thwarted the irritation-induced visceral hyperreflexia, suggesting that spinal NR2B phosphorylation plays a crucial role in visceral pain pathology.

Although the underlying mechanism remains unclear, epidemiological studies demonstrate patients with inflammatory pain-associated gynecological problems, such as

What We Already Know about This Topic

- Cross-organ sensitization has been inferred from epidemiological data and has been studied in animals
- In particular, there may be an association between pain-related gynecological problems and irritable bowel syndrome

What This Article Tells Us That Is New

- Rats were used to show that irritation of the uterus with mustard oil provoked colonic hypermotilty
- These effects were shown to be dependent on local transient receptor potential subfamily ankyrin 1 channel activation and spinal NR2B receptor stimulation

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endometriosis or dysmenorrhea, often complain of irritable bowel syndrome (IBS).[5–7] Cross-organ sensitization, a phenomenon in which inflammation/injury enhances the NMDAR-mediated neurotransmission both in the dorsal horn of the damaged viscera itself and in unaffected neighboring organs, has recently emerged as an important theme in researches investigating the concurrence of problems in pelvic viscera.[8–10] Our laboratory demonstrated that acute uterus irritation dynamically enhances pain-related urethra electromyogram activity *via* spinal NR2B phosphorylation.[1,11] Nevertheless, whether damages in the uterus could also reflexively affect bowel functions, demonstrating the comorbidity of gynecological problems and IBS, and the possible role of spinal NR2B subunit has not yet been clearly investigated.

Although there is sparse direct immunohistochemical evidence demonstrated the transient receptor potential subfamily ankyrin 1 ($TRPA_1$)[12]–expressing afferents innervate the pelvic viscera,[13] pharmacological activation of $TRPA_1$ modifies the function of the urinary bladder,[14] urethra,[15] colon,[16,17] and uterus.[10] This suggests a role of $TRPA_1$ in the neural regulation of lower urogenital and gastrointestinal tracts. Knockout of mouse $TRPA_1$ prevents the enhanced visceromotor response caused by 2,4,6-trinitrobenzene sulfonic acid–induced colitis and colon irritation induced by acute mustard oil (MO; a selective $TRPA_1$ agonist) instillation.[18] Moreover, $TRPA_1$ activation caused by a brief intracolonic MO application increased spontaneous excitatory postsynaptic potentials in a spinal slice through the selective potentiation of glutamate-mediated neurotransmission in the substantia gelatinosa, a key site for noxious input integration.[19] Interestingly, studies in which MO was applied into the descending colon have demonstrated the MO-induced spinal NR2B phosphorylation is an important determinant for the cross-organ sensitization among pelvic viscera.[3,4,20,21] These studies prompt us to investigate whether and how the activation of nociceptive $TRPA_1$-expressing afferents and the subsequent spinal NR2B phosphorylation participate in uterus–colon crosstalk, a possible mechanism underlying the concurrence of gynecological pain and IBS.

We hypothesized that acute uterus irritation provokes colonic hypermotility *via* the spinal NR2B phosphorylation. Thereby, we recorded colon motility and analyzed the spinal expression and location of phosphorylated NR2B in response to intrauterine MO instillation. Moreover, uterine $TRPA_1$ and spinal NR2B were pharmacologically antagonized to elucidate their roles in the uterus–colon crosstalk.

Materials and Methods

Animal Preparations

All procedures for animal studies were reviewed and approved by the Institutional Review Board of National Chung-Hsing University, Taichung, Taiwan. Two hundred sixty-three female Sprague–Dawley rats, weighing 250–300 g, were randomly assigned to groups and used throughout this study. Animals were individually housed in wood chip–lined plastic cages, had free access to water and food, and were maintained on a 12:12-h light–dark cycle with lights on at 07:00 AM. The estrous stage was assessed daily at 9:00 AM by vaginal lavage, using the traditional stage nomenclature.[22] Rats that had two complete, regular 4-day estrous cycles before the day of the experiment were used for this study. Measurements were made approximately 5–8 h after the lights were turned on and when the rats were in the diestrus stage (when both estradiol and progesterone are low).[22]

Surgical Preparations

In order to quickly achieve adequate anesthesia for surgical procedures, animals were first anesthetized with isoflurane (5% for induction and 2% for surgery). A PE-50 catheter (Portex, Hythe, Kent, United Kingdom) was placed in the left jugular vein for anesthetic administration. A midline abdominal incision was made to expose the pelvic viscera. Two wide-bore uterine cannulae, one for drug injections and the other for fluid drainage, were inserted into the lumen of the right lower uterine horn through small incisions made on the uterine horn and secured with cotton thread. After all surgical procedures were completed, urethane (1.2 g/kg; Sigma-Aldrich, St. Louis, MO) was injected intravenously to maintain anesthesia, as this reagent produces a long duration of anesthesia after single bolus while causing minimal changes in the visceral and reflex response.[23]

Colonic Motility Recording

The motility of the descending colon was continuously recorded as changes in intracolonic pressure (ICP) using an approach modified from the studies by Gourcerol *et al.*[24] and Carini *et al.*[25] In brief, a catheter with a 4-cm deflated flexible latex balloon lubricated with medical-grade lubricant at the tip was inserted intraanally into the descending colon such that the end of the balloon was 1 cm proximal to the anus. The balloon was then filled with normal saline (approximately 0.5 ml) and the ICP was continuously recorded *via* a catheter connected to a pressure transducer (P23 ID; Gould-Statham, Quincy, IL) on a computer system (MP30; Biopac, Santa Barbara, CA) through a preamplifier (7P1; Grass, Cleveland, OH). Because our pilot experiments showed the maximal ICP provoked by the colonic irritation was approximately 30 mmHg (28.15 ± 3.21 mmHg), the motility of the descending colon was then quantified offline by the number of contractions in which the amplitude was higher than 15 mmHg (about half the amplitude of the maximal contraction), using a program built in the recording software (Student Lab BSL PRO 3.7; Biopac; fig. 1A). Because balloon distension initially provoked rhythmic colonic contractions (RCCs) that gradually subsided, drug pretreatments, including intrauterine HC-030031 (a selective $TRPA_1$ antagonist) or intrathecal Ro 25–6981 (a NMDAR NR2B subunit selective antagonist) were injected after a equilibrium period of 90 min after balloon

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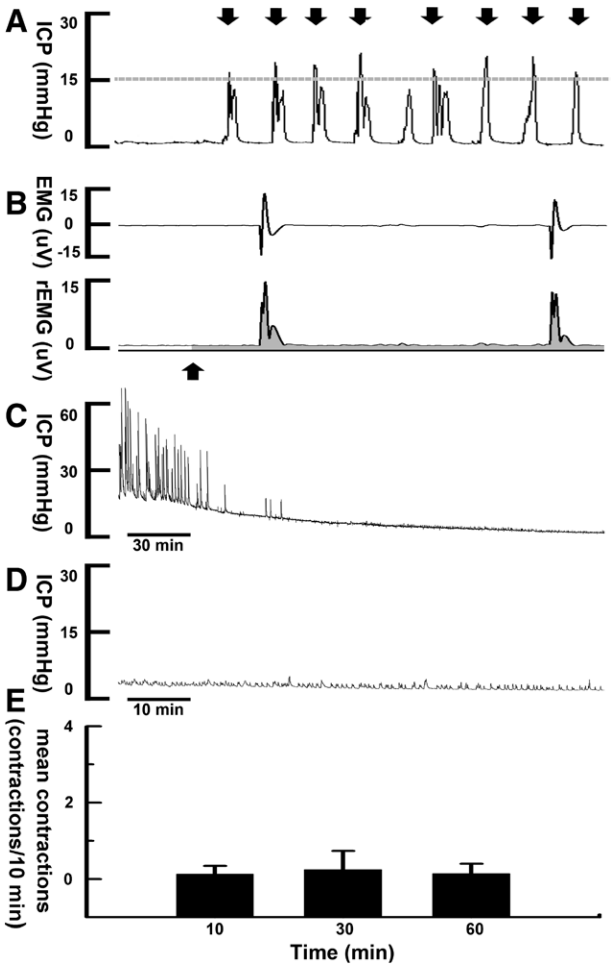

Fig. 1. Baseline intracolonic pressure (ICP) recordings. (*A*) An example of ICP recording. The peaks in ICP with amplitudes higher than 15 mmHg (marked by *arrows*) were identified as contractions. (*B*) An example of abdominal muscle electromyogram (EMG) and rectified EMG (rEMG) recordings. The integrated area under the rEMG is marked by the *gray area*. (*C*) Balloon distension provoked rhythmic colonic contractions in the ICP that subsided gradually during the equilibrium period. (*D*) After equilibrium, the manometry recording showed a stable ICP tracing. (*E*) Summarized data demonstrate no difference between the contraction count in the ICP curve measured at 10, 30, and 60 min after equilibrium ($P > 0.05$ *vs.* each other, n = 7).

distension. In all the cases (including animals that received no pretreated reagents), the ICP in response to intrauterine instillation of either MO or corn oil (CO) was continuously recorded for 60 min after a period of pretreatment (30 min). Therefore, the experiment usually finished 180 min after the starting of balloon distension.

Electromyogram Recording

In some experiments, electromyogram activity was picked up by Teflon-coated stainless steel wire electrodes stitched into the external oblique musculature immediately superior to the inguinal ligament. The electromyogram signals were continuously recorded on a computer system (MP30; Biopac) through a preamplifier (P511AC; Grass) using a band-pass filter with a frequency range of 30–3,000 Hz. The electromyogram activity was quantified off-line by integrating the area under the rectified electromyogram signal using a program built in the recording software (Student Lab BSL PRO 3.7; Biopac; fig. 1B).

Intrathecal Catheter

Implantation of an intrathecal cannula was performed as described in our previous study.[4] Briefly, 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. At the end of the experiment, a laminectomy was performed to verify the location of the cannula tip. In 5 of 64 rats, the cannula tip deviated by more than 0.5 mm from the target structure, and the data from these five cases were excluded from the statistical analysis.

Drug Administration

Drugs that were administered by intrauterine, intracolonic, intrathecal, or intravenous injections included the following: MO (allyl isothiocyanate; 0.05, 0.1, and 0.5% dissolved in 0.2 ml CO; intrauterine; Sigma-Aldrich), a pungent component causing acute colon inflammation;[3] CO (0.2 ml; intrauterine; Sigma-Aldrich), a control solution for MO;[3] HC-030031 (HC, 30 mg/kg, 0.2 ml; intrauterine; Sigma-Aldrich), a $TRPA_1$ receptor antagonist;[26] acetic acid (1, 3, and 10%, 0.2 ml; intracolonic; Sigma-Aldrich), an irritant inducing colonic motility;[23] Ro 25–6981 (RO, 10 μM, 10 μl; intrathecal; Sigma-Aldrich), a selective NMDAR NR2B subunit antagonist;[2] ghrelin (1, 3, and 10 μg/kg, 10 μl; intrathecal; Sigma-Aldrich), an agent causing rhythmic colonic motility;[27] and Evans Blue (50 mg/kg; intravenous; Sigma-Aldrich), a dye to quantify plasma extravasation.[28] In all cases, solvent solutions of volume identical to those of the tested agents served as vehicle controls.

Western Blotting

The rats were deeply anesthetized, and the spinal cords were quickly obtained before (preinjection control) and 10, 30, or 60 min after intrauterine MO injections. The dorsal horn of the right lumbosacral spinal cord (L6-S1) was dissected for Western blot analysis using methods described previously.[29] In brief, protein samples (20 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8 and 12%), transferred to a nitrocellulose membrane, blocked in 5% nonfat milk and probed with antibodies against β-actin (1:8,000; Santa Cruz, Santa Cruz, CA) total and phosphorylated NR2B (tNR2B and

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pNR2B, respectively, 1:1,000; Millipore, Billerica, MA). The blots were incubated with horseradish peroxidase–conjugated antibody (1:2,000) for 1 h at room temperature and visualized with an enhanced chemiluminescence solution (5 min) followed by film exposure (2 min). Densitometric analysis of the western blot membrane was performed with Science Lab 2003 (LAS-300; Fuji, Kanagawa, Japan).

Quantitation of Plasma Protein Extravasation

A subset of animals was prepared to evaluate the effect of intrauterine MO on plasma protein extravasation in the uterus and colon.[28] Evans Blue (50 mg/kg; Sigma-Aldrich) was injected intravenously 30 min before the removal of tissue. At the end of the experiments, the animals were decapitated and exsanguinated. The uterus and the descending colon were removed, blotted on wet filter paper, weighed, and stored in formamide at room temperature for 72 h to extract Evans Blue from the tissue. The amount of extracted Evans Blue was quantified by measuring the optical density of the extracted dye at a wavelength of 620 nm with the use of a spectrophotometer, and the value was expressed as micrograms per gram of wet tissue weight.[28] The concentration was estimated from a standard regression curve for Evans Blue concentrations ranging from 0.1 to 5 μg/ml.

Immunofluorescence

The rats were deeply anesthetized and perfused with 100 ml of 0.01 M phosphate-buffered saline (pH 7.4), followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The right lumbosacral spinal cord (L6-S1) was then harvested, postfixed at 4°C for 4 h, and cryoprotected in 30% sucrose overnight. For double-labeling immunohistochemistry analyses, the spinal cord sections were incubated overnight at 4°C with a mixture of rabbit antiphosphorylated NR2B (1:200; Abcam, Cambridge, United Kingdom) and mouse monoclonal antineuronal nuclear antigen (NeuN; a neuronal marker, 1:1,000; Chemicon, Billerica, MA), mouse antiglial fibrillary acidic protein (a marker of astroglial cells, 1:500; Millipore), or mouse antiintegrin αM (OX-42; a marker of microglia, 1:400; Santa Cruz). The sections were then incubated with Alexa Flour 488 (1:1,500) and Alexa Flour 594 (1:1,500; Invitrogen, Carlsbad, CA) for 1 h at 37°C.

Statistical Analysis

A total of 263 rats were used in this study, including 142 rats for manometry recording and 121 rats for Western blot (70 rats), immunohistochemistry (26 rats), and plasma protein extravasation (25 rats) analyses. The density of specific bands from Western blot analysis was measured using computer-assisted imaging analysis (LAS-300; Fuji) and normalized against corresponding loading control bands. The motility of the descending colon was quantified by counting the ICP contractions, and electromyogram activity was quantified by integrating the area under the rectified electromyogram signal using programs built in the recording software (Student Lab BSL PRO 3.7; Biopac). All data were analyzed using SigmaPlot (version 10.0; Systat Software, Inc., San Jose, CA), and are presented as the mean ± SD. For serial measurements over time, one-way ANOVA was used to assess changes in values, and a Tukey *post hoc* test was used to compare means for groups. In experiments evaluating the effect of administration of different reagents, paired, two-tailed Student *t* test was used to compare means for groups. In all cases, significance was assigned at a P value less than 0.05.

Results

Baseline Colonic Motility

To test the validity of the modified balloon method, we added normal saline (approximately 0.5 ml) into the balloon, which had been intraanally inserted into the descending colon of urethane-anesthetized rats, and the ICP was then continuously recorded. In all 142 rats tested, balloon distension provoked RCCs characterized by periodic peaks in the ICP, which gradually subsided during an equilibrium period (typically 90 min; fig. 1C). After the equilibrium period, the ICP maintained a relatively constant level without significant RCCs in 135 of 142 rats, and these animals were then used for further study. The manometric tracing in figure 1D demonstrated a stable ICP throughout the recording period, and summarized data showed no difference between the contraction count in the ICP curve measured at 10, 30, and 60 min after equilibrium (fig. 1E; $P = 0.631$, 0.642, and 0.913 in 10 *vs.* 30, 30 *vs.* 60, and 10 *vs.* 60 min, respectively, n = 7), indicating this modified balloon method reflects baseline colonic motility in routine circumstances.

Effects of Intrathecal Ghrelin

In experiments that tested the reliability of the modified balloon method in response to physiological challenges, cumulative administration of ghrelin (fig. 2A; 1, 3, and 10 μg/kg, 10 μl; intrathecal), but not of the vehicle solution (veh; 10 μl), enhanced colonic motility as characterized by provoking RCCs in the manometric tracing. Figure 2B shows that when compared with the preinjection control, spinal ghrelin administration at 1, 3, and 10 μg/kg increased the contraction count in the ICP curve in a dose-dependent manner ($P = 0.021$, $P < 0.001$, and $P = 0.001$ *vs.* control, respectively, n = 7), whereas no difference was found between the preinjection control and vehicle solution injections ($P = 0.962$ *vs.* control, n = 7). These results that imply the balloon technique used in this study responds to physiological impacts on colonic motility.

Effects of Intracolonic Acetic Acid

In contrast to the vehicle solution, which failed to affect colonic motility in the experiment testing the reliability of the modified balloon method in response to pathophysiological challenges (fig. 2C; veh, 0.2 ml), cumulative acetic acid injections (1, 3, and 10, 0.2 ml, intracolonic) enhanced colonic motility by provoking RCCs in the manometric

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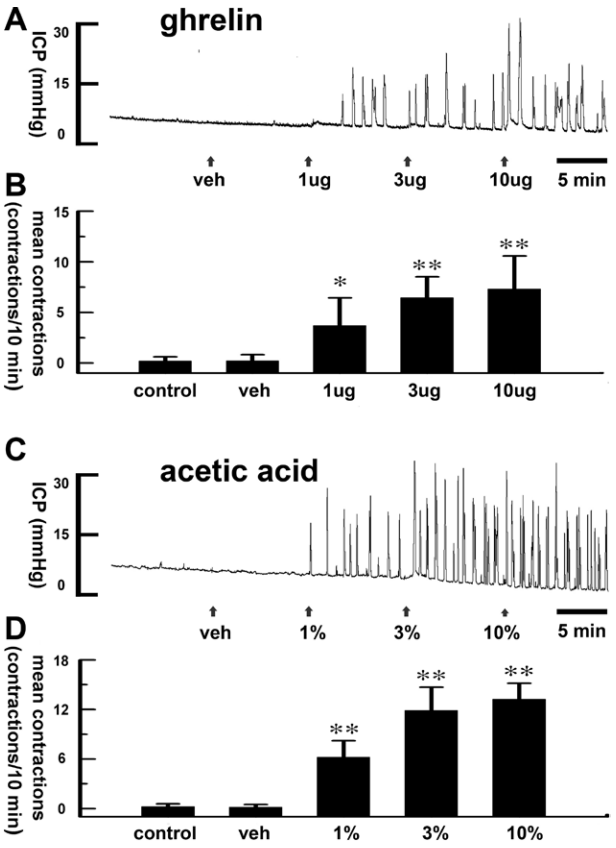

Fig. 2. Intrathecal ghrelin and intracolonic acetic acid administrations both provoke colonic motility. (*A*) Intrathecal (L6-S1) administration of ghrelin (1, 3, and 10 μg/kg, 10 μl), but not the vehicle solution (veh), provoked colonic motility in a dose-dependent manner as shown by the induced rhythmic colonic contractions in the intracolonic pressure (ICP) tracings. (*B*) When compared with that of the preinjection control, the contraction count in the ICP curve was increased by intrathecal ghrelin injections in a dose-dependent manner (*$P < 0.05$, ** $P < 0.01$, *vs.* control, n = 7). (*C*) Although the vehicle solution failed to affect the colon motility, cumulative acetic acid injections (1, 3, and 10%, 0.2 ml) into the lumen of the descending colon induced colonic motility that was observed as rhythmic colonic contractions in the ICP tracing. (*D*) When compared with the preinjection control, the contraction count in the ICP curve was increased in a dose-dependent manner after the injections of acetic acid (**$P < 0.01$, *vs.* control, n = 7). veh = vehicle solution.

tracing. The summarized data showed that when compared with the preinjection controls, intracolonic acetic acid injections increased the contraction count in the ICP curve in a dose-dependent manner (fig. 2D; $P = 0.002$, $P < 0.001$, and $P < 0.001$ *vs.* control in 1, 3, and 10, respectively, n = 7), suggesting this modified balloon technique responds to pathophysiological conditions.

MO-induced Uterus–Colon Cross-organ Sensitization

Next, colon manometry and the electromyogram activity of the rectus abdominis in response to uterine MO (0.1%, 0.2 ml) or CO (0.2 ml) injections were continuously recorded in anesthetized rats. We observed that in comparison with the preinjection control, CO injections into the uterine horn neither induced RCCs in the manometric recording nor triggered firing in the electromyogram tracing (fig. 3A). In contrast, MO administration (fig. 3B) dramatically provoked RCCs in the manometry and elicited synchronized burst electromyogram discharges. Figure 3, C and D, shows that uterine MO injections, but not CO injections, significantly increased the mean contraction count in the ICP curve ($P = 0.002$ and $P = 0.778$ *vs.* control, repectively, n = 7) and the mean area of integrated electromyogram ($P = 0.003$ and $P = 0.716$ *vs.* control, respectively, n = 7) compared with the preinjection control. These data imply that acute irritation of the uterus provokes colonic motility in a cross-organ manner.

MO-induced Spinal NR2B Phosphorylation

We next tested whether uterine MO administration could also induce spinal NMDAR NR2B subunit phosphorylation by using Western blot analysis of spinal dorsal horn samples (L6-S1) obtained at 10, 30, and 60 min after MO injections. We found that in correlation with the colonic hypermotility MO administration (0.1%, 0.2 ml) increased the band intensity labeled by the phosphorylated NR2B-specific antibody in a time-dependent manner (fig. 3E; pNR2B, normalized by the β-actin; $P = 0.033$, 0.008, and 0.002, *vs.* control in 10, 30, and 60 min, respectively, n = 7) when compared with the preinjection control. In contrast, this treatment failed to affect the band intensity labeled by the total NR2B-specific antibody at these time points (tNR2B; normalized by the β-actin; $P = 0.864$, 0.350, and 0.925 *vs.* control in 10, 30, and 60 min, respectively, n = 7), implying that spinal NR2B activation plays a key role in uterine–colon sensitization. We therefore focused our investigations on dorsal horn NR2B phosphorylation.

Uterus MO Instillation Induced No Colon Extravasation

We next tested whether acute intrauterine MO injections induce inflammation in the uterus or descending colon, by measuring the extravasation of Evans Blue dye in CO- or MO-treated animals. Figure 3F shows that in comparison to the preinjection control, it was MO (0.2 ml) but rather than CO (0.2 ml) administration into the uterine horn caused an increase in Evans Blue extravasation in the uterus extraction ($P = 0.547$ in CO and $P = 0.001$ in MO *vs.* control, both n = 7). In contrast, there was no difference found in the optical density of the colon extraction of CO- or MO-treated animals compared with the preinjection control ($P = 0.578$ in CO and $P = 0.974$ in MO *vs.* control, both n = 7), indicating that acute uterine MO injections provoke extravasation in the uterus itself rather than in the descending colon.

MO-induced NR2B Phosphorylation in Dorsal Horn Neurons

We used immunohistochemistry analysis to examine the cellular localization of the MO-induced NR2B

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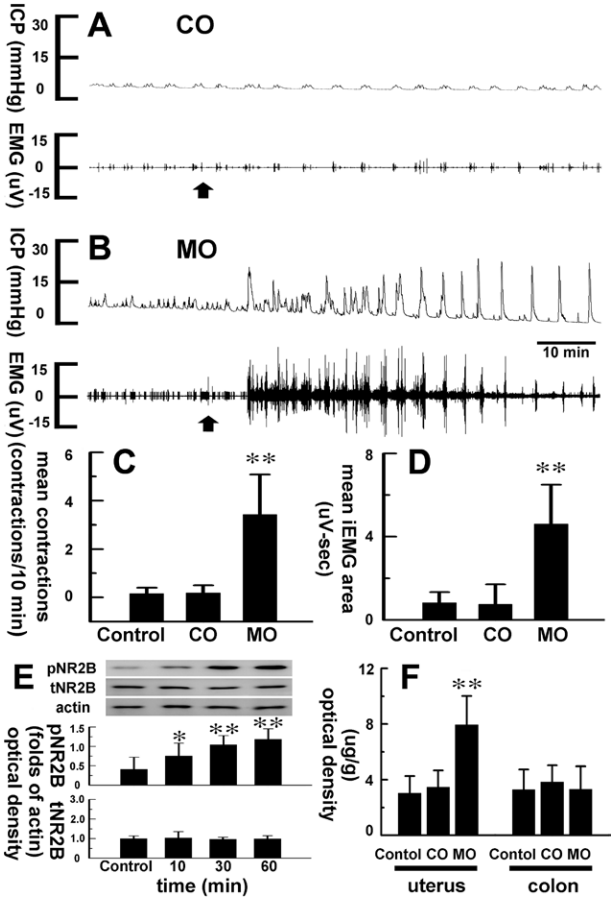

Fig. 3. Uterine mustard oil (MO) administration provokes painful colonic contractions and spinal NR2B phosphorylation. (*A*) When compared with the preinjection control, corn oil (CO; 0.2 ml; indicated by an *arrow* at the bottom) administration into the uterine horn neither induced colonic motility in the intracolonic pressure (ICP) nor triggered firing in the electromyogram (EMG) tracings. (*B*) Intrauterine MO (0.1%, 0.2 ml; indicated by an *arrow* at the bottom) dispensing dramatically provoked rhythmic colonic contractions with synchronized burst EMG discharges. (*C* and *D*) Intrauterine administration of MO, but not CO, significantly increased the contraction count in the ICP curve and the area of integrated EMG (iEMG) compared with the preinjection control (**P < 0.01, *vs.* control, both n = 7). (*E*) When compared with the preinjection control, MO administration into the uterine horn increased the band intensity of phosphorylated NR2B in a time-dependent manner (normalized by the β-actin) in dorsal horn samples (L6-S1) at 10, 30, and 60 min after administration (*P < 0.05, ** P < 0.01, *vs.* control, n = 7). However, this treatment failed to affect the band intensity of total NR2B (normalized by the β-actin; P > 0.05, *vs.* control, n = 7) or β-actin (P > 0.05, *vs.* control, n = 7). (*F*) When compared with the preinjection control, uterine MO injections, but not CO injections, significantly increased the optical density of the Evans Blue extravasation in the uterus (**P < 0.01, *vs.* control, both n = 7). There was no difference in the optical density of the Evans Blue extravasation in the descending colon between the preinjection control and uterine CO or MO injections (both P > 0.05 *vs.* control, n = 7). pNR2B = phosphorylated NR2B; tNR2B = total NR2B.

phosphorylation. Staining of spinal cord sections (L6-S1; obtained 60 min after reagent instillation) for phosphorylated NR2B immunoreactivity demonstrated that in comparison with CO (fig. 4A), uterine administration of MO (fig. 4B) increased the immunoreactivity of phosphorylated NR2B in the dorsal horn. Moreover, double staining for phosphorylated NR2B and NeuN (neuron-specific nuclear protein, a neuronal marker), OX-42 (integrin αM, a microglial marker) or glial fibrillary acidic protein (an astrocyte marker) showed the MO-enhanced phosphorylated NR2B immunoreactivity overlapped with NeuN (fig. 4C) but not OX-42 (fig. 4D) or glial fibrillary acidic protein (fig. 4E), implying that the MO-enhanced NR2B phosphorylation occurs in dorsal horn neurons but not in glial cells.

MO Induced Uterus–Colon Crosstalk in a Dose-dependent Manner

Next, MO at concentrations of 0.05, 0.1, and 0.5% (fig. 5A; 0.2 ml) or CO (0.2 ml) was instilled into the uterine horn of urethane-anesthetized rats. *In vivo* manometric recordings demonstrated that CO instillation exhibited no effect whereas administration of MO induced colonic motility in a dose-dependent manner as evidenced by provoking RCCs when compared with the preinjection control. Figure 5B shows that MO administration at concentrations of 0.05, 0.1, and 0.5% increased the contraction count in the ICP curve in a dose-dependent manner when compared with the preinjection control (P = 0.009, 0.002, and 0.002, *vs.* control, respectively, n = 7).

Effects of Ro 25–6981 Pretreatment

We next used a selective antagonist to pharmacologically block spinal NR2B phosphorylation to further elucidate its role in the MO-induced uterus–colon crosstalk. As anticipated, uterine MO injections induced colonic motility (fig. 6, A and B; MO; 0.1%, 0.2 ml), as shown by significantly enhancing the contraction count in the ICP curve (fig. 6C; P < 0.001 and P = 0.001 *vs.* control in the left and right, respectively, n = 7) associated with an increase in the band intensity of the pNR2B in dorsal horn samples (fig. 6D; P < 0.001 *vs.* control, n = 7). The pretreated Ro 25–6981 (Ro + MO, an NR2B antagonist; 10 μM, 10 μl; intrathecal), unlike the vehicle solution (veh + MO, 10 μl, intrathecal), attenuated MO-induced hypermotility as shown by a significant decrease in the contraction count in the ICP curve compared with the MO-treated animals (fig. 6C; P = 0.007 in Ro + MO and P = 0.506 in veh + MO *vs.* MO, n = 7). Additionally, spinal Ro 25–6981 injections also prevented MO-induced dorsal horn NR2B phosphorylation because it significantly decreased the band intensity of pNR2B when compared with that in MO-treated animals (fig. 6D; P < 0.001 *vs.* MO, n = 7). Moreover, neither the contraction count in the ICP curve nor the intensity of the pNR2B band was affected by the injection of Ro 25–6981 during baseline control before MO instillation (data not

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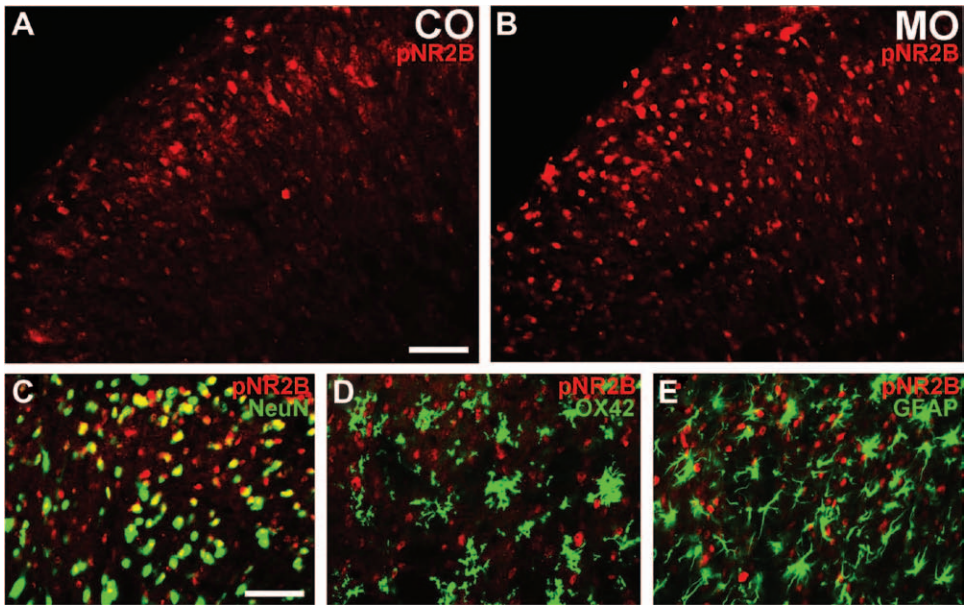

Fig. 4. Uterine mustard oil (MO) administration provokes NR2B phosphorylation in dorsal horn neurons. When compared with corn oil (*A*), intrauterine administration of MO (*B*) notably enhanced the immunoreactivity of phosphorylated NR2B (*red*) in the dorsal horn of spinal sections obtained at 60 min after injection. Amplified images showed that the immunoreacticity of NeuN (*C*, *green*), but not OX-42 (*D*, *green*) or glial fibrillary acidic protein (GFAP; *E*, *green*), was colocalized with the enhanced phosphorylated NR2B immunoreactivity (*yellow*). *Scale bar* = 50 μm, thickness = 50 μm. Each of these immunofluorescence images was replicated in seven sample preparations with similar results each time. NeuN = neuron-specific nuclear protein; OX-42 = integrin αM.

shown). These results provide pharmacological support for the pivotal role of phosphorylation of spinal NR2B subunits in the uterus–colon crosstalk.

Effects of HC-030031 Pretreatment

As shown in figure 6B, uterine MO injections induced colon hypermotility by a significant increase in contraction count in the ICP curve (fig. 6C; $P = 0.001$ *vs.* control, n = 7) and an increase in the band intensity of pNR2B in the dorsal horn sample (fig. 6D). The administration of HC-030031 (30 mg/kg, 0.2 ml, HC + MO), but not the vehicle solution (veh + MO), into the uterine horn before MO injections attenuated MO-induced hypermotility (fig. 6B) as shown by a significant decrease in the contraction count in the ICP curve when compared with that in the MO-treated animals (fig. 6C; $P = 0.002$ in HC + MO and $P = 0.924$ in veh + MO *vs.* MO, n = 7). In addition, pretreatment with HC-030031 prevented MO-elicited spinal NR2B phosphorylation as demonstrated by a significant decrease in the intensity of the pNR2B band compared with the same in the MO-treated group (fig. 6D; $P = 0.001$ *vs.* MO, n = 7). Conversely, when HC-030031 was injected during baseline control before MO instillation, HC-030031 *per se* exhibited no effect on the contraction count in the manometric recording or in the level of dorsal horn pNR2B expression (data not shown). These results provide evidence that the activation of $TRPA_1$ in the uterus could reflexively induce colon hypermotility *via* a mechanism involving the phosphorylation of the spinal NMDAR NR2B subunit.

Discussion

Through a complex neural network innervating viscera,[8,10,30] pathological alterations in one pelvic organ could lead to modifications in the function of others in a cross-organ manner.[31–33] In the current study, acute MO dispensing into the uterus reflexively enhanced colonic motility by provoking RCCs. The possibility that MO directly established an organic injury in the descending colon and thereby resulted in colon hypermotility is unlikely because we administered MO into the lumen of the uterus rather than in the descending colon. This proposal is further supported by the fact that uterine MO administration led to Evans Blue extravasation in the uterus itself but not in the descending colon, indicating our procedure did not result in colon inflammation. That is, the enhanced colon motility demonstrated in this study is a phenomenon resembling functional bowel disorders, such as IBS, in which colon functions are altered without a known pathological etiology. Moreover, in accompanied with colon hypermotility, uterine MO injections provoked abdominal electromyogram discharges that were synchronized with the RCCs. Because it is well established that colon–rectal distension provokes visceromotor response characterized by enhanced electromyogram activity of abdominal striated muscle,[17] abdominal electromyogram firing is used as an index of visceral pain, particularly in colon/rectum case. Our data suggest acute uterus irritation provokes colon hypermotility in a cross-organ manner; a phenomenon that could underlie the clinical finding that IBS, which is characterized

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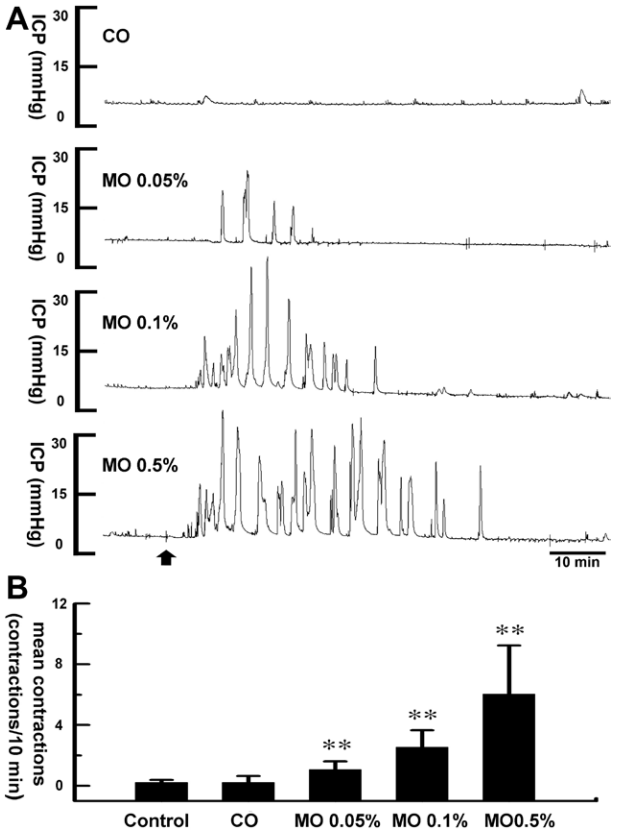

Fig. 5. Uterine mustard oil (MO) instillation provokes colonic motility in a dose-dependent manner. (*A*) In contrast to corn oil (CO; 0.2 ml), which did not affect colonic motility, intrauterine dispensing of MO (0.2 ml) at concentrations of 0.05, 0.1, and 0.5% provoked rhythmic colonic contractions in the intracolonic pressure (ICP) tracings. The *arrow* at the bottom indicates the timepoint for corn/MO instillation. (*B*) Summarized data showing that uterine MO, but not CO, injections increased the contraction count in the ICP curve in a dose-dependent manner compared with the preinjection controls (***P* < 0.01, *vs.* control, n = 7).

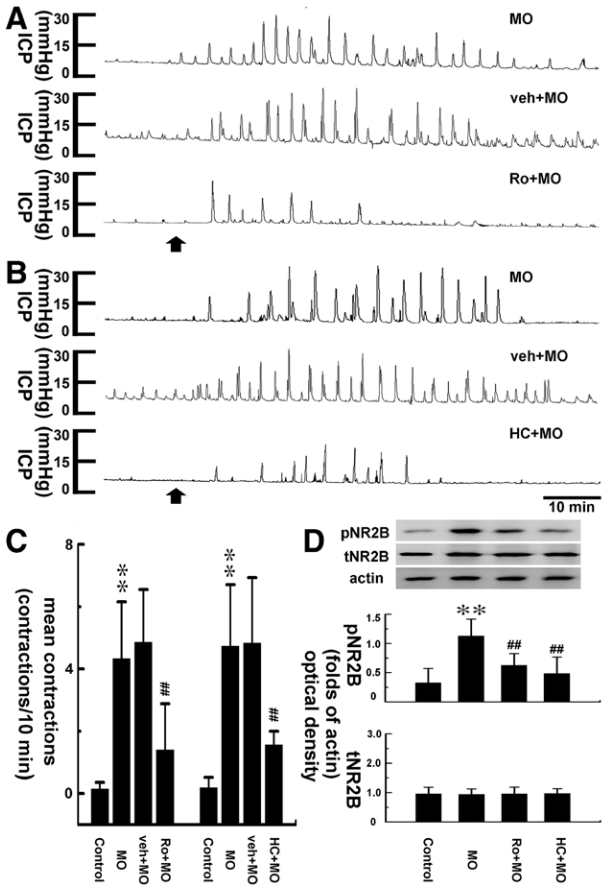

Fig. 6. Ro 25–6981 and HC-030031 both antagonizes mustard oil (MO)-induced uterine–colon crosstalk and spinal NR2B phosphorylation. (*A*) In contrast to the vehicle solution, which exhibited no effect on the MO (0.1%, 0.2 ml; intrauterine)-induced colonic motility, spinal pretreatment with Ro 25–6981 (10 μM, 10 μl; intrathecal) partially prevented the MO-induced rhythmic colonic contractions in the intracolonic pressure (ICP) tracing. The *arrows* at the bottom indicate the timepoint for corn oil instillation. (*B*) Pretreatment with HC-030031 (30 mg/kg, 0.2 ml; intrauterine), but not a vehicle solution, also attenuated the intrauterine MO-induced colon hypermotility. The *arrows* in the bottom indicate the timepoint for MO instillation. (*C*) When compared with the preinjection control, uterine MO injections enhanced the contraction count in the ICP curve (***P* < 0.01, *vs.* control, n = 7 both), and this enhancement was significantly reversed by pretreatment with Ro 25–6981 and HC-030031 (##*P* < 0.01, *vs.* MO, n = 7) but not vehicle solution (*P* > 0.05, *vs.* MO, n = 7). (*D*) When compared with control, uterine injections of MO increased the band intensity labeled by the phosphorylated NR2B-specific antibody in dorsal horn samples (L6-S1; ** *P* < 0.01, *vs.* control, n = 7), and this increase in band intensity was diminished by pretreatment with intrathecal Ro 25–6981 and intrauterine HC-030031 injection (##*P* < 0.01, *vs.* MO, n = 7). HC = HC-030031; pNR2B = phosphorylated NR2B; Ro = Ro 25–6981; tNR2B = total NR2B; veh = vehicle solution.

by hyperactivity in the distal gastrointestinal tract in association with abdominal pain, often occurs in conjunction with obstetrical/gynecological inflammation.[34–36]

The understanding of the $TRPA_1$ in pain-related behavior has advanced rapidly in recent years,[18,19,37] and $TRPA_1$ activation is currently recognized as necessary for the somatic pain provoked by intraplantar complete Freund adjuvant injection[38] as well as the visceral pain caused by trinitrobenzene sulfonic acid- and dextran sulphate sodium–induced colitis.[18,39] Although the therapeutic doses are still too high for drug development, known $TRPA_1$ antagonist(s) play a promising role *in vivo* activity against cystitis-associated hyperalgesia,[15] carrageenan-induced inflammatory pain,[40] and postoperative guarding pain.[41] By instilling a $TRPA_1$ agonist, MO, into the lumen of uterine horn, we provoked cross-organ sensitization between the uterus and colon in this study. Conversely, pharmacological antagonism of uterine $TRPA_1$ using HC-030031 abolished MO-induced uterus–colon crosstalk. Our result is the first demonstration that activation of uterine $TRPA_1$ modified the function of pelvic viscera. This finding implies $TRPA_1$ participates in the uterus physiology/pathophysiology and suggests that the development of pharmacological

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agents targeting $TRPA_1$ is a possible therapeutic strategy for the treatment of pelvic visceral pain.

In the current study, we showed that activation of uterine $TRPA_1$ induced colonic motility in a cross-organ manner. Considering that our previous investigation demonstrated that activation of uterus $TRPV_1$ sensitized the urethra reflex activity,[1] it would be interesting to test the likely interaction between these receptors because an electrophysiological study analyzed the $TRPA_1$-mediated current in mouse trigeminal ganglion neurons and demonstrated synergic interactions between $TRPA_1$ and $TRPV_1$.[42] Our unpublished data displayed that pretreated capsazepine, a selective $TRPV_1$ antagonist, partially attenuated MO-induced uterus–colon crosstalk. However, whether this observation resulted from $TRPA_1$–$TRPV_1$ interactions or other mechanisms needs further study to be fully elucidated.

NR2B-containing NMDARs have been proposed to participate in the plasticity underlying hyperalgesia/allodynia[43–49] because phosphorylation of NR2B tyrosine residues modulates NMDAR-mediated currents.[50] In addition to its role in the development of spinal nerve ligation-induced neuropathic pain,[48,51] the contribution of spinal NR2B to visceral pain was investigated in this study. Our results demonstrated that intrauterine MO instillation provoked colonic motility in association with NeuN-colocalized NR2B phosphorylation, both of which were thwarted by intrathecal dispensing of selective NR2B antagonist, Ro 25–6981. These findings suggest that activation of NR2B-containing NMDARs in dorsal horn neurons is crucially involved in the pain-related uterus–colon crosstalk. Though spinal NR2B subunits have been demonstrated to participate in the urethra[3,20,21,52] and bladder hyperreflexia[4] caused by acute colon irritation, our results are the first demonstration of a role of spinal NR2B in the cross-organ sensitization between the uterus and colon. With regard to a complex convergent neural network innervating and integrating the function of pelvic organs, including the urinary bladder, urethra, uterus, and colon,[8,10,30] our findings suggest that spinal NR2B phosphorylation could be a common mechanism shared by the crosstalk between pelvic organs, in which irritation of one pelvic viscera induced spinal NR2B phosphorylation, thus provoking crosstalk with another viscera.

In the current study, a modified balloon manometric analysis was used to record colon motility. We observed that after an equilibrium period of stress-relaxation, a tracing with a relatively constant ICP was recorded before physiological/pathological manipulations, indicating this technique is a valid measurement of colonic motility under baseline physiological conditions. To test the reliability of this method in response to physiological and pathophysiological challenges, we intrathecally injected ghrelin and intracolonically infused acetic acid to the animal because *in vivo* studies have demonstrated that spinal ghrelin injection elicits propulsive colonic contractions[27] and acute colonic irritation caused by acetic acid triggers colonic motility.[23] Our results showed both these treatments provoked RCCs in a dose-dependent manner, indicating this modified manometry is a reliable measurement of motility changes in response to physiological and pathological perturbations. Although studies are needed to further verify the reliability and validity of the manometric analysis used in this study, this method provides an available technique for investigating colonic motility. Additionally, using this manometry technique, the design of this study offers an *in vivo* animal model to investigate the interaction between the colon and uterus, which elucidates the mechanism underlying the comorbidity of gynecological problems and IBS.

In summary, our findings not only established the feasibility of a unique model to study the impact of various treatments on colonic motility but also enabled an eventual characterization of the pathophysiological mechanisms involved in the development and comorbidity of uterus inflammation and IBS. Our data support the notion that the comorbidity of gynecological/obstetrical and gastrointestinal problems is not coincidental but rather causal in nature. *Via* the activation of uterine $TRPA_1$, spinal NMDAR NR2B phosphorylation triggered by nociceptive afferents may set the foundation for the pathophysiological uterus–colon crosstalk. Perhaps leading to sensitization caused by the release of neurotrophic factors and other mediators,[8] long-term or ongoing stimulation of these pathways may possibly lead to permanent sensory changes in the descending colon. Although further researches are warranted to expand on the current findings, we suggest that clinicians consider gynecological or urological disease in cases of bowel disease with no known pathological etiology.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Lin: Department of Physiology, School of Medicine, College of Medicine, Taipei Medical University, No. 250, Wu-Hsing Street, Taipei, Taiwan 11031. tblin2@gmail.com; and Dr. Peng: Department of Medicine, Mackay Medical College, No. 46, Sec. 3, Zhongzheng Rd., Sanzhi Dist., New Taipei, Taiwan 25245. hsien.yu@gmail.com. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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References

1. Peng HY, Chang HM, Lee SD, Huang PC, Chen GD, Lai CH, Lai CY, Chiu CH, Tung KC, Lin TB: TRPV1 mediates the uterine capsaicin-induced NMDA NR2B-dependent cross-organ reflex sensitization in anesthetized rats. Am J Physiol Renal Physiol 2008; 295:F1324–35
2. Peng HY, Chen GD, Tung KC, Chien YW, Lai CY, Hsieh MC, Chiu CH, Lai CH, Lee SD, Lin TB: Estrogen-dependent facilitation on spinal reflex potentiation involves the Cdk5/ERK1/2/NR2B cascade in anesthetized rats. Am J Physiol Endocrinol Metab 2009; 297:E416–26
3. Peng HY, Chen GD, Tung KC, Lai CY, Hsien MC, Chiu CH, Lu HT, Liao JM, Lee SD, Lin TB: Colon mustard oil instillation induced cross-organ reflex sensitization on the pelvic-urethra reflex activity in rats. Pain 2009; 142:75–88
4. Peng HY, Hsieh MC, Lai CY, Chen GD, Huang YP, Lin TB: Glucocorticoid mediates water avoidance stress-sensitized colon-bladder cross-talk *via* RSK2/PSD-95/NR2B in rats. Am J Physiol Endocrinol Metab 2012; 303:E1094–106
5. Chang L, Heitkemper MM: Gender differences in irritable bowel syndrome. Gastroenterology 2002; 123:1686–701
6. Issa B, Onon TS, Agrawal A, Shekhar C, Morris J, Hamdy S, Whorwell PJ: Visceral hypersensitivity in endometriosis: A new target for treatment? Gut 2012; 61:367–72
7. Altman G, Cain KC, Motzer S, Jarrett M, Burr R, Heitkemper M: Increased symptoms in female IBS patients with dysmenorrhea and PMS. Gastroenterol Nurs 2006; 29:4–11
8. Pezzone MA, Liang R, Fraser MO: A model of neural cross-talk and irritation in the pelvis: Implications for the overlap of chronic pelvic pain disorders. Gastroenterology 2005; 128:1953–64
9. Qin C, Malykhina AP, Akbarali HI, Foreman RD: Cross-organ sensitization of lumbosacral spinal neurons receiving urinary bladder input in rats with inflamed colon. Gastroenterology 2005; 129:1967–78
10. Winnard KP, Dmitrieva N, Berkley KJ: Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: Modulation by estrous stage and involvement of the hypogastric nerve. Am J Physiol Regul Integr Comp Physiol 2006; 291:R1592–601
11. Peng HY, Huang PC, Liao JM, Tung KC, Lee SD, Cheng CL, Shyu JC, Lai CY, Chen GD, Lin TB: Estrous cycle variation of TRPV1-mediated cross-organ sensitization between uterus and NMDA-dependent pelvic-urethra reflex activity. Am J Physiol Endocrinol Metab 2008; 295:E559–68
12. Yu YB, Yang J, Zuo XL, Gao LJ, Wang P, Li YQ: Transient receptor potential vanilloid-1 (TRPV1) and ankyrin-1 (TRPA1) participate in visceral hyperalgesia in chronic water avoidance stress rat model. Neurochem Res 2010; 35:797–803
13. Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, Högestätt ED, Zygmunt PM: Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 2008; 53:391–9
14. Meotti FC, Forner S, Lima-Garcia JF, Viana AF, Calixto JB: Antagonism of the transient receptor potential ankyrin 1 (TRPA1) attenuates hyperalgesia and urinary bladder overactivity in cyclophosphamide-induced haemorrhagic cystitis. Chem Biol Interact 2013; 203:440–7
15. Gratzke C, Weinhold P, Reich O, Seitz M, Schlenker B, Stief CG, Andersson KE, Hedlund P: Transient receptor potential A1 and cannabinoid receptor activity in human normal and hyperplastic prostate: Relation to nerves and interstitial cells. Eur Urol 2010; 57:902–10
16. Hughes PA, Harrington AM, Castro J, Liebregts T, Adam B, Grasby DJ, Isaacs NJ, Maldeniya L, Martin CM, Persson J, Andrews JM, Holtmann G, Blackshaw LA, Brierley SM: Sensory neuro-immune interactions differ between Irritable Bowel Syndrome subtypes. Gut 2012; [Epub ahead of print]
17. Malin S, Molliver D, Christianson JA, Schwartz ES, Cornuet P, Albers KM, Davis BM: TRPV1 and TRPA1 function and modulation are target tissue dependent. J Neurosci 2011; 31:10516–28
18. Cattaruzza F, Spreadbury I, Miranda-Morales M, Grady EF, Vanner S, Bunnett NW: Transient receptor potential ankyrin-1 has a major role in mediating visceral pain in mice. Am J Physiol Gastrointest Liver Physiol 2010; 298:G81–91
19. Uta D, Furue H, Pickering AE, Rashid MH, Mizuguchi-Takase H, Katafuchi T, Imoto K, Yoshimura M: TRPA1-expressing primary afferents synapse with a morphologically identified subclass of substantia gelatinosa neurons in the adult rat spinal cord. Eur J Neurosci 2010; 31:1960–73
20. Peng HY, Chen GD, Lai CY, Hsieh MC, Hsu HH, Wu HC, Lin TB: PI3K modulates estrogen-dependent facilitation of colon-to-urethra cross-organ reflex sensitization in ovariectomized female rats. J Neurochem 2010; 113:54–66
21. Peng HY, Chen GD, Lai CH, Tung KC, Chang JL, Lin TB: Endogenous ephrinB2 mediates colon-urethra cross-organ sensitization *via* Src kinase-dependent tyrosine phosphorylation of NR2B. Am J Physiol Renal Physiol 2010; 298:F109–17
22. Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, Herman JP, Marts S, Sadee W, Steiner M, Taylor J, Young E: Strategies and methods for research on sex differences in brain and behavior. Endocrinology 2005; 146:1650–73
23. el-Mas MM, Abdel-Rahman AA: Contrasting effects of urethane, ketamine, and thiopental anesthesia on ethanol-clonidine hemodynamic interaction. Alcohol Clin Exp Res 1997; 21:19–27
24. Gourcerol G, Wu SV, Yuan PQ, Pham H, Miampamba M, Larauche M, Sanders P, Amano T, Mulak A, Im E, Pothoulakis C, Rivier J, Taché Y, Million M: Activation of corticotropin-releasing factor receptor 2 mediates the colonic motor coping response to acute stress in rodents. Gastroenterology 2011; 140:1586–96.e6
25. Carini F, Lecci A, Tramontana M, Giuliani S, Maggi CA: Tachykinin NK(2) receptors and enhancement of cholinergic transmission in the inflamed rat colon: An *in vivo* motility study. Br J Pharmacol 2001; 133:1107–13
26. da Costa DS, Meotti FC, Andrade EL, Leal PC, Motta EM, Calixto JB: The involvement of the transient receptor potential A1 (TRPA1) in the maintenance of mechanical and cold hyperalgesia in persistent inflammation. Pain 2010; 148:431–7
27. Shimizu Y, Chang EC, Shafton AD, Ferens DM, Sanger GJ, Witherington J, Furness JB: Evidence that stimulation of ghrelin receptors in the spinal cord initiates propulsive activity in the colon of the rat. J Physiol 2006; 576(Pt 1):329–38
28. Chuang YC, Yoshimura N, Wu M, Huang CC, Chiang PH, Tyagi P, Chancellor MB: Intraprostatic capsaicin injection as a novel model for nonbacterial prostatitis and effects of botulinum toxin A. Eur Urol 2007; 51:1119–27
29. Wu HC, Chang CH, Peng HY, Chen GD, Lai CY, Hsieh MC, Lin TB: EphrinB2 induces pelvic-urethra reflex potentiation *via* Src kinase-dependent tyrosine phosphorylation of NR2B. Am J Physiol Renal Physiol 2011; 300:F403–11
30. Berkley KJ: A life of pelvic pain. Physiol Behav 2005; 86:272–80
31. Cason AM, Samuelsen CL, Berkley KJ: Estrous changes in vaginal nociception in a rat model of endometriosis. Horm Behav 2003; 44:123–31
32. Dalrymple A, Slater DM, Beech D, Poston L, Tribe RM: Molecular identification and localization of Trp homologues, putative calcium channels, in pregnant human uterus. Mol Hum Reprod 2002; 8:946–51
33. Dmitrieva N, Berkley KJ: Contrasting effects of WIN 55212-2 on motility of the rat bladder and uterus. J Neurosci 2002; 22:7147–53

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34. Alagiri M, Chottiner S, Ratner V, Slade D, Hanno PM: Interstitial cystitis: Unexplained associations with other chronic disease and pain syndromes. Urology 1997; 49(5A suppl):52–7
35. Prior A, Wilson K, Whorwell PJ, Faragher EB: Irritable bowel syndrome in the gynecological clinic. Survey of 798 new referrals. Dig Dis Sci 1989; 34:1820–4
36. Whorwell PJ, McCallum M, Creed FH, Roberts CT: Non-colonic features of irritable bowel syndrome. Gut 1986; 27:37–40
37. Streng T, Axelsson HE, Hedlund P, Andersson DA, Jordt SE, Bevan S, Andersson KE, Högestätt ED, Zygmunt PM: Distribution and function of the hydrogen sulfide-sensitive TRPA1 ion channel in rat urinary bladder. Eur Urol 2008; 53:391–9
38. Fernandes ES, Russell FA, Spina D, McDougall JJ, Graepel R, Gentry C, Staniland AA, Mountford DM, Keeble JE, Malcangio M, Bevan S, Brain SD: A distinct role for transient receptor potential ankyrin 1, in addition to transient receptor potential vanilloid 1, in tumor necrosis factor α-induced inflammatory hyperalgesia and Freund's complete adjuvant-induced monarthritis. Arthritis Rheum 2011; 63:819–29
39. Mitrovic M, Shahbazian A, Bock E, Pabst MA, Holzer P: Chemo-nociceptive signalling from the colon is enhanced by mild colitis and blocked by inhibition of transient receptor potential ankyrin 1 channels. Br J Pharmacol 2010; 160:1430–42
40. Bonet IJ, Fischer L, Parada CA, Tambeli CH: The role of transient receptor potential A 1 (TRPA1) in the development and maintenance of carrageenan-induced hyperalgesia. Neuropharmacology 2013; 65:206–12
41. Wei H, Karimaa M, Korjamo T, Koivisto A, Pertovaara A: Transient receptor potential ankyrin 1 ion channel contributes to guarding pain and mechanical hypersensitivity in a rat model of postoperative pain. Anesthesiology 2012; 117:137–48
42. Salas MM, Hargreaves KM, Akopian AN: TRPA1-mediated responses in trigeminal sensory neurons: Interaction between TRPA1 and TRPV1. Eur J Neurosci 2009; 29:1568–78
43. Ali DW, Salter MW: NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. Curr Opin Neurobiol 2001; 11:336–42
44. Berberich S, Punnakkal P, Jensen V, Pawlak V, Seeburg PH, Hvalby Ø, Köhr G: Lack of NMDA receptor subtype selectivity for hippocampal long-term potentiation. J Neurosci 2005; 25:6907–10
45. Liu F, Day M, Muñiz LC, Bitran D, Arias R, Revilla-Sanchez R, Grauer S, Zhang G, Kelley C, Pulito V, Sung A, Mervis RF, Navarra R, Hirst WD, Reinhart PH, Marquis KL, Moss SJ, Pangalos MN, Brandon NJ: Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. Nat Neurosci 2008; 11:334–43
46. Luque JM, Bleuel Z, Malherbe P, Richards JG: Alternatively spliced isoforms of the N-methyl-D-aspartate receptor subunit 1 are differentially distributed within the rat spinal cord. Neuroscience 1994; 63:629–35
47. Peng HY, Chen GD, Hsieh MC, Lai CY, Huang YP, Lin TB: Spinal SGK1/GRASP-1/Rab4 is involved in complete Freund's adjuvant-induced inflammatory pain *via* regulating dorsal horn GluR1-containing AMPA receptor trafficking in rats. Pain 2012; 153:2380–92
48. Peng HY, Chen GD, Lai CY, Hsieh MC, Lin TB: Spinal SIRPα1-SHP2 interaction regulates spinal nerve ligation-induced neuropathic pain *via* PSD-95-dependent NR2B activation in rats. Pain 2012; 153:1042–53
49. Petralia RS, Wang YX, Wenthold RJ: The NMDA receptor subunits NR2A and NR2B show histological and ultrastructural localization patterns similar to those of NR1. J Neurosci 1994; 14:6102–20
50. Moon IS, Apperson ML, Kennedy MB: The major tyrosine-phosphorylated protein in the postsynaptic density fraction is N-methyl-D-aspartate receptor subunit 2B. Proc Natl Acad Sci U S A 1994; 91:3954–8
51. Peng HY, Chen GD, Lai CY, Hsieh MC, Lin TB: Spinal serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates neuropathic pain *via* kalirin and downstream PSD-95-dependent NR2B phosphorylation in rats. J Neurosci 2013; 33:5227–40.
52. Peng HY, Chang CH, Tsai SJ, Lai CY, Tung KC, Wu HC, Lin TB: Protein kinase A-dependent spinal α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate-receptor trafficking mediates capsaicin-induced colon-urethra cross-organ reflex sensitization. Anesthesiology 2011; 114:70–83

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