Region-specific changes in the phosphorylation of ERK1/2 and ERK5 in rat micturition pathways following cyclophosphamide-induced cystitis

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Qiao LY, Gulick MA. Region-specific changes in the phosphorylation of ERK1/2 and ERK5 in rat micturition pathways following cyclophosphamide-induced cystitis. Am J Physiol Regul Integr Comp Physiol 292: R1368–R1375, 2007.—Chronic inflammation of the urinary bladder generates hyperalgesia and allodynia. Growing evidence suggests a role of ERK in mediating somatic and visceral pain processing. In the present studies, we characterized and compared the activation of two ERK isoforms, ERK1/2 and ERK5, in micturition pathways, including the urinary bladder, lumbosacral dorsal root ganglia (DRG), and spinal cord in adult female and male rats before and after cyclophosphamide (CYP)-induced bladder inflammation. Results showed differential activation of ERK1/2 and ERK5 in these regions following cystitis. The level of phospho-ERK1/2 but not phospho-ERK5 was increased in the urinary bladder; the level of phospho-ERK5 but not phospho-ERK1/2 was increased in DRG; and the level of phospho-ERK1/2 but not phospho-ERK5 was increased in lumbar spinal cord following cystitis compared with control. Cystitis-induced upregulation of phospho-ERK1/2 and phospho-ERK5 was time dependent and showed similar patterns in female and male rats. The level of phospho-ERK1/2 in bladder was increased at 2 and 8 h after CYP injection; the level of phospho-ERK5 in DRG was increased at 8 and 48 h after CYP injection; and the level of phospho-ERK1/2 in lumbar spinal cord was increased at 48 h after CYP injection. The result that phospho-ERK5 was exclusively increased in DRG neurons, while phospho-ERK1/2 was increased in the spinal cord and the urinary bladder after cystitis, suggests a region-specific effect of neurotrophins on micturition pathways following bladder inflammation.

bladder inflammation; mitogen-activated protein kinase; dorsal root ganglia; spinal cord; lower urinary tract

EXTRACELLULAR SIGNAL-REGULATED kinases (ERK), a subfamily of mitogen-activated protein kinases (MAPK), are activated by neurotrophins or neuronal activities and play an important role in neural plasticity in the central or peripheral nervous systems by modulating gene expression and ion channel activation (15, 16, 41). Recent studies suggest that ERK may play a role in regulating nociceptive activities in primary sensory pathways after pathologic irritation of the peripheral system, such as peripheral nerve injury or inflammation (17, 24). Increased phosphorylation of ERK1/2 has been observed in rat spinal cord dorsal horn neurons in response to noxious stimulation of the peripheral tissue or electrical stimulation to the peripheral nerve (18). ERK1/2 was also phosphorylated in dorsal root ganglia (DRG) neurons following peripheral inflammation or nerve injury, which led to the activation and hypersensitivity of primary afferents (31, 32). In a murine model of visceral pain and hyperalgesia, intracolonic instillation of either capsaicin or mustard oil significantly induced the activation of ERK1/2 in lumbosacral spinal cord (12). Intrathecal injection of MEK inhibitor U-0126 or PD-98059, which specifically attenuates ERK activity, alleviated pain behavior induced by inflammation to hind paw (18, 31), viscera (4, 12), or joint (41). Taken together, these studies suggest an essential role of ERK1/2 in the generation and maintenance of inflammation-induced hyperalgesia.

Cyclophosphamide (CYP)-induced chronic bladder inflammation resembles the clinical syndrome interstitial cystitis, a painful urinary symptom that affects both men and women. Interstitial cystitis can alter the properties of sensory pathways and influence the micturition reflex circuitry, including alterations in neurochemical (38, 39, 48) and electrophysiological (52) properties of bladder afferent neurons. Altered visceral sensation from the urinary bladder with bladder inflammation may be mediated by at least two factors: 1) changes in the properties of the urinary bladder and 2) changes in the properties of peripheral bladder afferent pathways, such that bladder afferent neurons and/or spinal neurons respond in an exaggerated manner to normally innocuous stimuli. Recent studies with CYP-induced cystitis in female rats showed that innocuous and noxious bladder distension significantly increased the number of spinal neurons immunoreactive to phospho-ERK1/2 in CYP-inflamed animals; intrathecal blockade of ERK1/2 activity with PD-98059 significantly decreased micturition frequency in inflamed animals but had no effect on bladder reflex contractions of noninflamed bladder (4). However, it is not clear whether CYP-cystitis also induces phospho-ERK1/2 or other isoforms of ERK, such as ERK5 in the DRG neurons that are also affected by intrathecal inhibition.

Previous studies showed increased expression of neurotrophins in the urine of patients with cystitis (35), as well as in CYP-induced inflamed rat bladder (34). Neurotrophins influence neuronal cell biological function through two mechanisms: 1) activation of signal transduction cascades at nerve terminals and 2) activation of signal transduction cascades in the neuronal cell body through retrograde transport (3). Both mechanisms involve binding of receptor tyrosine kinase (Trk) and activation of ERK and/or phosphatidylinositol 3-kinase (PI3-kinase), which leads to activation of transcription factor and gene expression (40, 50). Upon stimulation of nerve terminals with neurotrophins in cultured neurons, ERK5 was activated in the neuronal cell body and led to phosphorylation of transcription factor CREB (p-CREB), a cAMP-responsive element binding protein (51). Previously, we identified that

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neurotrophin receptors TrkA and TrkB were significantly increased in the inflamed rat bladder and bladder afferent neurons after CYP injection (28, 38). The level of p-CREB was also increased in DRG after cystitis (39). The increased expression of Trk in the inflamed bladder and DRG may lead to the activation of ERK1/2 and/or ERK5, which further contributes to the activation of p-CREB.

In the present study, we aim to characterize the changes in phospho-ERK1/2 and phospho-ERK5 in the urinary bladder, lumbosacral DRG, and spinal cord following CYP-induced bladder inflammation in rat to determine the correlation between ERK activation and CYP-induced bladder overactivity. The resulting region-specific and time-dependent changes in phospho-ERK1/2 and phospho-ERK5 in bladder, DRG, and spinal cord in both female and male animals implied that retrograde neurotrophin signaling is most likely involved in primary afferent activation following cystitis. Part of the data has been reported as an abstract form in Society for Neuroscience Annual Meeting (37).

MATERIALS AND METHODS

Experimental Animals and Reagents

Adult male and female rats (150–200 g) from Harlan Sprague Dawley (Indianapolis, IN) were used. All experimental protocols involving animal use were approved by the Institutional Animal Care and Use Committee at the Virginia Commonwealth University. Animal care was in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care and National Institutes of Health guidelines. All efforts were made to minimize the potential for animal pain, stress, or distress, as well as to reduce the number of animals used. CYP and other chemicals used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO). Antibodies against total ERK1/2, total ERK5, phospho-ERK1/2, and phospho-ERK5 were from Cell Signaling Technology (Danvers, MA). Western blot secondary antibody was from the enhanced chemiluminescence (ECL) kit purchased from Pierce Biotechnology (Rockford, IL).

CYP-Induced Cystitis

CYP-cystitis was induced in rats by the technique previously documented and described in detail (38, 39, 48). Briefly, acute cystitis was induced in rats by injecting CYP intraperitoneally at a single dose of 150 mg/kg for 2, 8, or 48 h. To induce chronic cystitis, rats were injected with CYP intraperitoneally at a dose of 75 mg/kg every third day for 10 days (38). Control rats received volume-matched injections of saline. All injections were performed under isoflurane (2%) anesthesia. After CYP treatment, the urinary bladder increased in weight (2- to 3-fold, $P < 0.05$) and showed changes including regions of mucosal erosion, ulceration, and edema. Chronic cystitis also showed petechial hemorrhages and increase in the thickness of bladder wall, consistent with our previous results (38, 39).

Protein Extraction

The urinary bladder, lumbosacral DRG (combined L1, L2, L6, and S1) and L1, L6, and S1 spinal cord segments were freshly dissected out and homogenized in solubilization buffer containing 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 100 mM NaF supplemented with protease and phosphatase inhibitor cocktails (Sigma). The homogenate was centrifuged at 20,200 $g$ for 10 min at 4°C, and the supernatant was removed to a fresh tube for further analysis. The protein concentration was determined using a Bio-Rad DC protein assay kit.

Western Blot Analysis

Proteins were separated on a 7.5–10% SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk in Tris-buffered saline for 1 h and then incubated with phospho-ERK1/2 (1:1,000) or phospho-ERK5 (1:1,000) antibody followed by horseradish peroxidase-conjugated secondary antibody. The bands were identified by ECL. For internal loading control, the same membrane was stripped and reprobed with antibody against the nonphosphorylation form of ERK1/2 (1:1,000) or ERK5 (1:1,000); or the same amount of proteins was loaded on two separate gels for detection of phosphoprotein and a nonphosphorylation form of ERK1/2 or ERK5.

Quantification and Statistics

Western blot analysis. The ECL-exposed films were digitized, and densitometric quantification of immunoreactive bands was performed by using the software FluorChem 8800 (Alpha Innotech, San Leandro, CA). The density of the specific bands for phospho-ERK1/2 was normalized with the density of the bands for nonphosphorylation form of ERK1/2. The density of the specific band for phospho-ERK5 was normalized with the density of the band for the nonphosphorylation form of ERK5. The expression level of the phosphoprotein in the control animal from each independent experiment was considered as one, and the relative expression level of the phosphoprotein in experimental animals was adjusted as a ratio to its control in each independent experiment. The results were presented as means ± SE with $n$ being number of animals.

Statistical analysis. Comparison between control and experimental groups was made by using one-way ANOVA followed by Dunnett’s test. Differences between means at a level of $P \leq 0.05$ were considered to be significant.

RESULTS

Time-Dependent Upregulation of Phospho-ERK1/2 but not Phospho-ERK5 in Rat Urinary Bladder Following CYP-Induced Cystitis

Changes in the urinary bladder following intraperitoneal injection of CYP were consistent for both female and male rats. These changes include increased bladder weight and signs of inflammation, such as petechial hemorrhages on the bladder surface (2, 38). After the extraction of protein from the urinary bladder of control and animals treated with CYP for 2, 8, or 48 h or 10 days, the expression levels of phospho-ERK1/2 or phospho-ERK5 were examined in the extracts via Western blot analysis (Figs. 1A and 2).

In the urinary bladder of female rats, the level of phospho-ERK1/2 was dramatically increased at 2 h after CYP-injection (3.8-fold, $P < 0.05$) and gradually declined to near normal level at 48 h after inflammation (Fig. 1). Within the time course examined, the peak expression of phospho-ERK1/2 was shown at 2 h after CYP-injection (Fig. 1).

In the urinary bladder of male rats, we examined the expression level of phospho-ERK1/2 at 2, 8, and 48 h and 10 days following CYP-induced cystitis. The results confirmed an increased expression level of phospho-ERK1/2 in the urinary bladder at 2 and 8 h after CYP injections. As in female rat bladder, the level of phospho-ERK1/2 declined at 48 h and 10 days after CYP injection (Fig. 2A).

In both the female and male rat bladder, Western blot analysis of phospho-ERK5 showed that there was no detectable level of phospho-ERK5 expressed in the urinary bladder in...
control animals, whereas the urinary bladder expressed non-phosphorylated form of ERK5 before and after CYP treatment (Fig. 2B). Cystitis did not cause changes in the level of phospho-ERK5 in the inflamed bladder at any time point examined in either male or female rats (Fig. 2B). Figure 2B shows an example from male rats. Positive control for phospho-ERK5 was from a sample from DRG (see Figs. 3 and 4).

**Time-Dependent Upregulation of Phospho-ERK5 but not Phospho-ERK1/2 in Lumbosacral DRG Following CYP-Induced Cystitis**

After CYP treatment, lumbosacral DRG L1, L2, L6, and S1 were collected from female and male rats, respectively, and processed for Western blot analysis of phospho-ERK5 (Figs. 3 and 4) or phospho-ERK1/2 (Fig. 5).

In the female rat DRG, there were no significant changes in the level of phospho-ERK5 detected at 2 h after CYP treatment (Fig. 3). However, significant increases in the level of phospho-ERK5 were observed at 8 h and then gradually declined during the time course. Results were presented as means ± SE from 4–5 animals at each time point. *P < 0.05.

**Fig. 3.** Expression of phospho-ERK5 in female rat lumbosacral DRG following CYP-induced cystitis. Western blot analysis (A) shows that the activation of ERK5 in the DRG was initiated at 8 h after CYP treatment. A histogram (B) shows the time course changes in ERK5 phosphorylation in DRG after CYP injection. Results were presented as means ± SE from 3–5 animals at each time point. *P < 0.05.

In the male rat DRG, the activation of ERK5 was initiated at 8 h after CYP treatment (Fig. 4). The same experiment was performed for 3 more animals at each time point, and consistent results were observed but not shown here.
pho-ERK5 in the DRG were detected at 8 and 48 h (1.4–1.5 fold, \( P < 0.05 \)) after cystitis (Fig. 3). At 10 days after cystitis, the level of phospho-ERK5 returned to near normal in DRG (Fig. 3B). Similar experiments from male rats showed that the level of phospho-ERK5 was increased at 8 h after CYP-induced cystitis and then gradually declined until 10 days after inflammation (Figs. 4 and 6).

The expression of phospho-ERK1/2 in lumbosacral DRG was examined at 8 and 48 h and 10 days following CYP-induced cystitis. In both male and female rats, no significant changes in the level of phospho-ERK1/2 were detected in the lumbosacral DRG at any time point following CYP treatment compared with control when normalized with total ERK1/2 protein level (Figs. 5 and 6).

![Fig. 5](image1)

**Fig. 5.** Expression of phospho-ERK1/2 in male (A) and female (B) rat lumbosacral DRG following CYP-induced cystitis. Western blot analysis shows that the expression of phospho-ERK1/2 was not changed in both male (A) and female (B) rat DRG with CYP treatment at any time point examined.

**Differential Changes in Phospho-ERK1/2 and Phospho-ERK5 in Lumbosacral Spinal Cord Following Cystitis**

The changes in phospho-ERK5 and phospho-ERK1/2 were examined from L1, L6, and S1 spinal cord with Western blot analysis (Fig. 7) in both female and male rats. In L1 spinal cord, the upregulation of phospho-ERK1/2 was observed at 48 h and declined to near normal level by 10 days after cystitis (Fig. 7A). Phospho-ERK5 was not detected in L1 spinal cord at any time point following cystitis, while these spinal cord segments expressed nonphosphorylated form of ERK5 (Fig. 7B). In L6 and S1 spinal cord, no significant changes in phospho-ERK1/2 were observed at any time point examined following cystitis (Fig. 7, C–E). As well, phospho-ERK5 was not detected in L6 and S1 spinal cord at any time point following cystitis (data not shown). Positive control for phospho-ERK5 was from a sample from DRG (see Figs. 3 and 4).

**DISCUSSION**

The aim of this study was to determine whether CYP-induced cystitis involved changes in the phosphorylation of ERK1/2 and ERK5 in micturition pathways, including the urinary bladder, lumbosacral DRG, and spinal cord. Results demonstrated that cystitis induced differential activation of ERK1/2 and ERK5 in a region and time-dependent manner in both female and male rats. First, cystitis induced ERK1/2 phosphorylation in the urinary bladder at as early as 2 h and was sustained until 8 h following CYP injection in female rats. The increased expression of phospho-ERK1/2 in the urinary bladder was also observed in male rats at 2 and 8 h following cystitis. There were no changes in the level of ERK5 phosphorylation at any time point examined in both female and male rat urinary bladder after cystitis. Secondly, cystitis induced ERK5 phosphorylation in lumbosacral DRG at a relatively later time phase of 8 to 48 h following cystitis in both female and male rats. The level of phospho-ERK5 peaked at 8 h and gradually declined during chronic state of cystitis. There were no significant changes in ERK1/2 phosphorylation in the DRG after cystitis compared with control. Finally, we characterized the changes in ERK1/2 phosphorylation and ERK5 phosphorylation in lumbosacral spinal cord that receives the central projections of bladder primary afferent fibers (29, 30). We found that phospho-ERK1/2 was increased in L1 spinal cord at 48 h following cystitis compared with control in both male and female rats. There were no changes in phospho-ERK1/2 in L6 and S1 spinal cord following cystitis. There were no detectable levels of phospho-ERK5 in the L1, L6, and S1 spinal cord from both control and experimental animals when Western blot analysis was applied. These results suggested that bladder inflammation initially triggered ERK1/2 activation at the inflammatory site, and then resulted in the activation of ERK5 in the primary afferent cell body in the DRG, which may then trigger the activation of ERK1/2 in primary afferent nerve terminal located in the spinal cord.

![Fig. 6](image2)

**Fig. 6.** Histogram showing the expression levels of phospho-ERK1/2 and phospho-ERK5 in DRG after CYP-induced cystitis. After CYP treatment, significant increases in the phospho-ERK5 was observed in male rat DRG. ERK5 was activated in the DRG at 8 h after CYP injection and then gradually declined. This was consistent with the results from female rat DRG. However, there were no significant changes in the level of phospho-ERK1/2 in the DRG from both male and female animals. Results were presented as means ± SE from 3–5 animals at each time point. *\( P < 0.05 \).
Interstitial cystitis is a chronic inflammatory condition of the urinary bladder that affects an estimated one million people in the United States, both men and women. In this study, we injected CYP intraperitoneally to induce cystitis in adult male and female rats due to the urotoxicity effects of CYP metabolite acrolein. CYP cystitis resembles many symptoms of interstitial cystitis patients including 1) a reduction in the volume threshold to elicit micturition, 2) a reduction in the mean amplitude of bladder contractions, and 3) an increase in micturition frequency. In man, the most common urological complication associated with CYP treatment is cystitis (22, 46). In this study, we injected rats with a single dose of CYP (150 mg/kg ip) for 2, 8, and 48 h or multiple doses of CYP (75 mg/kg ip) every third day for 10 days to induce cystitis (38, 39, 48). This treatment regimen produces significant changes in the urinary bladder, including an increase in the bladder weight, increase in the thickness of bladder wall, signs of inflammation, such as mucosal erosion, and petechial hemorrhages on the bladder surface.

Previous studies demonstrated that CYP-cystitis in rat induced considerable secondary neurological responses, including changes in neurochemicals in the inflamed bladder and primary sensory pathways (28, 38, 39, 48). Primary afferent sensitization, in return, can contribute to hyperactivity of visceral organs (7, 10, 26). In the present study, we determined that these changes also involved the activation of ERK in the inflamed bladder, lumbosacral DRG, and spinal cord. Recent studies demonstrated that ERK1/2 was rapidly activated in the spinal cord neurons following peripheral inflammation or nociceptive stimuli of visceral organs and may participate in the modulation of somatic and visceral pain processing (4, 12, 17). In the chronic state of bladder inflammation induced by CYP, although no changes were noted in the spinal phospho-ERK1/2 in the inflamed animal with sham manipulation of the urinary bladder, normal innocuous bladder distension significantly increased spinal phospho-ERK1/2 in the inflamed animals but not in the noninflamed animals (4), together with our results, suggesting that ERK may participate in mediating bladder reflex overactivity accompanying bladder inflammation.

Bladder physiology is sensitive to numerous factors and changed within hours to weeks in response to peripheral organ disorders or injury and disease of the nervous system (5, 14, 36). Urodynamic studies demonstrated increased bladder activity at 30 min after colitis and 4 h or may be earlier after CYP injection (14, 36). In this study, we showed that the expression of phospho-ERK1/2 in the inflamed bladder peaked at 2 to 8 h.
after CYP injection. We did not examine the kinase activity in the period prior to 2 h after CYP treatment, but this does not exclude the possibility of ERK1/2 activation at an earlier time point. After 48 h of cystitis, the level of phospho-ERK1/2 in the inflamed bladder started to decline although, at this time point, the bladder still showed inflammation and hypertrophy. The mechanism is unknown, but the results suggest that cystitis induced a transient rather than a sustained activation of ERK1/2 in the bladder. Multiple doses of CYP injection (chronic cystitis) did not further change the level of phospho-ERK1/2 in the inflamed bladder compared with control. The long-term contribution of phospho-ERK1/2 to bladder overactivity may be due to phospho-ERK1/2-mediated ion channel (such as potassium channel) regulation (13, 41, 47) and gene expression.

Following cystitis, there were considerable changes in the expression of growth factors (20) and neurotrophins (23, 34, 35, 49) in the inflamed bladder. Cystitis also involves changes in cytokines (25) and neurotransmitters, such as glutamate and receptor (27) in the lower urinary tract pathways. These changes may contribute to the activation of intracellular signaling molecules, such as ERK-MAPK, as well as changes in the electrophysiological properties in the inflamed bladder and bladder afferent neurons (47, 52). For example, increases in the level of nerve growth factor (NGF) and its high-affinity receptor TrkA have been demonstrated in the inflamed bladder of animals or urine of the patients with cystitis (23, 28, 34, 35). In the inflamed bladder, NGF may bind to TrkA and initiate signaling transduction cascades, including activation of ERK1/2 or PI3-kinase/Akt pathways. In the dorsal horn neurons, glutamate and brain-derived neurotrophic factor play an important role in ERK activation (19, 21, 53). Administration of NMDA receptor antagonists dose-dependently reduced CYP-induced bladder overactivity (27).

Activation of ERK5 in the DRG was initiated at 8 h after CYP injection. At 2 h after CYP injection, there was no change in the level of phospho-ERK5 in DRG, while the level of phospho-ERK1/2 was increased in the bladder at this time point. This suggests a delayed activation of ERK5 in the DRG compared with the onset of ERK1/2 phosphorylation in the inflamed bladder. In cultured neurons, ERK5 was activated in the remote neuronal cell body via retrograde neurotrophin signaling (51). This may explain why ERK5 was not activated in the inflamed bladder and the temporal pattern of ERK1/2 and ERK5 activation following bladder inflammation. The role of neurotrophins in target organ to neuron interaction has been investigated in several animal models. For example, the hypertrophied bladder after bladder outlet obstruction exhibits markedly increased levels of NGF, and autoimmunization against NGF reduces the pelvic ganglia neuronal hypertrophy (11, 43–45). This suggests that neurotrophins released in the hypertrophied bladder are partly responsible for the change in neuronal morphology. Increased access to bladder neurotrophins and/or increased retrograde transport may increase the expression of Trks, phospho-ERK, and p-CREB phosphorylation in the bladder afferent neurons. Thus, neurotrophic factors expressed in the urinary bladder after cystitis may contribute to ERK5 activation and further induce p-CREB expression in DRG.

Several researchers have reported increased phospho-ERK1/2 in DRG following peripheral inflammation or nerve injury. Hind paw inflammation evoked increased phospho-ERK1/2 in DRG (1). Phospho-ERK1/2 was not only expressed in DRG neurons but also in the surrounding satellite neurons following inflammation or nerve injury (1, 8, 32). Intense noxious peripheral stimuli or electrical stimuli on C-fibers activated ERK1/2 in the spinal cord and DRG within a few minutes (6, 17), suggesting a rapid response in ERK1/2 activity in the DRG. In the present study, the earliest time point we examined was 2 h after cystitis. At this time point, phospho-ERK1/2 peaked in the inflamed bladder but not in the DRG (data not shown). Therefore, there was a possibility that phospho-ERK1/2 could be activated in bladder and DRG at the time prior to 2 h after CYP injection. Considering that a small percentage of DRG neurons expressing phospho-ERK1/2 after inflammation near the DRG somata (32), it is likely that other components of the MAPK pathways, such as ERK5, p38 MAPK (33), and/or c-Jun NH2-terminal kinase (9), may be activated in the DRG after inflammation. This is particularly true considering that activation of ERK5 was observed in the lumbarosacral DRG following cystitis in the current study.

Activation of ERK1/2 in the spinal cord was demonstrated in many pathophysiological conditions. In a recent study with CYP-induced cystitis in female rats, Cruz et al. (4) demonstrated that phospho-ERK1/2 immunoreactivity was exhibited in neurons in laminae I-II and dorsal commissure, and a few in the intermediolateral gray matter, areas that receive projections from primary afferent neurons and show responsiveness to bladder stimulation. Consistent with our current findings that cystitis did not cause significant changes in the level of phospho-ERK1/2 in L6 and S1 spinal cord, Cruz et al. (4) reported that sham manipulation of the bladder had no effects on the level of phospho-ERK1/2 in L6 spinal cord in inflamed animals compared with controls. However, phospho-ERK1/2 was rapidly increased in the L6 spinal cord in response to both innocuous (15 cmH2O) and noxious (60 cmH2O) pressure in the inflamed animals and only increased in the L6 spinal cord in noninflamed rats in response to noxious distension, suggesting a hypersensitivity of the inflamed bladder following CYP-injection.

In conclusion, we have found that bladder inflammation induced differential changes in phospho-ERK1/2 and phospho-ERK5 in the primary afferent cell body and in the target organ and dorsal horn of the spinal cord where the axonal terminals locate. Time-dependent changes in these components in these regions suggested a sequential activation of neurotrophin signaling cascades that were bladder inflammation per se caused a rapid local activation of ERK1/2 in the urinary bladder, followed by activation of ERK5 in the neuronal cell body located in the DRG, and then an increased expression of phospho-ERK1/2 in the L1 spinal cord. The location-specific and time-dependent changes in the phospho-ERK1/2 and phospho-ERK5 may be a result of target-to-neuron and neuron-to-neuron interaction mediated by neurotrophins and/or neurotransmitters.

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