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Neurotrophins: Peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity

R. J. Mannion*†, M. Costigan*†, I. Decosterd*†, F. Amaya*, Q.-P. Ma‡, J. C. Holstege§, R.-R. Ji*, A. Acheson¶, R. M. Lindsay¶, G. A. Wilkinson‖, and C. J. Woolf*‡**

*Department of Anesthesia and Critical Care, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129; ‡Department of Anatomy and Developmental Biology, University College, London, United Kingdom WCIE6BT; §Department of Anatomy, Erasmus University Medical School, 3000 DR Rotterdam, The Netherlands; ¶Regeneron Pharmaceutical Inc., Tarrytown, NY 10591; and ‖European Molecular Biology Laboratory, D69117 Heidelberg, Germany

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ABSTRACT **Brain-derived neurotrophic factor (BDNF) is expressed in nociceptive sensory neurons and transported anterogradely to the dorsal horn of the spinal cord where it is located in dense core vesicles in C-fiber terminals. Peripheral inflammation substantially up-regulates BDNF mRNA and protein in the dorsal root ganglion (DRG) in a nerve growth factor-dependent fashion and results in novel expression of BDNF by DRG neurons with myelinated axons. C-fiber electrical activity also increases BDNF expression in the DRG, and both inflammation and activity increase full-length TrkB receptor levels in the dorsal horn. Sequestration of endogenous BDNF/neurotrophin 4 by intraspinal TrkB-Fc fusion protein administration does not, in noninflamed animals, change basal pain sensitivity nor the mechanical hypersensitivity induced by peripheral capsaicin administration, a measure of C fiber-mediated central sensitization. TrkB-Fc administration also does not modify basal inflammatory pain hypersensitivity, but does block the progressive hypersensitivity elicited by low-intensity tactile stimulation of inflamed tissue. BDNF, by virtue of its nerve growth factor regulation in sensory neurons including novel expression in A fibers, has a role as a central modulator of tactile stimulus-induced inflammatory pain hypersensitivity.**

The neurotrophin brain-derived neurotrophic factor (BDNF) is expressed in the adult by TrkA-positive primary sensory neurons (1), most of which are nociceptive C fibers. It is anterogradely transported to the central terminals of these afferents in the superficial laminae of the spinal dorsal horn (2–4), where it is localized in dense core synaptic vesicles (4). BDNF in the brain regulates synaptic efficacy (5, 6) and may, if released from afferent terminals in the spinal cord, act as synaptic modulator either presynaptically on primary afferent TrkB-positive terminals or postsynaptically on TrkB-expressing neurons in the spinal cord.

BDNF in the dorsal horn could have three roles. It might contribute to the transfer of information related to the intensity and duration of noxious stimuli from primary afferent to second-order neurons, i.e., determine basal pain sensitivity. Alternatively it may be involved in the stimulus-induced activity-dependent plasticity of somatosensory pathways, central sensitization (7). Central sensitization is a C fiber-mediated increase in excitability of dorsal horn neurons that generates hypersensitivity by recruiting previously subthreshold mechanoreceptors inputs to pain transmission pathways (8). Finally, BDNF may have a role in generating inflammatory pain hypersensitivity. Basal inflammatory hyperalgesia reflects changes in sensitivity to transient thermal or mechanical stimuli and is caused by both an altered transduction sensitivity of nociceptor peripheral terminals and an increased excitability of central neurons (9). In noninflamed animals stimulus-evoked hypersensitivity or central sensitization can be produced only by intense C-fiber stimuli (10), but after inflammation it can be elicited by light touch, the phenomenon of progressive tactile hypersensitivity (11–13).

After inflammation, BDNF mRNA is increased in dorsal root ganglion (DRG) neurons in a nerve growth factor (NGF)-dependent fashion (14), in keeping with the up-regulation of BDNF mRNA and protein in these neurons after systemic NGF administration (1). NGF levels increase in inflamed tissue (15) and contributes to basal (16–18) and stimulus-induced inflammatory hyperalgesia (19).

We have investigated the central actions of BDNF in the spinal cord by scavenging endogenous released BDNF with a TrkB-Fc fusion protein (20, 21), looking at basal pain sensitivity and noxious stimulus-evoked hypersensitivity in noninflamed animals as well as basal sensitivity and tactile stimulus-evoked hypersensitivity in inflamed animals. TrkB-Fc also will sequester neurotrophin 4, which binds with high affinity to the TrkB receptor (22), but has not been shown in the dorsal horn.

METHODS

Experiments were performed on adult male Sprague–Dawley rats. Chronic intrathecal cannulae (32G, Micor, PA) were inserted under halothane anesthesia (2%) into the subarachnoid space via a posterior thoracic laminectomy and threaded to the lumbar enlargement. After recovery (48 h), the animals were divided into three groups: basal sensitivity ($n = 14$), intraplantar capsaicin ($n = 10$), and inflamed ($n = 21$).

Behavior. All animals were habituated to the behavioral tests before surgery. After surgery any animal with a neurological deficit was excluded. Mechanical sensitivity on the midplantar or dorsal surface of the foot was assessed with von Frey hairs (0.03–95.5 g). Each filament was applied five times, and the lowest threshold that evoked paw withdrawal was recorded. The basal sensitivity group was tested for mechanical (dorsal) and thermal sensitivity (hot plate, 49°C paw jump or lick latency). Intraplantar capsaicin (30 μg) was injected, and mechanical sensitivity (plantar) was tested at 15-, 30-, and 45-min postinjection. Inflammation was generated by intraplantar 1 complete Freund's adjuvant (CFA). In seven

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Abbreviations: BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; DRG, dorsal root ganglion; CFA, complete Freund's adjuvant.
†M.C., I.D., and R.J.M. contributed equally to this study.
**To whom reprint requests should be addressed at: Neural Plasticity Research Group, Department of Anesthesia and Critical Care, Massachusetts General Hospital and Harvard Medical School, 149 13th Street, Room 4310, Charlestown, MA 02129. E-mail: woolf.clifford@mgh.harvard.edu.

animals dorsal mechanical sensitivity tests were made at 3, 6, 12, 24, and 30 h. In 14 animals dorsal mechanical threshold was measured at 24- and 48-h post-CFA injection followed by tactile conditioning stimuli. Eight standard light strokes were applied at 1 Hz to the dorsum of the hindpaw moving from the ankle to the toes, repeated every 5 min for 2 h, with dorsal mechanical threshold measurements after each train (11). The tester was blinded to the intrathecal treatments. Intrathecal injections were made 30 min before the capsaicin administration and the tactile conditioning stimulation.

Inflammation. Intraplantar CFA 100 μl (Sigma) was injected into the left hindpaw under halothane (2%) anesthesia (16). A neutralizing anti-NGF sheep antiserum (16) was administered 1 h before the CFA and again at 24 h. In nine animals a nerve block was produced by using 0.5% bupivicaine (0.5 ml) applied to the exposed sciatic nerve 30 min before capsaicin or CFA injection.

Electrophysiological Studies. Recordings from single biceps femoris/semitendinosis motoneurons in decerebrate-spinal rats (six animals per treatment group) were performed as described (23). The von Frey threshold for activating the motoneurons was established on the plantar surface of the hindpaw, and the touch responsiveness of the motoneurons was measured by integrating the activity generated by eight standard light strokes of the dorsum of the hindpaw applied at 1 Hz.

BDNF ELISA. Immulon plates were coated with a monoclonal anti-BDNF antibody (1.25 μg/ml in sodium carbonate buffer). Tissues were homogenized in 50 mM Tris·HCl, pH 7.4, 600 mM NaCl, 0.2% Triton X-100, 1% BSA (fraction V), 200 kallikrein units/ml aprotonin, 0.1 mM benzethonium chloride, 1 mM benzamidine, and 0.1 mM PMSF. After blocking (5% BSA in PBS) and washing, samples and standards were incubated with a biotinylated polyclonal anti-BDNF (50 ng/ml), and after 1 h avidin D-horseradish peroxidase complex was added (1:10,000; 1 h), followed by development with 3,3′,5,5′-tetramethylbenzidine and peroxide. After 20 min, the reaction was stopped with 1 M H_3PO_4. All samples were assayed in duplicate.

Northern Blots. Total cellular RNA was extracted and blotted as described (24) by using the following probes. A rat BDNF cDNA comprising the entire coding sequence (1.1 kb) provided by P. Emson (The Babraham Institute, Cambridge, U.K.) cloned into Bluescript II KS (Stratagene). A 602-bp fragment of the full-length rat TrkB cDNA (25) was generated by *Apa*I/*Sma*I restriction digestion and subcloned into Bluescript II KS (Stratagene). This probe codes for the kinase region of the transcript and lacks homology to the truncated T1 and T2 isoforms (positions 2154–2756 of the full-length sequence (GenBank accession no. M55291). A 240-bp rat cyclophilin PCR product was produced from rat DRG cDNA by using primers Cyc1 (5′-TTGGGTCGCGTCTGCTTCGA-3′) and Cyc2 (5′-GCCAGGACCTGTATGCTTCA-3′) and subsequently cloned into pGEM-T PCR cloning vector (Promega). At least two Northern blots were used for each observation, and fold change was determined with respect to cyclophilin-loading controls.

***In Situ* Hybridization/Immunohistochemistry/Western Blots.** *In situ* hybridization was carried out by using digoxygenin-labeled riboprobes (24) generated as described above. Western blots used a polyclonal anti-TrkB antibody (26) and standard techniques (24). The blots were reprobed with a loading control (anti-mitogen-activated protein kinase 1:1,000; New England Biolabs), and fold changes were expressed relative to these levels. For an analysis of the size frequency distribution of BDNF mRNA-labeled neuronal profiles, five 20-μm sections (separation of 100 μm) were randomly chosen from the L4 DRG from three animals. Profile areas were calculated from 600 nucleated cells, by using the National Institutes of Health IMAGE package, and area-frequency histograms were plotted. This analysis assumes that the nucleus is located in the center of the cell body. Relative changes in the proportion of BDNF mRNA profiles after inflammation were calculated by measuring the % of BDNF mRNA-labeled nucleated profiles/total number of neuronal profiles per section. Double labeling for BDNF mRNA and NF200 staining was performed by doing the *in situ* first, taking a photomicrograph, and then staining the same section with a monoclonal anti-NF200 antibody (1:400; Sigma) by using standard techniques.

Fusion Proteins. Fusion protein dimers consisting of the extracellular binding domain of TrkB or TrkC fused to the Fc portion of a human Ig were produced in insect cells by using baculovirus as an expression system and were tested in *in vitro*-dissociated nodose ganglion cell assays for their ability to neutralize BDNF (TrkB-Fc) or neurotrophin 3 (TrkC-Fc)-mediated neuronal survival. The TrkB-Fc fusion protein was stable *in vivo* as tested by a ligand-capture ELISA of central nervous system tissue, with BDNF as capture and anti-human Fc as reporter, after intracerebral administration of the protein. This TrkB-Fc fusion protein has been shown to inhibit

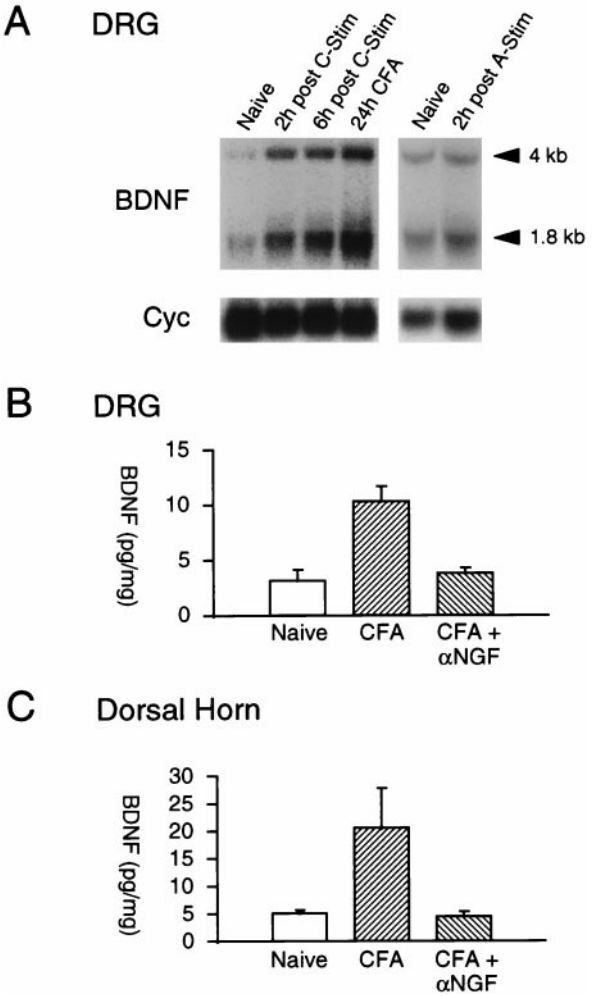

FIG. 1. Change in BDNF mRNA and protein levels in L4 and L5 DRGs and lumbar dorsal horn. (*A*) Northern blots for BDNF and cyclophilin mRNA in L4 and L5 DRGs showing increased BDNF mRNA after stimulation of the sciatic nerve at C- but not A-fiber strength and 24 h after inflammation of the hindpaw. (*B*) BDNF protein levels, measured by ELISA, in L4 and L5 DRGs ($n = 6$) in naïve and inflamed rats (48 h post-CFA). Inflammation increased BDNF in the ipsilateral DRG ($P < 0.01$), which was prevented by anti-NGF ($P < 0.01$). (*C*) The elevation of BDNF in the dorsal horn 48 h after inflammation ($n = 6$, $P < 0.05$) also was prevented by anti-NGF pretreatment ($n = 6$, $P < 0.05$).

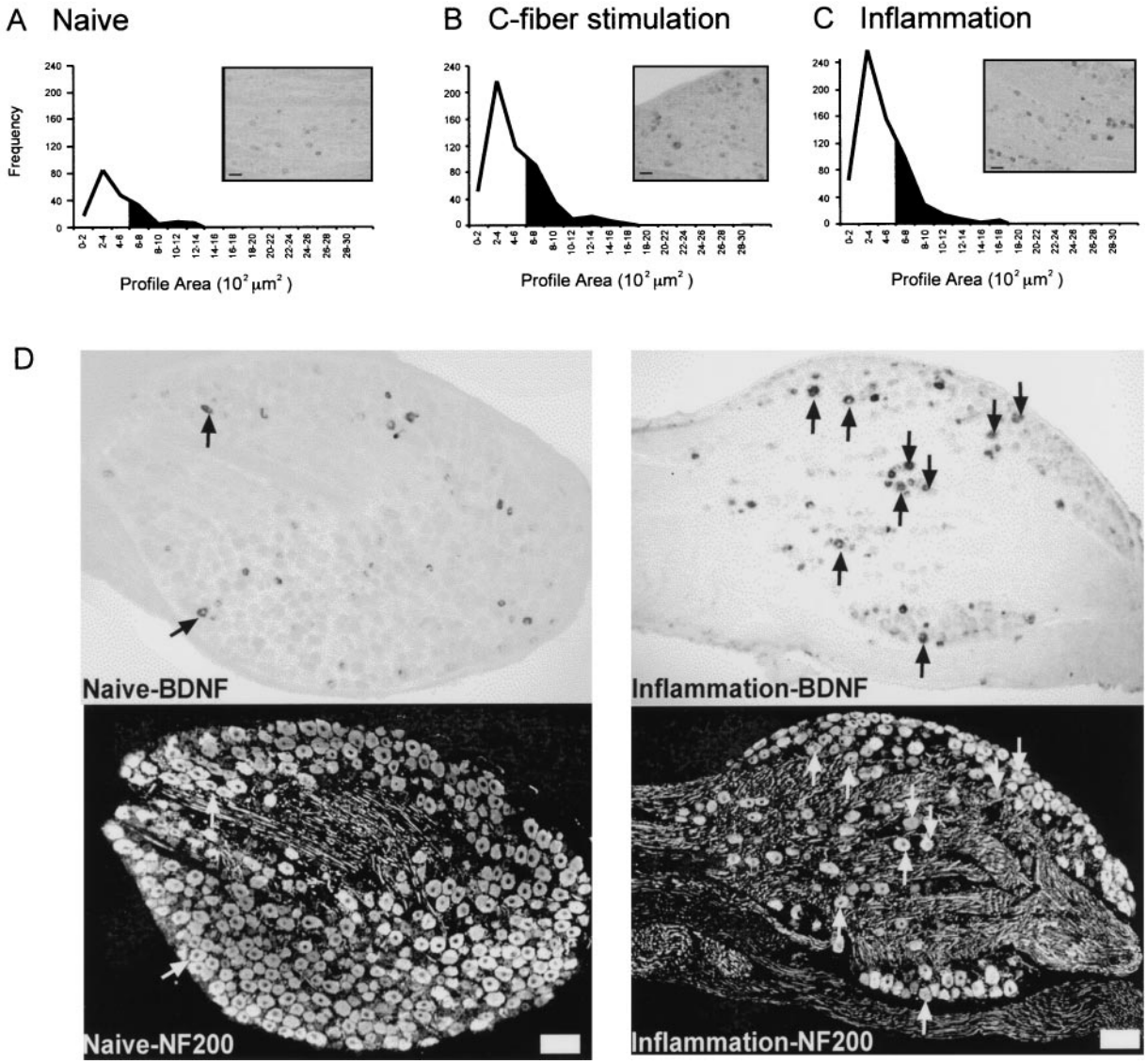

FIG. 2. Increase and phenotypic change in BDNF expression in the DRG. BDNF mRNA labeled neuronal profiles increase 2 h after stimulation of the sciatic nerves (*B*) and 24 h after inflammation (*C*). The number of labeled profiles/total neuronal profiles per section increased from 11.1 ± 1.7 (naives, *A*) to 31.4 ± 2.4 (C-fiber stimulation, *B*) and 35.9 ± 9.2 (inflammation, *C*) ($n = 3$, $P < 0.05$). Scale (*A–C*): 100 μm. Profile area-frequency histograms constructed from 600 profiles in three animals show an increase in the number of small and medium sized neuronal profiles expressing BDNF mRNA. Cells >600 μm^2 (shaded) are A fibers (29). (*D*) Double staining for BDNF mRNA and NF200 immunohistochemistry shows only a small proportion of BDNF mRNA-expressing cells are NF200 positive in naives (arrows), but after inflammation the proportion of double-labeled cells increases 2-fold. Scale (*D*): 200 μm.

BDNF in brain slices (21). Staining for the distribution of Fc 1 h after TrkB-Fc intrathecal injection revealed label over 70% of the lumbar spinal cord, indicating good penetration.

Statistical Analysis. The following tests were used: electrophysiology (ANOVA with Tukey's test for multiple comparisons and analysis of covariance), histochemistry (ANOVA with Fischer probable least-squares difference), and behavior (Wilcoxon signed-rank, log rank test, and ANOVA). Results are expressed as mean ± SEM.

RESULTS

TrkB-Fc Does Not Alter Basal Sensitivity in Naive Animals. The effect of sequestering endogenous BDNF in the spinal cord on the response of awake behaving rats to acute noxious stimuli was tested by the intrathecal administration of a high dose of a TrkB-Fc fusion protein into the lumbar subarachnoid space (10.8 μg in 10 μl), with either TrkC-Fc or vehicle as a control. Neither fusion protein had any detectable effect on dorsal mechanical threshold for eliciting a withdrawal response (65 ± 6 g TrkB-Fc, $n = 4$; 68.5 ± 3.5 g TrkC-Fc, $n = 4$; 63 ± 5 g, saline, $n = 6$) nor on thermal response latency (21.6 ± 2 s TrkB-Fc, $n = 4$; 20 ± 3 TrkC-Fc, $n = 4$; 23.2 ± 2 saline, $n = 6$).

TrkB-Fc Does Not Alter Stimulus-Induced Hypersensitivity in Noninflamed Animals. BDNF's role in C fiber-mediated stimulus-evoked pain hypersensitivity in naïve animals was investigated by examining the effect of intrathecal TrkB-Fc on the secondary mechanical hypersensitivity evoked by a localized intraplantar injection of capsaicin (30 μg) (27). Intraplantar capsaicin in the presence of intrathecal saline transiently reduced the mechanical threshold for eliciting a flexor withdrawal on the plantar skin at a distance from the capsaicin injection from 10.5 ± 8.1 g to 1.6 ± 1.1 g, 45 min postcapsaicin, $n = 4$, with a return to baseline at 90 min. This secondary mechanical hyperalgesia was not altered by intrathecal TrkB-Fc administration (16.2 ± 6.5 g precapsaicin to 1.0 ± 0.2 g 45 min postcapsaicin, $n = 6$).

Inflammation and Electrical Activity Increase BDNF Expression in DRG Neurons. A Northern blot of the ipsilateral

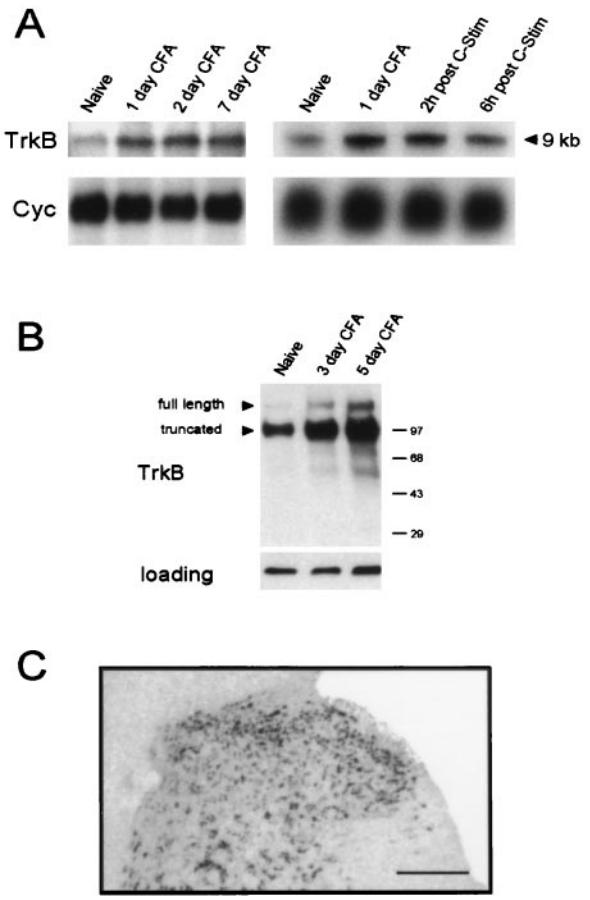

FIG. 3. TrkB mRNA and protein up-regulation in the dorsal horn. (*A*) Northern blots for full-length TrkB mRNA show up-regulation of this transcript after inflammation (CFA). Electrical stimulation (Stim) also induces up-regulation of full-length TrkB mRNA. Cyc, cyclophilin. (*B*) Western blot for TrkB protein shows up-regulation of both full-length and truncated TrkB in the dorsal horn at 3 and 5 days post-CFA. (*C*) *In situ* hybridization for full-length TrkB mRNA in the dorsal horn shows labeling in all laminae. Scale: 200 μm.

L4 and L5 DRGs showed a 6-fold elevation of both the 1.6- and 4.0-kb BDNF mRNA transcripts 24 h after CFA-induced inflammation of the hindpaw (Fig. 1). *In situ* hybridization detected a 3-fold increase in the relative proportion of BDNF mRNA-labeled neuronal profiles in the DRG after inflammation (Fig. 2). BDNF protein levels in the DRG and the dorsal horn (Fig. 1) were also significantly increased 48 h after the inflammation ($n = 6$, $P < 0.01$). Pretreatment of animals before the inflammation with anti-NGF prevented the elevation in BDNF in the DRG and dorsal horn ($P < 0.01$, $n = 6$) (Fig. 1). The inflammation resulted in a shift in the size-frequency distribution of BDNF mRNA-labeled neuronal profiles to the right, in keeping with larger cells expressing BDNF mRNA (Fig. 2). That an increase in the proportion of BDNF mRNA-expressing A fibers occurred after inflammation was shown by double labeling for NF200, which recognizes a heavy chain neurofilament found only on cells with myelinated axons (28). In naïve animals 9.4 ± 1.9% of BDNF mRNA-positive profiles were NF200 positive. This colocalization increased to 23.1 ± 3.7% 48 h after CFA ($n = 3$, $P < 0.01$, Fig. 2).

To test whether the BDNF up-regulation after inflammation might include an activity-dependent component, we investigated the effect of electrical activation of sensory neurons on BDNF mRNA expression levels. Activation of the L4 and L5 DRG neurons was produced by stimulation of the sciatic nerve for 30 min at Aβ fiber strength (100 μA, 50 μs at 50 Hz) or at strengths that also recruited C fibers (5 mA, 500 μs at 20 Hz). A-fiber stimulation did not change the levels of BDNF mRNA significantly (Fig. 1), but C-fiber strength stimulation resulted in a 2.5-fold increase in BDNF mRNA at 2 h and maintained at 6 h (Fig. 1). In sham animals, where the peripheral nerve was exposed but not stimulated, the levels of BDNF mRNA were identical to those in naïve rats. *In situ* hybridization for BDNF mRNA after sciatic nerve C-fiber stimulation showed increased labeled profiles, with a size-frequency distribution similar to that seen after inflammation (Fig. 2). No increase was seen after A-fiber stimulation ($n = 3$, $P = 0.36$). Intraplantar capsaicin (60 μg) increased the proportion of BDNF mRNA-labeled L4 DRG neuronal profiles 1.8-fold 2 h later, ($n = 3$), which was prevented by a precapsaicin local anesthetic block of the sciatic nerve with bupivicaine ($n = 3$). At 4 h after CFA, which is too early for a retrograde NGF-mediated change in transcription in the DRG, there was a slight increase in BDNF mRNA in the L4 and L5 DRGs (1.5-fold), also prevented by sciatic nerve block (data not shown).

Regulation of TrkB in the Dorsal Horn After Stimulation and Inflammation. *In situ* hybridization with a probe specific for full-length TrkB mRNA revealed labeled neurons throughout the dorsal horn with particularly high density in laminae I/II outer and laminae III/IV (Fig. 3). Peripheral inflammation induced a 3-fold up-regulation of the full-length 9-kb TrkB mRNA in the dorsal horn, at 1 day postinjury, maintained for a week (Fig. 3). Western blots showed that protein levels were up-regulated for both full-length (4-fold at 3 days and 9-fold at 5 days) and truncated TrkB (2-fold at 3 days and 5 days) (Fig. 3). C-fiber strength electrical stimulation of the sciatic nerve increased TrkB mRNA levels 2-fold 2 h after stimulation, with recovery by 6 h (Fig. 3).

TrkB-Fc Does Not Change Baseline Inflammatory Hypersensitivity. To test BDNF's contribution to the increase in baseline sensitivity produced by inflammation, two approaches were used. The first involved an examination of whether an intrathecal injection of TrkB-Fc in animals inflamed 48 h earlier modified basal cutaneous mechanical hypersensitivity. Baseline mechanical sensitivity (dorsal) after inflammation was substantially increased measured by a fall in threshold (68.3 ± 7.5 g to 23.9 ± 2.8 g, $n = 14$). This sensitivity was not modified by intrathecal TrkB-Fc (27.6 ± 5.6 vs. 24.7 ± 4.7 g, $n = 7$) or TrkC-Fc (26.4 ± 5.9 vs. 23.1 ± 3.4 g, $n = 7$). The second approach involved six injections of either saline or 10 μl of TrkB-Fc at 2, 5, 11, 23, 26, and 29 h post-CFA injection, measuring mechanical threshold (dorsal) at 3, 6, 12, 24 and 30 h. In both TrkB-Fc- ($n = 4$) and saline-injected ($n = 3$) rats an inflammatory mechanical hyperalgesia was detectable at 3 h, peaked at 12 h, and remained unchanged at 24 and 30 h, with no difference between the two groups.

TrkB-Fc Reduces Tactile Stimulus-Induced Inflammatory Hypersensitivity. Noxious stimuli sufficient to activate C fibers are required in noninflamed animals to elicit central sensitization. In inflamed animals, however, repeated intermittent low-intensity stimuli of the inflamed skin induces an incremental hypersensitivity, progressive tactile hypersensitivity (11, 12, 19). Such progressive hypersensitivity was tested in electrophysiological and behavioral experiments by a standard protocol of tactile stimulation of the inflamed hindpaw in animals treated with either intrathecal TrkB-Fc or TrkC-Fc (Fig. 4 *A* and *B*). A progressive reduction in threshold and increase in responsiveness to touch and pinch stimuli of electrophysiologically recorded single flexor motor neurons occurred after TrkC-Fc administration that was identical in amplitude and time course to that previously reported in the absence of the fusion protein (11). There was a reduction in the cutaneous mechanical threshold (dorsal) from 30 ± 9 g to 8 ± 2 g ($n = 6$) and an increased action potential response to the standard train of low-intensity touch stimuli from 44 ± 22 spikes per stimulus train to 175 ± 28 (Fig. 4). TrkB-Fc

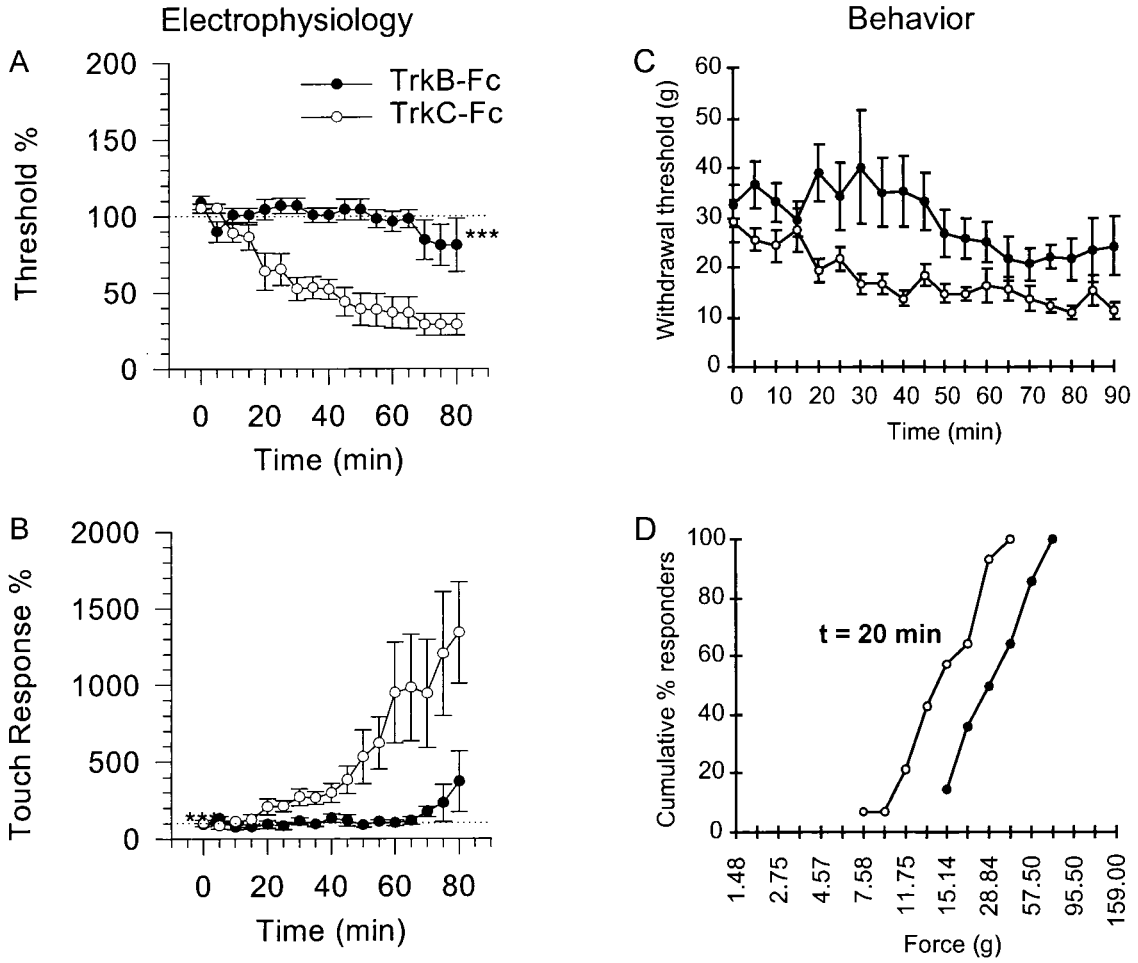

FIG. 4. Intrathecal TrkB-Fc, but not TrkC-Fc, fusion protein prevents the establishment of progressive tactile hypersensitivity. (*A* and *B*) Electrophysiological recordings from single flexor motoneurons showing that in TrkC-Fc-, but not TrkB-Fc-, treated animals tactile stimulation of the inflamed paw progressively reduces mechanical threshold and touch responsiveness ($n = 6, P < 0.001$). In behaving animals TkB-Fc attenuates the reduction in mechanical threshold for flexion withdrawal during the period of intermittent tactile stimulation ($n = 7$) compared with TrkC-Fc-treated animals ($n = 7$) (*C*) A cumulative response plot of the thresholds in the two treatment groups 20 min after commencement of the tactile stimuli shows a significant shift of the curve to the left ($P = 0.005$) (*D*).

administration significantly blocked the development of the progressive tactile hypersensitivity (Fig. 4 *A* and *B*). The threshold 60 min after the repeated tactile stimulation in this group was 35 ± 12 g compared with 32 ± 9 g before the tactile stimuli, and the number of spikes evoked by the standard tactile stimulus train did not increase (47 ± 29 at the start and 53 ± 39 at 60 min, $n = 6$).

In behaving animals, repeated tactile stimulation of the inflamed hindpaw at 5-min intervals, beginning 30 min after an injection of the TrkC-Fc fusion protein, reduced the mechanical threshold for initiating a withdrawal response by 66% from the pretactile stimulation baseline (Fig. 4*C*). The intrathecal injection of TrkB-Fc, however, significantly attenuated the effect of the repeated tactile stimulation of the inflamed hindpaw, with no reduction in threshold over the first 45 min of testing (Fig. 4*C*).

DISCUSSION

BDNF, although present in nociceptor central terminals in the spinal cord in naïve animals, does not appear to contribute to the signaling of the onset, duration, and intensity of noxious peripheral stimuli, i.e., to basal pain sensitivity. This function is subserved by glutamate-mediated fast synaptic potentials in dorsal horn neurons (29). Similarly, central sensitization in naïve animals, which involves glutamate acting on postsynaptic NMDA receptors (30), does not appear to involve BDNF (27). Substance P, like BDNF, is stored and released from dense-core vesicles in C fibers, and greater stimulation intensities are required to elicit release of the peptide than glutamate from primary afferent central terminals (31). The release mechanisms for dense core vesicles may differ from those of the clear vesicles, which contain glutamate, and in this way encode stimulus intensity.

Central sensitization is an activity-dependent increase in membrane excitability of dorsal horn neurons triggered normally only by C-fiber inputs and manifests as alterations in pain-related behavior (32) and flexion reflexes (7) in animals, as well as pain hypersensitivity in humans (8). Although BDNF is not involved in this phenomenon in naïve animals, the up-regulation of BDNF in DRG cells during inflammation, and the associated change in TrkB expression in the dorsal horn, provides a mechanism whereby sensory processing in the spinal cord can be boosted, such that BDNF now contributes to tactile stimulus-induced pain hypersensitivity. The BDNF up-regulation is mediated by an increase in peripheral levels of NGF. NGF is induced at the site of inflammation (33) and alters the sensitivity peripheral terminals (17, 34), which may lead to increased action potential traffic in the neurons contributing to the up-regulation of BDNF in the DRG, because activity increases BDNF mRNA in the DRG, as in the cortex (35, 36). NGF is also retrogradely transported from the

periphery to the cell body (37), activating specific signal transduction pathways (38) that may, via the cAMP response element binding protein (39), contribute to increased BDNF expression. Whether neurotrophin 4, another ligand for TrkB, contributes needs to be determined.

The full-length TrkB receptor also is up-regulated in the dorsal horn after peripheral inflammation in ways that parallel the change in BDNF levels in the DRG. There are then complementary presynaptic and postsynaptic changes of a neurotrophin ligand and its high-affinity receptor in the dorsal horn during inflammation, which resemble changes in substance P and its receptor (16, 40).

The attenuating effect of TrkB fusion protein administration on the development of tactile stimulus-induced inflammatory hypersensitivity, in contrast to the absence of an effect on basal inflammatory hypersensitivity, points to a particular role for BDNF in stimulus-induced pain in inflamed animals. Low-intensity stimulation of inflamed skin could induce BDNF-mediated pain hypersensitivity in two ways, either by activating peripherally sensitized C nociceptors (41) or by activating low-threshold Aβ fiber mechanoreceptors. Aβ fibers never normally induce pain hypersensitivity but after inflammation there is a phenotypic switch with the novel appearance of substance P in some Aβ fibers (12), and similar changes occur for BDNF.

The central actions of BDNF on membrane excitability may be mediated by tyrosine phosphorylation of the NMDA receptor, either directly via the TrkB receptor, or indirectly via other tyrosine kinases. *Src*, for example, increases channel open time in dorsal horn neurons (42). BDNF also increases open probability of the NMDA receptor by increasing the frequency of entry of the channel into the open state (43). BDNF in the hippocampus produces phosphorylation of the NR1 subunit of the NMDA receptor (44), and the TrkB receptor is associated with *src* as well as protein kinase C (45, 46), providing several potential different ways in which BDNF, once released in the dorsal horn, could induce a use-dependent increase in excitability.

BDNF, in addition to its highly specialized role as a growth factor during development, contributes to synaptic plasticity at multiple sites in the brain. This neurotrophin, by virtue of its up-regulation in DRG neurons by activity and NGF, and as a result of its transport to the spinal cord, release, and action on TrkB-expressing cells in the dorsal horn, contributes to stimulus-induced hypersensitivity after inflammation.

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1. Apfel, S. C., Wright, D. E., Wiideman, A. M., Dormia, C., Snider, W. D. & Kessler, J. A. (1996) *Mol. Cell. Neurosci.* **7,** 134–142.
2. Yan, Q., Rosenfeld, R. D., Matheson, C. R., Lopez, O. T., Bennett, L. & Welcher, A. A. (1997) *Neuroscience* **78,** 431–448.
3. Zhou, X. F. & Rush, R. A. (1996) *Neuroscience* **74,** 945–953.
4. Michael, G. J., Averill, S., Nitkunan, A., Rattray, M., Bennett, D. L. H., Yan, Q. & Priestley, J. V. (1997) *J. Neurosci.* **17,** 8476–8490.
5. Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H. & Bonhoeffer, T. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8856–8860.
6. Kang, H. & Schuman, E. M. (1996) *Science* **273,** 1402–1406.
7. Woolf, C. J. (1983) *Nature (London)* **306,** 686–688.
8. Torebjork, H. E., Lundberg, L. E. R. & LaMotte, R. H. (1992) *J. Physiol. (London)* **448,** 765–780.
9. Ren, K., Hylden, J. L. K., Williams, G. M., Ruda, M. A. & Dubner, R. (1992) *Pain* **50,** 331–344.
10. Woolf, C. J. & Wall, P. D. (1986) *J. Neurosci.* **6,** 1433–1443.
11. Ma, Q.-P. & Woolf, C. J. (1996) *Pain* **67,** 97–106.
12. Neumann, S., Doubell, T. P., Leslie, T. A. & Woolf, C. J. (1996) *Nature (London)* **384,** 360–364.
13. Petersen, K. L., Brennum, J. & Dahl, J. B. (1997) *Pain* **70,** 167–174.
14. Cho, H.-J., Kim, S.-Y., Park, M.-J., Kim, D.-S., Kim, J.-K. & Chu, M.-Y. (1997) *Brain Res.* **749**, 358–362.
15. Safieh-Garabedian, B., Poole, S., Allchorne, A., Winter, J. & Woolf, C. J. (1995) *Br. J. Pharmacol.* **115,** 1265–1275.
16. Woolf, C. J., Safieh-Garabedian, B., Ma, Q.-P., Crilly, P. & Winter, J. (1994) *Neuroscience* **62,** 327–331.
17. Lewin, G. R., Rueff, A. & Mendell, L. M. (1994) *Eur. J. Neurosci.* **6,** 1903–1912.
18. McMahon, S. B., Bennett, D. L. H., Priestley, J. V. & Shelton, D. L. (1995) *Nat. Med.* **1,** 774–780.
19. Ma, Q.-P. & Woolf, C. J. (1997) *NeuroReport* **8,** 807–810.
20. Cabelli, R. J., Shelton, D. L., Segal, R. A. & Shatz, C. J. (1997) *Neuron* **19,** 63–76.
21. McAllister, A. K., Katz, L. C. & Lo, D. C. (1997) *Neuron* **18,** 767–778.
22. Lindsay, R. M. (1996) *Philos. Trans. R. Soc. London B* **351,** 365–373.
23. Ma, Q.-P. & Woolf, C. J. (1995) *J. Physiol. (London)* **486,** 769–777.
24. Costigan, M., Mannion, R. J., Kendall, G., Lewis, S. E., Campagna, J. A., Coggeshall, R. E., Meredith-Middleton, J., Tate, S. & Woolf, C. J. (1998) *J. Neurosci.* **18,** 5891–5900.
25. Middlemas, D. S., Lindberg, R. A. & Hunter, T. (1991) *Mol. Cell. Biol.* **11,** 143–153.
26. Farinas, I., Wilkinson, G. A., Backus, C., Reichardt, L. F. & Patapoutian, A. (1998) *Neuron* 21, 325–334.
27. Gilchrist, H. D., Allard, B. L. & Simone, D. A. (1996) *Pain* **67,** 179–188.
28. Bennett, D. L., Michael, G. J., Ramachandran, N., Munson, J. B., Averill, S., Yan, Q., McMahon, S. B. & Priestley, J. V. (1998) *J. Neurosci.* **18,** 3059–3072.
29. Willis, W. D. & Coggeshall, R. E. (1991) *Sensory Mechanisms of the Spinal Cord* (Plenum, New York).
30. Woolf, C. J. & Thompson, S. W. N. (1991) *Pain* **44,** 293–299.
31. Cao, Y. Q., Mantyh, P. W., Carlson, E. J., Gillespie, A. M., Epstein, C. J. & Bausbaum, A. I. (1998) *Nature (London)* **392,** 390–394.
32. Coderre, T. J. & Melzack, R. (1992) *J. Neurosci.* **12,** 3665–3670.
33. Woolf, C. J., Allchorne, A., Safieh-Garabedian, B. & Poole, S. (1997) *Br. J. Pharmacol.* **121,** 417–424.
34. Woolf, C. J., Ma, Q.-P., Allchorne, A. & Poole, S. (1996) *J. Neurosci.* **16,** 2716–2723.
35. Patterson, S. L., Grover, L. M., Schwartzkroin, P. A. & Bothwell, M. (1992) *Neuron* **9,** 1081–1088.
36. Rocamora, N., Welker, E., Pascual, M. & Soriano, E. (1996) *J. Neurosci.* **16,** 4411–4419.
37. Hendry, I. A., Stockel, K., Thoenen, H. & Iversen, L. L. (1974) *Brain Res.* **68,** 103–121.
38. Greene, L. A. & Kaplan, D. R. (1995) *Curr. Opin. Neurobiol.* **5,** 579–587.
39. Tao, X., Finkbeiner, S., Arnold, D. B., Shaywitz, A. J. & Greenberg, M. E. (1998) *Neuron* **20,** 709–726.
40. Krause, J. E., DiMaggio, D. A. & McCarson, K. E. (1995) *Can. J. Physiol. Pharmacol.* **73,** 854–859.
41. Steen, K. H., Reeh, P. W., Anton, F. & Handwerder, H. O. (1992) *J. Neurosci.* **12,** 86–95.
42. Yu, X. M., Askalan, R., Keil, G. J. & Salter, M. W. (1997) *Science* **275,** 674–678.
43. Levine, E. S., Crozier, R. A., Black, I. B. & Plummer, M. R. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 10235–10239.
44. Suen, P.-C., Wu, J.-Y., Levine, E. S., Mount, H. T., Xu, J.-L., Lin, S.-Y. & Black, I. B. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 8191–8195.
45. Zirrgiebel, U., Ohga, Y., Carter, B., Berninger, B., Inagaki, N., Thoenen, H. & Lindholm, D. (1995) *J. Neurochem.* **65,** 2241–2250.
46. Iwasaki, Y., Gay, B., Wada, K. & Koizumi, S. (1998) *J. Neurochem.* **71,** 106–111.