Endogenous ephrinB2 mediates colon-urethra cross-organ sensitization via Src kinase-dependent tyrosine phosphorylation of NR2B

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Peng H, Chen G, Lai C, Tung K, Chang J, Lin T. Endogenous ephrinB2 mediates colon-urethra cross-organ sensitization via Src kinase-dependent tyrosine phosphorylation of NR2B. Am J Physiol Renal Physiol 298: F109–F117, 2010.—Recently, the role of Eph receptor (EphR) tyrosine kinase and their ephrinB ligands in spinal pain-related neural plasticity has been identified. To test whether Src-family non-receptor tyrosine kinase-dependent glutamateric N-methyl-D-aspartate receptor (NMDAR) NR2B subunit phosphorylation underlies lumbosacral spinal EphR activation to mediate cross-organ sensitization between the colon and the urethra, external urethra sphincter electromyogram activity evoked by pelvic nerve stimulation and protein expression in the lumbosacral (L6–S2) dorsal horn were studied before and after intracolonic mustard oil (MO) instillation. We found MO instillation produced colon-urethra reflex sensitization along with an upregulation of endogenous ephrinB2 expression as well as phosphorylation of EphB1/2, Src-family kinase, and NR2B tyrosine residues. Intrathecal immunoglobulin fusion protein of EphB1 and EphB2 as well as PP2 reversed the reflex sensitization and NR2B phosphorylation caused by MO. All these results suggest that EphBR-ephrinB interactions, which provoke Src-family kinase-dependent NMDAR NR2B phosphorylation at the lumbosacral spinal cord level, are involved in cross-organ sensitization, contributing to the development of viscero-visceral referred pain between the bowel and the urethra.

pelvic pain syndrome; urethra; irritable bowel syndrome; colon; NMDA

ALTHOUGH THE ETIOLOGY IS NOT fully understood, patients with irritable bowel syndrome (IBS) have a higher prevalence of lower urinary tract dysfunction with pelvic pain compared with control groups (15). In addition, pelvic visceral pain might not only arise from an inflamed/injured organ itself but might also be referred from other diseased viscera, and thus results in viscero-visceral referred pain (6). Despite the fact that bowel-to-urethra cross-organ sensitization has been demonstrated previously (25), little information is available to explain the underlying mechanism because it could involve complicated interactions in afferent inputs arising from different viscera, known as central sensitization (33).

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It is established that spinal glutamatergic N-methyl-D-aspartate receptor (NMDAR)-dependent neurotransmission underlies pain-related central sensitization (10, 11). The NMDAR NR2B subunit, as a major unit controlling NMDAR activity via phosphorylation mechanisms, was found to play a pivot role in the NMDA-dependent form of neural plasticity at the spinal cord level (10, 11, 17, 19, 20). Chronic inflammatory pain caused by Freund’s adjuvant injections was shown to be associated with increases in spinal NR2B subunit phosphorylation (10). Moreover, Slack and colleagues (28) revealed that intrathecal administration of Src-family kinase inhibitors prevented hyperalgesia-related NR2B subunit phosphorylation in the spinal dorsal horn. These findings imply that, at the spinal cord level, Src-family kinase could modulate NR2B subunits to mediate postinflammatory/neuropathic pain and/or hyperalgesia.

EphB receptors (EphBRs) are transmembrane molecules acting as guidance cues during neural development (32). Studies demonstrated that in the central nervous system (CNS), EphBRs and their ephrinB ligands play modulating roles in neural plasticity induction, mainly but not exclusively via interactions with NMDAR (9, 12). EphB tyrosine kinases were identified as a possible modulator of NMDAR. EphBR activation caused by immunoglobulin fusion protein of ephrinB2 (ephrinB2-Fc) induced Src kinase-dependent NR2B phosphorylation (31). Moreover, intrathecal injections of ephrinB2-Fc induced Src phosphorylation in the spinal cord. Together, these results suggest that ephrinB2 and its EphBR tyrosine kinase may modulate pain signaling probably via NMDARs at the spinal cord level (3).

At the lumbosacral spinal cord, our laboratory has recently demonstrated a novel form of pain-related central sensitization, cross-organ sensitization, in which instillation of mustard oil (MO) into the descending colon could sensitize the reflex activity of the urethra (21, 22). Pharmacological investigations indicated spinal NR2B subunit phosphorylation is crucial in the induction of cross-organ sensitization (19, 20, 23). Although the physiological/pathophysiological relevance is still without final proof, cross-organ sensitization was linked to the development of viscero-visceral referred pain in the pelvic area, for it is characterized by pathological enhancement of urethra activity caused by activation of nociceptive afferent fiber arising from visceral organs (24). Therefore, we hypothesize that the interactions between ephrinB2 and EphBRs as well as the downstream intracellular cascade are involved in cross-
organ sensitization. We tested this hypothesis using intact animal preparations, in which we instilled MO into the descending colon to produce urethra reflex sensitization.

**MATERIALS AND METHODS**

**Animal Preparations**

Female Sprague-Dawley rats (*n = 160; 205–290 g) were used in this experiment, which was reviewed and approved by the Institutional Review Board of Chung-Shan Medical University (Taiwan). Rats were anesthetized with urethane (1.2 g/kg ip). A PE-50 intracolonic catheter was inserted into the descending colon (4 cm from the anus), and another PE-10 catheter was inserted through a slit made at the atlanto-occipital membrane and passed caudally to the T13 vertebrae (L6–S2 spinal cord) for the dispensing of test agents. The right pelvic nerve was dissected and mounted on a pair of wire electrodes for stimulation. Oligo-single-unit action potentials in external urethra sphincter electromyogram (EUSE) activity were recorded by a pair of epoxy-coated copper wire electrodes placed ∼1–2 mm lateral to the urethra and were continuously recorded on a recording system (MP30, Biopac, Santa Barbara, CA). Single shocks at a fixed suprathreshold strength were repeated at 1 stimulation/30 s (test stimulation; TS) and given through the stimulation electrodes. The protocols for assessing the effects of different kinds of reagents on reflex activity were the following.

**Protocol 1: colon irritation.** MO (0.5%, 0.1 ml) or corn oil (CO) was instilled into the lumen of the descending colon 1 min before TS onset through an intracolonic catheter to induce acute colon irritation. The excitability of reflex activity was evaluated by applying the TS to the pelvic afferent nerve that was continuously recorded from TS starting for 120 min.

**Protocol 2: intracolonic pharmacological tests.** Lidocaine (0.5%, 0.1 ml) was instilled into the descending colon via the intracolonic catheter 10 min before TS started.

**Protocol 3: intrathecal pharmacological tests.** EphB1-Fc (10 μg, 10 μl), EphB2-Fc (10 μg, 10 μl), PP2 (50 μM, 10 μl), and N-2-amino-5-phosphonovalerate (APV; 10 μM, 10 μl) were injected via the intrathecal catheter 10 min before TS started.

**Application of Drugs**

Drugs administered included NMDA (10 μM, 10 μl it, Sigma), a selective glutamatergic NMDAR agonist (APV, 10 μM, 10 μl it, Sigma), a glutamatergic NMDAR receptor antagonist; ephrinB2-Fc chimera (5 μg/ rat it, Sigma), EphB receptor ligand; EphB1-Fc chimera (10 μg/ rat it, Sigma), EphB1-selective antagonist; EphB2-Fc chimera (10 μg/ rat it, Sigma), EphB2-selective antagonist; 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolof[3,4-d]pyrimidine (PP2; 50 μM, 10 μl it, Tocris) a Src-family kinase inhibitor; lidocaine hydrochloride (0.5%, 0.1 ml, intracolonic, AstraZeneca), a nerve conduction blocker; MO (0.5% diluted in CO, 0.1 ml, intracolonic, Sigma), a component causing acute colon irritation; and CO (intracolonic, Sigma), a vehicle control for MO. In all cases, solvent solutions of identical volume to tested agents were dispensed to serve as the vehicle control.

**Western Blotting**

Two hours after intracolonic MO/CO instillation, the spinal cords were obtained after a laminectomy. The right lumbarosacral (L6–S2) dorsal horns were dissected out and were immediately snap-frozen on liquid nitrogen. The protocol for Western blotting has been described elsewhere (22, 23); in brief, the sample was homogenized and the extracts were centrifuged to retain supernatants. The supernatant was separated on a polyvinylidene difluoride membrane and then were incubated for 1 h at room temperature in either rabbit anti-ephrinB2 (1:2,000, Santa Cruz Biotechnology), anti-phosphorylated EphB1/2 (pEphB1/2 1:2,000, Millipore), rabbit anti-phospho-Src antibody (1:1,000, Millipore), or rabbit anti-phosphoryr1336 or anti-phosphoryr1472-NR2B (1:1,000 Millipore). Blots were washed and incubated in peroxidase-conjugated donkey anti-rabbit IgG (1:5,000, Santa Cruz Biotechnology) or donkey antimouse IgG (1:10,000, Santa Cruz Biotechnology) for 1 h at room temperature. Protein bands were visualized using an enhanced hemiluminescence detection kit (ECL Plus, Millipore), and then densitometric analysis of the Western blot membranes was done with Science Lab 2003 (Fuji). Results were normalized against β-actin and are presented as the means ± SD.

**RNA Extraction and Quantitative RT-PCR**

The dissected right lumbarosacral spinal dorsal horns (L6–S2) were placed in RNAlater solution (Applied Biosystems). Total RNA was extracted using RNA extraction kits (GeneMark). Reverse transcription was performed using cDNA reverse transcription kits (Applied Biosystems). cDNA samples underwent absolute quantitative real-time PCR on the OneStep instrument (Applied Biosystems) the default 40-cycle program. TaqMan gene expression assays were purchased from Applied Biosystems (assay RN_00667869_ml and RN_00680474_ml). For each amplicon, PCR efficiency was estimated near 1.0 by serial dilutions of cDNA. Relative quantities of mRNA were estimated using the ΔΔCt method. RT-PCR of the β-actin gene was also performed, and the expression level of β-actin mRNA was used to serve as a normalized control. All the results of RT-PCR are presented as the means ± SD.

**Data Analysis**

Comparisons across drug- and vehicle-treated groups were determined using one-way, repeated-measures ANOVA, followed by a post hoc test without correction. In all cases, differences of *P < 0.05* were considered as statistically significant differences.

**RESULTS**

**MO Induced Cross-Organ Sensitization**

CO instillation into the descending colon exhibited no effect on the TS-evoked baseline EUSE reflex activity (Fig. 1A; TS + CO). Despite the fact that it induced no spontaneous background discharge (MO), intracolonic MO instillation produced reflex sensitization characterized by a high-frequency elongated firing in the evoked activity starting at the onset, which peaked at 60 min and was sustained for 120 min following TS onset (TS + MO). Summarized data showed MO instillation significantly increased the mean spike numbers evoked by TS (Fig. 1B; TS + MO) compared with CO instillation (TS + CO).

**MO Provoked EphrinB2 and pEphB1/2 Expression**

In rats that received the TS with MO instillation (Fig. 1, C and D; TS + MO), the expression of ephrinB2 (% of β-actin) and EphB1/2 phosphorylation (pEphB1/2, % of β-actin) in the lumbarosacral dorsal horn both significantly increased compared with those receiving the TS with CO instillation (TS + CO), while levels of β-actin remained unchanged.

**Involvement of Colon Afferent Fibers**

Compared with MO instillation-induced reflex sensitization (Fig. 2A; TS + MO), intracolonic pretreatment with lidocaine but not vehicle solution (data not shown) reversed the MO-
elicited reflex sensitization (TS+LID+MO), although it did not affect the baseline reflex activity evoked by the TS (TS+LID). Summarized data show that, compared with CO (TS+CO), intracolonic MO instillation (TS+MO) significantly increased the mean spike number evoked by the TS (TS+MO) compared with CO instillation (TS+CO). **P < 0.01 vs. TS+CO; n = 7. C and D: in rats that received TS in association with colon MO instillation (TS+MO), the expression of endogenous ephrinB2 (% of β-actin) and phosphorylated EphB1/2 (pEphB1/2; % of β-actin) both increased significantly compared with those that received the TS with CO instillation (TS+CO). *P < 0.05 vs. TS+CO; n = 4.

Involvement of EphB1 and EphB2 Receptors

Intracolonic MO (Fig. 3A; TS+MO) but not CO (TS+CO) instillation induced reflex sensitization. Rather than vehicle solutions (data not shown), prior administration of selective EphB1 and EphB2 receptor antagonists, Eph1B-Fc and Eph2B-Fc, both attenuated the MO-elicited reflex sensitization (TS+Eph1B+MO and TS+Eph2B+MO, respectively), while these agents did not affect the baseline reflex activity evoked by the TS (TS+Eph1B and TS+Eph2B, respec-

Fig. 1. Cross-organ reflex sensitization caused by intracolonic mustard oil (MO) instillation. A: compared with corn oil (TS+CO), which did not elicit any effects on the baseline reflex activity caused by the test stimulation (TS; single action potentials are indicated by arrows), MO instillation into the descending colon produced reflex sensitization (TS+MO) of the external urethra electromyogram (EUSE) activity, while it did not induce any spontaneous firing (MO). B: intracolonic MO instillation significantly increased the mean spike numbers evoked by the TS (TS+MO) compared with CO instillation (TS+CO). **P < 0.01 vs. TS+CO; n = 7. C and D: in rats that received TS in association with colon MO instillation (TS+MO), the expression of endogenous ephrinB2 (% of β-actin) and phosphorylated EphB1/2 (pEphB1/2; % of β-actin) both increased significantly compared with those that received the TS with CO instillation (TS+CO). *P < 0.05 vs. TS+CO; n = 4.
Summarized data show MO instillation (Fig. 3C; TS+MO) but not CO instillation (TS+CO) significantly increased the mean spike number evoked by the TS (**P < 0.01 vs. TS+CO; n = 7) that was reversed by intrathecal pretreatment with lidocaine (TS+LID+MO). ##P < 0.01 vs. TS+MO; n = 7. C: Western blotting showed that in rats that received TS in association with intracolonic MO instillation (TS+MO), the expression of ephrinB2 (% of β-actin) and pEphB1/2 (% of β-actin) were both increased significantly compared with those that received TS with CO instillation (TS+CO). *P < 0.05 vs. TS+CO; n = 4. Moreover, intracolonic treatment with lidocaine before MO instillation reversed the MO-elicited increments in the expression levels of ephrinB2 and pEphB1/2 (TS+LID+MO). #P < 0.05 vs. TS+MO; n = 4.

Involvement of Src-Family Kinase

Compared with animals that received the TS with MO instillation (Fig. 3A; TS+MO), pretreatment with PP2, but not vehicle solution (data not shown) prevented the MO-elicited reflex sensitization (Fig. 3B; TS+PP2+MO), while it showed no effects on the baseline reflex activity (TS+PP2). Summarized data demonstrated MO instillation (Fig. 3D; TS+MO) significantly increased the mean spike number evoked by the TS (TS+CO) that was reversed by PP2 injection (TS+PP2+MO), despite the fact that this treatment exhibited no effect on the mean spike number evoked by the TS (Fig. 3C; TS+PP2).

Involvement of NMDAR

Rather than vehicle solution (data not shown), intrathecal APV injection reversed the reflex sensitization caused by MO instillation (TS+APV+MO) compared with animals that re-
ceived the TS with MO instillation (Fig. 3A; TS+MO), while it did not affect the baseline reflex activity (Fig. 3B; TS+CO). Summarized data indicated MO instillation (Fig. 3D; TS+MO) significantly increased the mean spike number evoked by the TS that was reversed by intrathecal pretreatment with APV (Fig. 3D; TS+APV+MO), despite the fact that it caused no effect on the baseline reflex activity (Fig. 3C; TS+APV).
EphrinB2/EphBR Underlies Reflex Sensitization

In rats that received the TS with MO instillation (Fig. 4A; TS+MO), the ephrinB2 expression (% of β-actin) was significantly increased in the dorsal horn, and prior intrathecal EphB1-Fc, EphB2-Fc, PP2, and APV failed to reverse the increment in eprinB2 expression caused by MO (TS+EphB1+MO, TS+EphB2+MO, TS+PP2+MO and TS+APV+MO, respectively). In addition, compared with animals that received the TS with CO instillation (TS+CO), EphB1/2 phosphorylation (% of β-actin) was significantly increased by MO instillation (TS+MO) that was reversed by prior treatment with EphB1-Fc and EphB2-Fc (TS+EphB1+MO, TS+EphB2+MO, respectively) but not with PP2 or APV (TS+PP2+MO and TS+APV+MO, respectively) and vehicle solution (data not shown).

Src-Family Kinase Downstream of EphB2

 Src phosphorylation (Fig. 4B; % of β-actin) increased significantly in animals that received the TS with MO instillation (TS+MO) compared with CO instillation (TS+CO). Pretreatment with EphB1-Fc, EphB2-Fc, and PP2 (TS+EphB1+MO, TS+EphB2+MO and TS+PP2+MO, respectively) but not APV (TS+APV+MO) or vehicle solutions (data not shown) reversed the increment in pSrc expression caused by MO instillation.

Role of NMDAR NR2B Subunit

Compared with rats that received the TS with CO instillation (Fig. 4C; TS+CO), pY1336 and pY1472 both increased significantly (% of β-actin) in animals that received the TS with MO instillation, which was reversed by prior treatment with EphB1-Fc, EphB2-Fc, PP2, and APV (TS+EphB1+MO, TS+EphB2+MO, and TS+PP2, respectively) but not APV (TS+APV+MO) or vehicle solutions (data not shown).
EphB2-Fc, and PP2 (TS+EphB1+MO, TS+EphB2+MO and TS+PP2+MO, respectively) but not vehicle solutions (data not shown). Next, we examined mRNA expression of the NR2B subunit by qPCR using specific probes. Figure 5A shows that, compared with CO (TS+CO), MO instillation into the descending colon significantly increased the mRNA expression of NR2B (TS+MO) that was reversed by prior treatment with lidocaine (TS+LID+MO), but not vehicle solution (data not shown). Moreover, intracolonic MO instillation significantly increased the mRNA level of NR2B subunit (% of β-actin) under 40 cycles of amplification (Fig. 5B; TS+MO) compared with rats that received TS with CO (TS+CO). Intrathecal pretreatment with EphB1-Fc, EphB2-Fc, and PP2, but not vehicle solution (data not shown) reversed the MO-elicited increment in mRNA (TS+EphB1+MO, TS+EphB2+MO and TS+PP2+MO, respectively).

DISCUSSION

In this study, we found intracolonic MO instillation induced cross-organ sensitization with corroborated increments of endogenous ephrin2B and the phosphorylation state of EphB1/2 that were both abolished by intrathecal pretreatment of EphBR selective antagonists. Moreover, MO instillation also induced a parallel increment in the phosphorylation of Src-family kinase. Intrathecal application of a Src-family kinase inhibitor blocked MO-elicited reflex sensitization accompanied by a reversal in Src-family kinase phosphorylation. Finally, in association with the MO-elicited reflex sensitization, the expression levels of NR2B Tyr1336 and Tyr1472 increased. Intrathecal pretreatment with a Src-family kinase inhibitor reversed the reflex sensitization of the increment in Tyr1336 and Tyr1472 phosphorylation. Taken together, these results suggest the development of cross-organ sensitization caused by intracolonic MO instillation may be due, at least in part, to the phosphorylation of specific tyrosine residues of the NMDAR NR2B subunit caused by Src-family tyrosine kinase downstream of the interactions between EphBR and its endogenous ephrinB2 ligand at the lumbosacral spinal cord level.

Recent studies have demonstrated that intrathecal administration of an ephrinB2-Fc chimera, which activates EphBRs, induced thermal hyperalgesia (28). Abe et al. (1) reported that expression of ephrinB2 in the dorsal root ganglion (DRG) neurons and EphB1 receptor in the dorsal horn were both enhanced by nerve injuries in adult rats. Administration of ephrinB2 small interfering RNA decreased expression of ephrinB2 and mechanical allodynia caused by crushed nerves (14). Furthermore, chronic constriction nerve injury induces an upregulation of ephrinB1 and EphBR in rat spinal cord and DRG (29, 30). In this study, we demonstrated that intracolonic MO instillation sensitized the urethral reflex activity in association with an upregulation of endogenous ephrinB2 expression and EphB1/2 phosphorylation. These results extend the role of the interaction between EphBRs and endogenous ephrinB2 at the spinal cord level as not only required for the onset of various form of pain/hyperalgesia arising from injured tissues themselves but also essential for the development of viscerovisceral referred pain between the bowel and the urethra.

It is established that NMDAR-mediated activity-dependent neural plasticity is essential for inflammatory/neuropathic pain at the spinal cord level (4). NMDAR function is modulated by phosphorylation mechanisms caused by neighboring activated receptors (16). Although the relative importance of the different pathways is still controversial, studies investigating pain processing have focused on the Src-dependent phosphorylation of the NR2B subunit, which is crucial for NMDAR localization and endocytosis (8). Studies investigating neuropathic pain revealed that NR2B subunit phosphorylation at residue Tyr1472 in dorsal horn neurons is essential for maintenance of neuropathic pain (1). NR2B subunit tyrosine phosphorylation was also found to play a pivotal role in postinflammatory hyperalgesia (10,11). Our result is analogous to these results showing that NR2B subunit tyrosine residue phosphorylation plays a role in pain processing. Moreover, this is the first study showing that intracolonic MO instillation, which elicited sensitization on urethra reflex activity, induced NR2B phosphorylation on more than one tyrosine residues, i.e., Tyr1336 and Tyr1472. However, there are 25 tyrosine residues in the carboxyl tail of the NR2B subunit that could be phosphory-
lated, including Tyr1252, Tyr1336, and Tyr1472 (18). Since only antibodies specific to pTyr1336 and pTyr1472 were used for immunoblotting in this study, we do not know how many of these residues phosphorylated in our model, in addition to Tyr1336 and Tyr1472. Further investigations are needed to clarify the precise sites and numbers of tyrosine residue phosphorylation. In addition, Ali and Salter (2) reported Src-family kinase may target subunits other than NR2B. Therefore, the possibility of Src-family kinase phosphorylating other NMDAR subunit(s), e.g., NR2A, to underlie pain onset, cannot be ruled out. Furthermore, since EphBRs could also potentially regulate AMPA receptor trafficking to underlie forms of activity-dependent neural plasticity (5), we cannot exclude the possibility that EphBR activation may regulate the function of other receptors, in addition to the NMDA receptor, to participate in the development of cross-organ sensitization.

EphBR tyrosine kinases and their ephrinB ligands are reported to be involved in the processing of pain input through Src-family kinase-dependent modulations of NMDAR (3, 28). Activation of EphBR induced behavioral thermal hyperalgesia and increments in the level of Src phosphorylation bound to EphBRs in the dorsal horns. This phenomenon could be counteracted by a prior injection of the NMDAR antagonist MK801 (28), implying that Src-family kinase-dependent NMDAR activation plays a role in EphBR-mediated pain processing. Both the thermal hyperalgesia and NR2B phosphorylation caused by intrathecal ephrinB2-Fc were reversed by the Src-family inhibitor (3). In addition, by analyzing subcellular fractions Goebel-Goody et al. (7) demonstrated Src-family kinase inhibits the NMDAR NR2B1472 phosphorylation and NMDA trafficking in hippocampal slices. In the ventral tegmentum area, cocaine induced NMDAR NR2 subunit phosphorylation that was reversed by Src-family kinase (27). Together, these results suggest that NMDAR phosphorylation downstream of the activation of Src-family kinase enhances neural activity.

Our results are consistent with these findings, and we suggest that, analogous to what is shown in cultured embryonic neurons (31) and in vivo preparations (10), Src-family kinases are responsible for tyrosine phosphorylation of the NR2B subunit in inflammatory pain. However, Salter and Kalia (26) indicated that both Fyn and Src play roles in NMDAR phosphorylation. In the present study, we cannot exclude that a combination of these two Src-family kinases may be responsible for ephrinB2-mediated NR2B phosphorylation presented in this study.

Conditions such as IBS, chronic pelvic pain, and chronic prostatitis all typically involve chronic pain. This study demonstrates a spinal EphBR-dependent cross-organ sensitization between the descending colon and urethra that commences almost immediately after MO instillation and lasts for at least 120 min. Although this study offers an animal model of the neural mechanism involved in the development of cross-organ sensitization underlying viscerovisceral pain, this model is limited by insult acuity and the subsequent measurement interval. To clarify the role of cross-organ sensitization in chronic pain, long-term study is needed. In fact, interactions between EphBR and NMDAR are involved in chronic neuropathic rather than acute pain (3). Behavior studies that investigated neuropathic pain caused by sciatic nerve ligation showed time-dependent upregulation of EphrinB and EphBR in DRG and dorsal horn neurons, which peaks at 7 and lasts for 21 days after nerve ligation. This suggests a role for the interactions between EphrinB/EphBR and NMDAR in the induction and maintenance of chronic pain (3, 29). An intrathecal EphBR activator also induces thermal hyperalgesia and mechanical allodynia correlated with a reduced long-term spinal potentiation threshold between nociceptive C-afferent fibers and dorsal horn neurons in naive animals. Conversely, pharmacological blocking of EphBR using EphB-Fc prevents nerve injury-induced thermohyperalgesia and mechanical allodynia, and produces a reversal of enhanced long-term potentiation.

This suggests that the effects of ephrinB2/EphB on induction/maintenance of neuropathic pain depend on regulation of both neuronal excitability and spinal synaptic plasticity (30). We propose that sustained irritation of pelvic viscera may establish pathological neural plasticity in the dorsal horn and eventually cause chronic pain. Unpublished data have shown that ephrinB2/EphB interactions modulate activity-dependent neural plasticity in naive animals, although a detailed mechanism remains elusive.

Because the anal sphincter and descending colon are essentially parts of the same organ system, colon-anal reflex sensitization simply involves the lower gastrointestinal tract itself and is not a cross-organ response. Using fluorescent retrograde tracers, Kane et al. (13) demonstrated that pudendal motor nerves bifurcate into separate fascicles that innervate the external anal sphincter (EAS) and external urethra sphincter (EUS). Because both are essentially innervated by the same nerve root, we propose that acute irritation of the descending colon sensitizes EAS by activating lower gastrointestinal tract peripheral nerves. The nerves innervating the EUS share a common origin with EAS nerves, so the reflex activity of EUS is thus sensitized. This proposal does not dilute the importance of cross-organ reflex sensitization, as complex communication within the nervous system is essential to referred pain induction. In contrast, our study provides a possible animal model for pathological cross-talk development between the anus and urethra, which suggests a higher prevalence of lower urinary tract pain associated with IBS. The limitation of this technique is that we failed to simultaneously record reflex activity of the EAS in response to intracolonic MO instillation in this study. However, in a separate experiment (data not shown), we did not detect any increased EAS response at up to 60 min after MO treatment. These results seem to be counterintuitive and cannot be explained neuroanatomically. Further studies that elucidate the reflex activity of EAS during EUS reflex sensitization should be performed to shore up this animal model.

In conclusion, our findings add to the understanding of the ephrin/Eph receptor system, which is an important player in cross-organ sensitization, acting as a modulator of NMDAR in the adult spinal cord in vivo. These results help bring to light the mechanisms underlying central sensitization in the spinal cord, which is considered essential to the onset and maintenance of different forms of referred pain.

REFERENCES


