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Colitis elicits differential changes in the expression levels of receptor tyrosine kinase TrkA and TrkB in colonic afferent neurons: A possible involvement of axonal transport

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Abstract

The role of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in colitis-induced hypersensitivity has been suggested. NGF and BDNF facilitate cellular physiology through binding to receptor tyrosine kinase TrkA and TrkB respectively. The present study by examining the mRNA and/or protein levels of TrkA and TrkB in the distal colon and in colonic primary afferent neurons in the dorsal root ganglia (DRG) during colitis demonstrated that colitis elicited location-specific changes in the mRNA and protein levels of TrkA and TrkB in colonic primary sensory pathways. In colitis both the TrkA and TrkB protein levels were increased in the L1 and S1 DRGs in a time-dependent manner; however, the level of TrkB mRNA but not TrkA mRNA was increased in these DRGs. Further experiments showed that colitis facilitated a retrograde transport of TrkA protein toward and an anterograde transport of TrkA mRNA away from the DRG, which may contribute to the increased TrkA mRNA level in the distal colon during colitis. Colitis also increased the level of NGF mRNA but not BDNF mRNA in the distal colon. Double staining showed that the expression of TrkA but not TrkB was increased in the specifically labeled colonic afferent neurons in the L1 and S1 DRGs during colitis; this increase in TrkA level was attenuated by pretreatment with resiniferatoxin. These results suggested that colitis-induced primary afferent activation involved retrograde transport of TrkA but not TrkB from the distal colon to primary afferent neurons in DRG.

INTRODUCTION

Sensory neurons located in the dorsal root ganglia (DRG) play an important role in the regulation of peripheral function. In turn, irritation of peripheral organs also has significant effects on the activity of sensory neurons. This is also true for colonic inflammation (colitis)—induced neurochemical (e.g. neurotrophins and neurotrophin signal transduction [11,32]) and electrophysiological changes in the primary sensory pathways [3,11,20,22,29,30,32]. Systemic drug intervention by injecting antiserum of either nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF) intraperitoneally to rats with colonic inflammation reversed the

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Conflict of Interest: none

associated colonic hypersensitivity [8], suggesting a prominent role of NGF and BDNF in mediating the activity of colonic sensory pathways.

NGF and BDNF are members of the neurotrophin family that also include neurotrophin-3 (NT-3) and NT-4/5 [36]. The cellular effects of neurotrophins require their binding to specific receptors namely tropomyosin-receptor-kinase (Trk) [24], a subfamily of receptor tyrosine kinase. Specifically, TrkA preferentially binds NGF; TrkB binds BDNF or NT-4/5; and TrkC binds NT-3 [36]. Recent studies demonstrate that NT-3 can also bind to TrkA and TrkB [36]. At embryonic stage, all sensory neurons contain TrkA; during development, some of the sensory neurons gradually lose TrkA and acquire different phenotypes [23]. At adulthood, TrkA and TrkB are mainly expressed in nociceptors [26,28,33,34,44] while TrkC is expressed in large proprioceptive sensory neurons [4,26], suggesting a distinct role of TrkA, TrkB and TrkC in mediating sensory activity.

Although all four neurotrophins are reported to play roles in neuronal growth, survival and differentiation [18], NGF and BDNF are extensively reported to be involved in mediating sensory hypersensitivity [7–9,12,19,33–35,40]. Recent studies also suggested the involvement of NT-3 in the regulation of sensory neuron functions, however, a study of hind paw inflammation demonstrated an antagonistic function of NT-3 to NGF in which NT-3 reversed NGF-induced hind paw hyperalgesia [41]. This was probably due to negative regulation of TrkA signaling by NT-3 in sensory neurons [41,42]. Spinal intrathecal injection of BDNF or TrkB antisense or antiserum, but not TrkC-Fc, significantly blocked inflammation-induced hypersensitivity [8,12,25,43]. Based on these studies showing that TrkA and TrkB but not TrkC play primary roles in inducing sensory hypersensitivity during inflammation, we hypothesize that TrkA and/or TrkB also have roles in the activation of colonic primary afferent pathways during colonic inflammation.

Our previous studies showed that both L1 and S1 DRGs contained colonic afferent neurons and exhibited considerable neurochemical changes during trinitrobenzene sulfonic acid (TNBS)-induced colitis [29,30,32]. In the present study, we aimed to characterize the changes in the expression levels of TrkA and TrkB in colonic afferent neurons in L1 and S1 DRGs during 3 and 7 days of colitis and investigate the mechanism underlying these changes in order to better understand the role of NGF and BDNF signaling in the visceral pain model induced by TNBS.

MATERIALS AND METHODS

Experimental animals

Adult male Sprague-Dawley rats (150–200 g) (Harlan Sprague Dawley, Inc.) were used in the current studies. All experimental protocols involving animal use were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Animal care was in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and National Institutes of Health guidelines. All efforts were made to minimize the potential for animal pain, stress or distress.

Induction of colonic inflammation

To induce inflammation in the distal colon, animals were anesthetized and TNBS was instilled into the lumen of the colon 6 cm proximal to the anus at a dose of 150 mg/kg (60 mg/mL solution in 50 % EtOH) through a syringe-attached polyethylene catheter. Animals that received similar volume of 50 % EtOH enema or saline served as control. All colonic instillations were performed under isoflurane anesthesia (2.5 %, SurgiVet, Smiths Medical PM, Inc. Waukesha, WI). Euthanasia of animals was performed at either 3 days or 7 days after

intracolonic injection. To ensure exposure of the distal colon to TNBS, rats were held head-down by lifting up the tail for 2 min. In some of the animals, colitis was induced four days after tracing dye injection for a proper transport of these dyes as described below. The effectiveness of TNBS in inducing colitis was assessed by H & E staining (supplemental data).

Retrograde labeling

Under anesthesia (2.5 % isoflurane), the rat distal colon was exposed under a sterile environment with a lower abdominal incision. Neuronal tracing agent Fast Blue (FB, 4 %, weight/volume; Polysciences, Inc. Warrington, PA) was injected into 10 sites (4 μ L per site) in the muscular wall of the descending colon (5–7 cm away proximal to the external anal sphincter) to label colonic afferent neurons innervating this area. Sterilized Hamilton syringe (10 μ L size) was used for injection. To prevent leakage and labeling of adjacent tissues, the needle was left in place for 30 sec after each injection and a cotton swab was held close to the injection site to wipe off any excess dye that might leak from the needle tip during the needle withdrawal. Injections into the lumen, major blood vessels, or overlying fascial layers were avoided. The incision was closed with 4-0 sutures, and the rats were allowed to survive until the harvest of the tissues.

Peripheral spinal nerve ligation

The peripheral (distal) end of the S1 DRG axon (innervating peripheral tissue) was ligated with a 6-0 suture for 30 min prior to harvest of tissue in order to block the interaction and cross-talk between the distal colon and the sacral DRG. The ligation was performed on both sets of the two DRG primary afferent nerve bundles in control animals and animals treated with TNBS for 3 days or 7 days. One set of the S1 DRG nerve was removed and post-fixed for immunostaining; for this purpose, 3–5 mm length of the nerve on both sides of the ligation site was removed but kept in intact with one side is longer than the other in order to label the orientation. Another set of the DRG primary afferent nerve was dissected out and divided into distal to the ligation site and proximal to the ligation site, and was subjected to mRNA extraction and real-time PCR.

Perfusion and tissue harvesting

Intracardiac perfusion was performed for euthanasia of animals. Under anesthesia (3–4 % isoflurane), animals were euthanized via perfusion first with oxygenated Krebs buffer (pH 7.4) (95 % O₂, 5 % CO₂) followed by 4 % paraformaldehyde. After perfusion, the spinal cord and DRG were quickly removed and postfixed for 6 h. Tissue was then rinsed in phosphate buffered saline (0.1 M PBS, pH 7.4) and placed in ascending concentrations of sucrose (20 %) for cryoprotection. DRGs and spinal cord from level L1-S1 were identified and DRG was sectioned parasagittally at a thickness of 20 μ m. Tissues from control and experimental animals were handled in an identical manner.

Immunostaining

DRG and axon sections from control and experimental animals were processed for TrkA and TrkB immunoreactivity using an on-slide immunostaining technique. Briefly, samples were incubated for 1 h at room temperature with blocking solution containing 3 % normal donkey serum (Jackson ImmunoResearch, West Grove, PA) in PBST (0.3 % Triton X-100 in 0.1 M PBS, pH 7.4). Samples were then incubated in primary antibodies diluted in PBST containing 5 % normal donkey serum overnight at 4 °C. The TrkA and TrkB antibodies (Santa Cruz, CA) were used at 1:750 (TrkA) and 1:500 (TrkB), respectively. After rinse (3 $\times$ 10 min with 0.1 M PBS), tissues were incubated with fluorescence-conjugated species-specific secondary antibody Alexa 594 (1:500, Molecular Probes, Eugene, OR) for 2 h at room temperature. Following wash, the slides were coverslipped with Citifluor (Citifluor Ltd., London). Control

sections incubated in the absence of primary or secondary antibody were also processed and evaluated for specificity or background staining levels. In the absence of primary antibody, no positive immunostaining for TrkA or TrkB was observed. We also have published results from western blot analysis indicating the specificity of the antibodies used (29, 33, 34), and these data will not be duplicated here. According to the manufacturer, the rabbit polyclonal TrkA antibody used in these studies (sc-118) shows no cross-reactivity with TrkB or TrkC. Similarly; the rabbit polyclonal TrkB antibody used in these studies (sc-8316) shows no cross-reactivity with TrkA or TrkC.

RNA extraction and quantitative real-time PCR

Total RNA was extracted using a RNA extraction kit RNAqueous (Ambion, TX). RNA concentration was determined spectrophotometrically. cDNA was synthesized using Cloned AMV First-Strand Synthesis Kit (Invitrogen) with random hexamers. Following reverse transcription, quantitative real-time PCR was performed for NGF, BDNF, TrkA and TrkB with specific Taqman probes (Catalog # for NGF: Rn01533872; BDNF: Rn00560868; TrkA: Rn00572130; and TrkB: Rn01441749) mixed with PCR Master-Mix for 40 cycles (95 °C for 15 sec, 60 °C for 1 min) on a 7300 real-time PCR system (Applied Biosystems). Quantitative real-time PCR of the same sample was performed for GAPDH expression as internal control. The level of target mRNA was normalized against GAPDH (Catalog # Rn01462662) expression in the same sample and was calculated with ΔC_t method. The expression level of target mRNA in control animal from each independent experiment was considered as 1, and the relative expression level of target mRNA in experimental animals was adjusted as a ratio to its control in each independent experiment and expressed as fold changes ($2^{\Delta\Delta C_t}$ -fold).

RTX treatment

Resiniferatoxin (RTX), an ultrapotent analog of capsaicin, was dissolved in 10 % Tween-80, 10 % ethanol, and normal saline and injected systemically at a dose of 300 μ g/kg body weight [46] one week prior to TNBS induction to produce long-lasting desensitization of unmyelinated nociceptive C-fiber afferents [6,10,38,39]. RTX-induced desensitization was examined by the eye movements in response to RTX solution dropped to the surface of the eye [38], and by the reduction of TRPV1 expressing neurons in DRG. The effect of RTX on the distal colon with or without colitis was assessed by H & E staining (supplemental data).

Animal treatment

Six animals were treated with EtOH and served as control for TNBS treatment. Ten animals were treated with TNBS. Each treatment was divided evenly into 3 days and 7 days. For FB injection, a total of 14 animals were injected and divided into EtOH-treated control (4) and TNBS-treated colitis (5 for each time point). An additional eight FB-labeled animals were treated with RTX and divided evenly into control and 3 day TNBS-treated. For real-time PCR, a total of 9 animals (3 were treated with EtOH, 3 with TNBS for 3 days and 3 with TNBS for 7 days) were used.

Data analysis

A Nikon fluorescence photomicroscope with a multiband filter set was used for simultaneous visualization of Alexa 594 (red, ex 590 ± 22.5 nm/em 660 ± 16 nm) and FB (UV, excitation wavelength of 340–380 nm). DRG cells with a visible nucleus were identified, and the cells that exhibited greater fluorescence than background level were counted as positive. We have chosen every third section for one specific antibody to avoid double counting. The percentage of total DRG neurons (labeled with DAPI) expressing TrkA or TrkB, or the percentage of colonic afferent neurons (FB labeled) expressing TrkA or TrkB was presented as mean $\pm$ SEM. 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

TrkA expression in DRG with colitis

The TrkA immunoreactivity was observed in L1 and S1 DRGs from both control and colitic animals. As previously reported [33,34], most of the TrkA immunoreactive cells had relatively small ($18 \pm 3 \mu\text{m}$) diameter (Fig. 1A–F, arrows). The basal level of TrkA expression in the adult DRG was averaged 15 % (L1 DRG) to 20 % (S1 DRG) of total DRG neurons in animals treated with EtOH for either 3 days or 7 days, thus they were pooled and presented as control (Fig. 1A, D, and G). TNBS colitis caused significant increases in the number of DRG cells immunoreactive to TrkA (Fig. 1B, C, E, F, and G). There were 1.5 to 3 fold increases ($P < 0.05$) in TrkA in the L1 and S1 DRGs at both 3 and 7 days post colitis induction (Fig. 1G).

Real-time PCR results showed that the level of TrkA mRNA was not changed in either L1 or S1 DRG at both 3 days and 7 days post colitis induction (Fig. 1H and I).

TrkB expression in DRG with colitis

The TrkB immunoreactivity was also observed in L1 and S1 DRGs from both control and colitic animals. As previously reported [33,34], TrkB immunoreactivity was mainly observed in medium sized ($35 \pm 7 \mu\text{m}$) DRG neurons with pericellular staining (Fig. 2A and B, arrows). The number of TrkB immunoreactive cells in DRG was around 20 % of total DRG neurons in animals treated with EtOH and there were no significant difference between 3-day treatment and 7-day treatment, thus they were pooled and presented as control (Fig. 2A, B, and C). During colitis, the number of DRG cells immunoreactive to TrkB was not changed at 3 days but significantly increased ($p < 0.05$) at 7 days post TNBS instillation (Fig. 2C). In contrast to TrkA, TrkB mRNA level was increased in L1 DRG at 7 days but not 3 days of colitis (Fig. 2D), and TrkB mRNA level was increased in S1 DRG at both 3 days and 7 days of colitis (Fig. 2E).

TrkA and TrkB mRNA expression in the distal colon with colitis

Because TrkA protein level was increased in the lumbosacral DRG; TrkA mRNA level was not changed in these DRGs during colitis, we examined the TrkA mRNA levels in the inflamed distal colon in order to identify the origin of the increased TrkA protein level in the DRG. Results showed that the level of TrkA mRNA was significantly increased in the distal colon (2–5 fold, $p < 0.05$) at both 3 and 7 days of colitis (Fig. 3A). We also found that the TrkB mRNA level was increased in the distal colon at 7 days but not 3 days of colitis (Fig. 3B).

NGF and BDNF mRNA expression in the distal colon with colitis

The level of NGF mRNA was significantly increased in the distal colon at 3 days (2.5-fold, $p < 0.05$) and 7 days (3-fold, $p < 0.05$) post colitis induction (Fig. 3C). When the same samples were examined for BDNF mRNA expression, the ct values for BDNF were greater than 40 in all samples examined, therefore we consider that there were undetectable value of BDNF mRNA in the distal colon before and during colitis. This is in contrast with that BDNF mRNA levels were greatly increased in L1 and S1 DRGs during colitis [32].

TrkA and TrkB immunoreactivity in colonic afferent neurons

Both TrkA (Fig. 4) and TrkB (data not shown) were expressed in FB-labeled colonic afferent neurons. Fig. 4 showed that not only there were more DRG cells expressing TrkA (Fig. 4A and D, red cells) but also more FB cells (Fig. 4B and E, blue cells) expressing TrkA at 7 days

of colitis when compared to control (Fig. 4C and F, merged purple cells). The percentage of FB cells expressing TrkA was significantly increased in L1 (control: $7.3 \pm 2.2\%$; colitis 3 days: $32.3 \pm 6.8\%$; colitis 7 days: $42.7 \pm 5.3\%$; $p < 0.05$) and S1 DRGs (control: $29.0 \pm 4.0\%$; colitis 3 days: $41.5 \pm 1.5\%$; colitis 7 days: $48.5 \pm 11.7\%$; $p < 0.05$) at both 3 and 7 days of colitis when compared to control (Fig. 5A). However, there were no changes in the percentage of colonic afferent neurons expressing TrkB in L1 (control: 23.1 ± 10.2 ; colitis 3 days: 31.1 ± 5.6 ; colitis 7 days: 22.7 ± 10.9) or S1 DRG (control: 26.2 ± 5.9 ; colitis 3 days: 27.1 ± 4.1 ; colitis 7 days: 25.4 ± 8.2) during colitis (Fig. 5B).

Attenuation of TrkA expression by RTX

Since colitis increased TrkA protein expression in L1 and S1 DRGs and in colonic afferent neurons at both 3 and 7 days post TNBS instillation (Figs. 1, 4 and 5), and TrkA mRNA was increased in the inflamed distal colon but not in the DRG (Figs. 1 and 3), we injected RTX one week prior to colitis induction in order to examine if the primary afferent activation is involved in colitis-induced changes in TrkA in DRG. We selected 3 days post TNBS treatment. Results showed that prolonged treatment with RTX completely reversed colitis-induced TrkA up-regulation in L1 (Fig. 6A) and S1 DRGs (Fig. 6B), as well as in colonic afferent neurons from L1 (Fig. 6C) and S1 DRGs (Fig. 6D). RTX had no effects on the expression level of TrkA in DRG from non-inflamed animals.

Increased transport of TrkA mRNA and protein between DRG and distal colon during colitis

To further examine if colitis facilitates TrkA transport between the distal colon (distal axonal terminal of the DRG) and the sensory neuronal cell body in the DRG, we performed S1 distal spinal nerve (contains primary afferent fibers) ligation *in situ* in order to block the cross-talk and interaction between the distal colon and the primary sensory neurons in S1 DRG. Immunostaining (Fig. 7) showed that TrkA protein was greatly accumulated at the distal side of the ligation site in animals with colitis (Fig. 7B, arrows) when compared to that in control animals (Fig. 7A). However, real-time PCR showed that TrkA mRNA was greatly accumulated at the proximal side of the ligation site during colitis (Fig. 8B) while TrkB mRNA was not (Fig. 8D). Both TrkA and TrkB mRNA levels were decreased at the distal side of the ligation site during colitis (Fig. 8A and C).

DISCUSSION

The contribution of Trk and Trk-mediated signaling pathways to inflammation-induced sensory hypersensitivity has been reported in several systems [8,12,16,25,31,43]. Activation of Trk and its signal transduction requires binding of neurotrophins to the receptors [36]. Previous studies showed that systemic injection of either NGF neutralizing antibody or BDNF neutralizing antibody reversed colitis-induced colonic hypersensitivity [8], suggesting a prominent role of the neurotrophin system in visceral pain. However, the expression profile of NGF, BDNF, TrkA and TrkB in the colonic sensory pathways is unknown; this limits the understanding of the molecular mechanism underlying neurotrophin-mediated colonic hypersensitivity caused by colitis. The limited information regarding the expression of NGF and BDNF systems in the primary sensory pathways during colitis includes 1) the expression of NGF and TrkA were increased in both neural and non-neural structures of bowel in patients with Crohn's disease or ulcerative colitis [9]; 2) the expression levels of BDNF mRNA and protein were increased in the primary sensory neurons in lumbosacral DRG at 3 and 7 days post TNBS-induced colitis [32], and 3) NGF mRNA was elevated in the epithelial cells of the gut at 6 h post colitis induction in rat [37]. To further understand the role of neurotrophins in inflammation-induced visceral pain, in the current study we characterized the expression of NGF, BDNF, TrkA and TrkB in the distal colon and/or colonic afferent neurons during TNBS-induced colitis and found that colitis elicited differential changes in the mRNA and/or protein

levels of NGF, BDNF, TrkA and TrkB in the inflamed colon and primary afferent neurons in lumbosacral DRG.

The distal colon receives dual spinal innervation from the hypogastric/splanchnic (L1-L2) and pelvic afferents (L6-S1 in rats) [1,2]. Spinal afferents arise from the target with cell bodies in the DRG projecting into the dorsal horn of the spinal cord [13], where they are thought to play a major role in nociception. Studies from our laboratory [29] and others [5] using retrograde tracing with the conventional neuronal tracing dye have demonstrated that primary afferent neurons with projection to colon are located in lumbosacral DRG including L1, L2, L6 and S1, representing innervation from the hypogastric/splanchnic and pelvic afferents [1] respectively. Our previous studies demonstrated that BDNF was up-regulated in L1 and S1 but not L6 DRG during colitis. Thus, in the present study we have chosen L1 and S1 DRGs for examination.

Examination of TrkA and TrkB immunoreactivity in DRG during colitis revealed that both TrkA and TrkB protein levels were up-regulated in the L1 and S1 DRGs by colitis but were affected in a time-dependent manner. TrkA protein level was increased at both 3 and 7 days post colitis induction; while TrkB protein level was only increased at 7 days post colitis induction. We did not examine the time point beyond 7 days at which colitis started to resolve but the hypersensitivity still existed [14,17], however, our data suggested that TrkB expression probably was a later event during colitis. To confirm this notion, we examined the mRNA levels of TrkA and TrkB in L1 and S1 DRGs in a time course of 3 to 7 days of colitis. We found that TrkA mRNA level was not changed in either L1 or S1 DRG, however, TrkB mRNA level was elevated in L1 and S1 DRGs at 7 days of colitis. These results further suggest that colitis stimulated TrkB but not TrkA gene expression in the lumbosacral DRG. Although the cellular response to BDNF requires binding to TrkB but does not necessarily require up-regulation of TrkB protein level, increases in TrkB expression in the DRG may enhance the sensory neurons responding to BDNF, and may play a role in the re-enforcement of colonic hypersensitivity at the resolving stage of colitis. Taken together with the recent studies demonstrating that spinal intrathecal injection of BDNF or TrkB antisense or antiserum significantly attenuated inflammation-induced hypersensitivity [12,25], it suggests a central modulatory role of BDNF and TrkB in mediating inflammatory pain.

Since we did not observe significant changes in the level of TrkA mRNA in DRG during colitis, we hypothesize that the sustained increases in TrkA protein level in DRG is due to retrograde transport of TrkA from the inflamed colon. This notion was supported by our ligation experiment (Fig. 7) showing that TrkA protein was greatly accumulated at the distal side of the ligation site (between the distal colon and the ligation site on S1 afferents) during colitis, suggesting that colitis facilitated an increased retrograde transport of TrkA protein toward DRG therefore caused the increased immunoreactivity of TrkA in colonic afferent neurons shown in the current study. The result that TrkA mRNA was not changed in the DRG during colitis may be due to the anterograde transport of TrkA mRNA away from DRG toward the peripheral axonal terminals (Fig. 8), where it triggers localized translation of TrkA to generate a retrograde signaling complex. The retrograde transport hypothesis has been reviewed previously [15, 27]. The anterograde transport of mRNA and localized translation of protein at the axon terminals are also seen during axonal growth [45]. Another possibility for failing to discriminate the changes in TrkA mRNA level in DRG during colitis may be due to the mixed neuronal population examined. Analysis of the specific population of the colonic afferent neurons such as with laser capture microdissection may improve the outcome. The decreased levels of TrkA and TrkB mRNA at the distal side of the ligation site at 7 days of colitis may be results from either further transport (e.g. for TrkA) or degradation. However, up-regulation of TrkB in the DRG was most likely not due to the retrograde transport because TrkB protein level was not increased in the colonic afferent neurons. We previously reported that TrkB

protein was increased in the bladder afferent neurons during colitis [29]; the latter is likely due to newly synthesized TrkB in DRG.

Another interesting aspect of the interaction between distal colon and colonic afferent neurons involves the possible anterograde transport of BDNF from the DRG to the distal colon. We have reported previously that both BDNF mRNA and protein levels were increased in the L1 and S1 DRGs with colitis [32]. In our other study, we found the increases in BDNF protein level in the inflamed distal colon (data not shown). However the current study showed that there was no detectable level of BDNF mRNA in the distal colon before and during colitis. These results suggest an extrinsic source of BDNF release into the distal colon during colonic inflammation. In DRG, BDNF may also bind to TrkB that was elevated in bladder afferent neurons and thereby, induce the activation of bladder afferent pathways [29]. Since TrkB expression was not changed in the colonic afferent neurons during colitis, TrkB up-regulation in DRG is most likely a secondary effect following the activation of colonic afferent neurons by colitis. The signaling pathways that lead to TrkB up-regulation in the DRG during colitis have not been studied. It is not known but it is interesting to investigate in the future if TrkA-mediated signaling in colonic afferent neurons would regulate TrkB expression in non-colonic afferent neurons through a paracrine mechanism. Up-regulation of TrkB could also be mediated by neuronal depolarization and contribute to neuronal hypersensitivity [21].

Our results with systemic administration of RTX suggested an involvement of unmyelinated C-fiber colonic afferents in the up-regulation of TrkA in DRG during colitis. RTX is an ultrapotent analog of capsaicin. Systemic RTX produces long-lasting desensitization of capsaicin sensitive primary afferents (presumptive C-fibers) and has been widely used to study the action of nociceptive C-fiber afferents [6,10,38,39]. RTX pretreatment in control animals had no effect on the expression levels of TrkA in DRG and in colonic afferent neurons, suggesting that the basal expression of TrkA in DRG was not mediated by C-fiber that might stay “silent” at the physiologic state. The results that RTX pretreatment completely reversed colitis-induced up-regulation of TrkA in DRG and in colonic afferent neurons provided additional information in support of the notion that TrkA might play a role in colon to DRG interaction during colitis.

In conclusion, our results demonstrate that colitis-induced primary sensory activation involves neurotrophin pathways. Colitis up-regulates NGF and TrkA transcripts in the inflamed colon or axonal terminals which may involve the recruitment of TrkA mRNA at the sensory axon terminals via anterograde transport and localized translation of TrkA to generate retrograde transport complex (Fig. 9). Colitis also up-regulates BDNF and TrkB expression in the DRG at a later phase (7 days versus 3 days post colitis induction), and facilitates anterograde transport of BDNF protein to the axonal terminals (may be secreted and bind to TrkB locally) in the distal colon (Fig. 9). The mechanistic study of NGF, BDNF, TrkA and TrkB expression in the distal colon and primary afferent neurons during colitis shown in the current paper, combining with the previous report that systemic injection of NGF neutralizing antibody or BDNF neutralizing antibody reversed colitis-induced colonic hypersensitivity [8], provides further evidence that NGF and BDNF play prominent but distinct roles in visceral pain associated with inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. TrkA expression in DRG with colitis

Immunostaining (A–F) of TrkA in L1 and S1 DRGs from control and colitic rats showed that TrkA was expressed in all the DRGs examined. Histogram (G) showed that the number of TrkA immunoreactive cells was significantly increased in L1 and S1 DRGs at both 3 and 7 days post TNBS treatment. Real-time PCR results showed that there were no changes in the level of TrkA mRNA in either L1 or S1 DRG at both 3 and 7 days of colitis (H and I). Calibration bar = 40 μ m. *, $p < 0.05$. Arrows indicate positively stained cells. Arrowheads indicate negative (background) cells.

Figure 2. Increases in TrkB immunoreactivity in DRG with colitis

Microscopic photograph (A–B) showed TrkB immunoreactivity in L1 DRG from control and colitic rats at 7 days post TNBS treatment. Histogram (C) showed that the number of TrkB immunoreactive cells was significantly increased in L1 and S1 DRGs at 7 days but not 3 days post TNBS treatment. Real-time PCR results showed that there were significant increases in TrkB mRNA level in L1 and S1 DRGs during colitis (D and E). Calibration bar = 60 μ m. *, $p < 0.05$. Arrows indicate positively stained cells. Arrowheads indicate negative (background) cells.

Figure 3. Changes in NGF, TrkA and TrkB mRNA levels in the distal colon during colitis
 Total RNA was extracted from the distal colon from animals treated with EtOH (control) and TNBS and subjected to examination of mRNA expression levels of TrkA, TrkB and NGF. There were significant increases in TrkA mRNA level at 3 and 7 days of colitis (A), increases in TrkB mRNA level at 7 days but not 3 days of colitis (B) and increases in NGF mRNA level at both 3 and 7 days of colitis (C). *, $p < 0.05$. $n = 3$.

Figure 4. Expression of TrkA in colonic afferent neurons

Colonic afferent neurons (B, E, blue cells) were labeled with retrograde tracing dye FB injected into the colonic wall of control (A, B, C) and colitic animals (D, E, F), and immunostained with TrkA (A, D, red cells). The merged photograph (C, F) showed co-localization of TrkA and FB-labeled cells in DRG (purple cells). The samples were L1 DRG from control and colitic animals treated with TNBS for 7 days. Calibration bar = 60 μ m. Arrows indicate cells co-expressing TrkA and FB.

Figure 5. Increases in TrkA but not TrkB immunoreactivity in colonic afferent neurons with colitis
 The percentage of colonic afferent neurons expressing TrkA was significantly increased in L1 and S1 DRGs at both 3 days and 7 days post TNBS-induced colitis (A). The percentage of colonic afferent neurons expressing TrkB was not changed by colitis in both L1 and S1 DRGs. *, $p < 0.05$.

Figure 6. RTX blocked colitis-induced TrkA up-regulation in L1 and S1 DRGs and colonic afferent neurons

Histograms A–D showed the effects of resiniferatoxin (RTX) pretreatment on the expression of TrkA immunoreactivity in L1 and S1 DRGs before and after colitis induction. Pretreatment with RTX had no effect on TrkA immunoreactivity in non-inflamed animals; however, it significantly suppressed the increased TrkA immunoreactivity in L1 DRG (A) and S1 DRG (B), and colonic afferent neurons in L1 DRG (C) and S1 DRG (D). *, $p < 0.05$.

Photomicrographs (E–H) showed the representative staining of TrkA in L1 DRG from animals with different treatment: E, control; F, TNBS 3d; G, RTX; H, RTX+TNBS 3d. Calibration bar = 80 μm. Arrows indicate positive cells.

Figure 7. Colitis increased TrkA protein retrograde transport toward DRG

Immunostaining showed that there was no difference in the density of TrkA-IR afferent fibers at both sides of the ligation site in control animals (A); however, TrkA protein was greatly accumulated at the distal side of the ligation site during colitis (B). Arrows indicate afferent nerves containing TrkA protein. Calibration bar = 150 μ m.

Figure 8. Colitis increased TrkA protein anterograde transport away from DRG

Real-time PCR analysis showed that the TrkA mRNA level was decreased at the distal side of the ligation site at 7 days post colitis induction (A). TrkA mRNA level was increased time-dependently at the proximal side of the ligation site during colitis (B). In contrast, TrkB mRNA level was decreased at the distal side of the ligation site (C) but was not changed at the proximal side of the ligation site (D). *, $p < 0.05$; # $p < 0.05$.

Figure 9. Schematic diagram illustrates the putative mechanism for the dynamic changes in NGF, BDNF, TrkA and TrkB expression levels in the distal colon and DRG during colitis

Colitis induces NGF expression in the distal colon. The sensory axon terminals innervating the distal colon recruit TrkA mRNA via anterograde transport and triggers localized translation to generate retrograde transport complex containing NGF and TrkA. In the primary afferent neurons, BDNF is synthesized and BDNF protein is anterogradely transported to the axonal terminals (may be secreted and bind to TrkB locally). In the nearby DRG neurons, TrkB is synthesized.