Effects of Analgesic or Antidepressant Drugs on Pain- or Stress-Evoked Hippocampal and Spinal Neurokinin-1 Receptor and Brain-Derived Neurotrophic Factor Gene Expression in the Rat

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ABSTRACT

Clinical studies show that people suffering from chronic pain are often also burdened by depression. Antidepressants are used to treat some types of chronic pain; however, little is known about their mechanisms of action. This study addressed the effects of a nonsteroidal anti-inflammatory drug and a tricyclic antidepressant drug on pain- and stress-evoked gene expression in the rat spinal cord dorsal horn and hippocampus. Rats were pretreated with either indomethacin or imipramine and then challenged with either intraplantar complete Freund’s adjuvant or a bout of immobilization stress. Results showed that indomethacin significantly reduced nociception-related peripheral edema, hyperalgesia, and reversed the pain-evoked up-regulation of neurokinin (NK)-1 receptor and brain-derived neurotrophic factor (BDNF) gene expression in the spinal cord to levels not statistically different from controls. However, indomethacin did not protect against significant pain-induced down-regulation of these genes in the hippocampus by approximately 50%, suggesting that although analgesic drug treatment reduces nociceptive sensory activation in the spinal cord, it is insufficient to prevent the impact of pain on the hippocampus. Conversely, although imipramine did not provide significant behavioral analgesia, it significantly blocked both pain- and stress-evoked alterations in hippocampal and spinal NK-1 and BDNF gene expression. Thus, these results show that application of either analgesic or antidepressant drugs alone does not fully protect against both the behavioral and molecular effects of persistent pain on both “sensory” and “affective” processing within the central nervous system.
rived neurotrophic factor (BDNF), as well as their preferred receptors, the neurokin (NK)-1 and tyrosine kinase B, respectively, are well characterized neuromodulators of nociceptive sensory processing and important contributors to development and maintenance of hyperalgesia and the central sensitization associated with persistent pain (Henry, 1993; Bennett, 2001; Malcangio and Lessmann, 2003). Their involvement in the neurobiology of mood disorders has also been demonstrated (Nibuya et al., 1995; Duman et al., 1997; Kramer et al., 1998; McLean, 2005), suggesting that the effects of pain and stress may converge and activate similar neuronal pathways in the higher brain centers. Moreover, we have shown previously that both peripheral inflammatory pain and immobility stress each have profound damaging effects on the limbic system, indicated by alterations in hippocampal volume and down-regulation of NK-1 receptor and BDNF gene expression (Duric and Mccarson, 2005, 2006).

Prior reports have indicated that administration of either an NSAID (indomethacin, a nonselective cyclooxygenase inhibitor) or a tricyclic antidepressant drug (imipramine, a nonselective monoamine reuptake inhibitor) produces dose-dependent antinociceptive effects in various rat models of inflammatory nociception (Yokogawa et al., 2002; Zarrindast and Sahebgharani, 2002; Nagakura et al., 2003; Bauero et al., 2004; Zhang et al., 2004). However, the effects of analgesics and antidepressants on the coordination of sensory and emotional aspects of pain are still poorly understood. Therefore, this study addressed the effects of indomethacin and imipramine pretreatments on pain- or stress-induced changes in NK-1 receptor and BDNF gene expression in “sensory” (spinal cord dorsal horn) versus “affective” (hippocampal) pain processing within the CNS.

Materials and Methods

Animal Housing and Handling. Young adult male Sprague-Dawley rats (Harlan Farms, Indianapolis, IN), used for all experiments, were age matched (7–8 weeks old) at the beginning of the drug and animal treatments. Rats were allowed at least 1 week of habituation before any treatments were applied. The maintenance of the rat colony and all of the animal handling were performed in accordance with National Institutes of Health laboratory care standards and approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee. Efforts were made to minimize animal suffering and to reduce the number of animals used in this study. Animals were housed (12-h light/dark cycle) in groups of three per cage with ad libitum access to food and water, and they were mixed together so there was one member of each treatment group in every cage. All rats, including the sham control group, were handled identically to reduce stress-associated variability.

Pain and Stress Treatments. Rats (200–300 g) were subjected to i.p. pretreatments with either a single dose of indomethacin (10, 20, or 30 mg/kg for 1 h) or repetitive imipramine doses (15 mg/kg for 21 days) and then challenged with either an inflammatory stimulus (s.c. injection of 50 μl of CFA) into the plantar aspect of the right hind paw or an acute (45 min) immobility stress. Sham control animals were not exposed to pain or stress paradigms but were administered i.p. vehicle (ethanol or saline) of the same volume as the drug injections. Otherwise, controls were handled identically to the treatment animals.

A schematic set of animals was added to avoid the potential effects of additional animal handling and stress associated with thermal and mechanical testing on the outcome of these experiments. Before the dissection of spinal and hippocampal tissues, both hind paws were removed and weighed to assess the effects of drug treatments on the nociception-related peripheral inflammation.

Thermal and Mechanical Testing. A Thermal Paw Analgesimeter (Department of Anesthesiology, University of California, San Diego, CA) was used to measure thermal withdrawal sensitivity. Rats were initially placed in Plexiglas chambers and allowed to acclimate for 15 to 20 min. A high-intensity light beam (CXL/CRX lamp bulb; 8; 50 W; Eiko, Tokaimura, Japan) was focused on the plantar surface of the hind paw as a noxious thermal stimulus; paw withdrawal latencies were measured with an automatic timer to the nearest 0.01 s (Hargreaves et al., 1988; Dirig et al., 1997). The intensity of the light was set to 4.25 A to produce the baseline withdrawal latency of approximately 10 s. For quantification of mechanical sensitivity thresholds, von Frey monofilaments (Stoelting, Inc., Wood Dale, IL) of graded bending forces (2.6–522 mN) were applied to the plantar aspects of the hind paw of unrestrained rats placed in an elevated Plexiglas chamber with a wire mesh grid bottom. Monofilaments were applied perpendicular to the hind paw surface with sufficient force to cause a slight bending of the filament in increasing order of intensity until the rat responded by vocalization or withdrawal of the paw. Mechanical stimulation was repeated three times at 5- to 10-min intervals, with randomization of order of testing for each paw (adapted from Brennan et al., 1996). Monofilament thresholds were converted to grams of force using the manufacturer’s table. Thermal and mechanical baseline measurements of both hind paws were taken for each animal before nociceptive inoculation with CFA. All behavioral measurements were conducted by an experimenter blind to animal treatments.

![Image](https://jpet.aspetjournals.org/static/figure1.png)

**Fig. 1.** Schematic diagram showing drug treatment paradigms. Rats were subjected to i.p. pretreatments with either a single dose of indomethacin (10, 20, or 30 mg/kg for 1 h; A) or repetitive imipramine doses (15 mg/kg for 21 days; B) and then challenged with either an inflammatory stimulus (s.c. injection of 50 μl of CFA) into the plantar aspect of the right hind paw or an acute (45 min) immobilization stress. Sham control animals were not exposed to pain or stress paradigms but were administered i.p. vehicle (ethanol or saline) of the same volume as the drug injections. Otherwise, controls were handled identically to the treatment animals.
Tissue Dissection. All rats were decapitated 24 h after receiving the pain or stress challenge. Immediately after decapitation, rat brains and spinal cords were removed. Rat brains were dissected along the sagittal midline, followed by bilateral removal of the hippocampus. Spinal cord tissues were rapidly removed using hydraulic pressure (a forceful injection of ice-cold isotonic saline) applied to the caudal end of the vertebral canal with a 60-ml syringe and a 16-gauge needle. The lumbar portions (L1-L4) of the vertebral column were then dissected. The dorsal horn regions were dissected by cutting the lumbar spinal cord along the sagittal axis and dividing it into quarters. Only the ipsilateral side of the spinal cord was assayed. The lumbar portions of the vertebral column were then cut at the lumbar spinal cord along the sagittal axis and dividing it into quarters. Only the ipsilateral side of the spinal cord was assayed. The time of sacrifice, hind paws were also removed just above the tibiotalar joint and weighed to measure edema.

Solution Hybridization-Nuclease Protection Assays. The NK-1 receptor and BDNF (BDNF cDNA plasmid was graciously provided by Dr. Ronald Duman, Yale University School of Medicine, New Haven, CT) sense and antisense cRNA probes were generated by an in vitro run-off transcription reaction (McCarson and Krause, 1994; Nibuya et al., 1995). Synthesis of the antisense \(^{32}\)P-labeled cRNA and antisense cRNA probes were generated into quadrants. Only the ipsilateral side of the spinal cord was assayed. The dorsal horn regions were dissected by cutting the lumbar spinal cord along the sagittal axis and dividing it into quarters. Only the ipsilateral side of the spinal cord was assayed. At the time of sacrifice, hind paws were also removed just above the tibiotalar joint and weighed to measure edema.

Statistical Analyses. Data from all of the experiments were analyzed using analysis of variance (ANOVA) with Student-Newman-Keuls statistical tests used for post hoc comparisons, except for the mechanical sensitivity experiment, where nonparametric Kruskal-Wallis analysis with Dunn’s post-test was used (InStat; GraphPad Software, Inc., San Diego, CA). Significance was considered to be \( p < 0.05 \).

Results

Analgesic and Anti-Inflammatory Effects of Indomethacin and Imipramine. The analgesic and anti-inflammatory properties of NSAID and antidepressant drugs were evaluated using paw sensitivity thresholds and levels of edema. In animals that did not receive a drug treatment (CFA alone group), 50-µl injection of CFA produced significant thermal (Fig. 2A) and mechanical (Fig. 2B) hyperalgesia of the ipsilateral paws at 24 h postinjection. Administration of ethanol (indomethacin vehicle) had no considerable effect on the lowered sensitivity. However, pretreatments with either 10 or 20 mg/kg indomethacin (INDO) fully inhibited both forms of the hyperalgesia because the thresholds recorded in these animals were similar to baseline latencies. The highest dose of indomethacin (30 mg/kg) attenuated only the mechanical hypersensitivity, whereas the mechanical thresholds were only partially affected. When given at 20 mg/kg, indomethacin seemed to exhibit optimal antinociceptive properties, so this dose was used throughout subsequent experiments. Pretreatment with 15 mg/kg/day imipramine (IMI) for 21 consecutive days showed no significant effects on mechanical hyperalgesia, whereas thermal sensitivity was only slightly decreased. Similar to ethanol, administration of saline (imipramine vehicle) did not affect CFA-induced hyperalgesia.

CFA produced robust local edema of the ipsilateral hind paw at 24 h postinjection, as shown by the significant increase in weight compared with the contralateral paw (Fig. 3). The anti-inflammatory effect of indomethacin was confirmed because edema of the ipsilateral paw was completely blocked at this time, and the ipsilateral paw weight was almost identical to that of the contralateral side (Fig. 3A). In contrast, imipramine showed no anti-inflammatory properties; ipsilateral paws in these animals were similar in weight and redness to the group that received CFA alone (Fig. 3B). In addition, acute immobilization stress had no effect on the appearance or weight of either paw.
lation of NK-1 receptor and BDNF gene expression in “sensory” (spinal cord) and the “affective” (hippocampus) regions of the CNS were assessed using solution hybridization-nuclease protection assays. As indicated in Figs. 4 and 5, 24 h after CFA injection into the hind paw, NK-1 receptor (Fig. 4A) and BDNF (Fig. 4B) mRNA levels were robustly increased in the ipsilateral spinal cord dorsal horn (by 45 and 67%, respectively) compared with sham-treated controls. Administration of indomethacin (20 mg/kg) 1 h before CFA injection completely inhibited the nociception-evoked up-regulation of both genes in the spinal cord (Fig. 4). Not surprisingly, a single 45-min bout of immobilization stress had no effect on either NK-1 receptor or BDNF mRNA levels in the dorsal horn (Figs. 4 and 5). Administration of imipramine (15 mg/kg/day) for 21 days did not alter spinal gene expression by itself (Fig. 5). Interestingly, imipramine pretreatment did diminish CFA-induced increases in NK-1 receptor and BDNF mRNA levels (Fig. 5) to an extent comparable with that produced by indomethacin (Fig. 4).

In the hippocampal formation, both inflammatory nociception and immobilization stress robustly decreased NK-1 receptor and BDNF gene expression, as shown in previous reports (Duric and McCarson, 2005). Neither pain nor stress produced significantly sided differences; thus, hippocampal
mRNA levels are shown as bilateral averages. Surprisingly, indomethacin pretreatment was unable to block the damaging modulatory effects of pain or stress on the hippocampus (Fig. 6). As shown in Fig. 6, hippocampal NK-1 receptor and BDNF mRNA levels are similar in vehicle- or indomethacin-treated rats receiving pain or stress model treatments. This contrasts with the ability of indomethacin to reverse the pain-evoked changes in gene expression in the spinal cord dorsal horn (Fig. 4). Administration of the antidepressant drug imipramine clearly prevented the immobilization stress-induced decreases in hippocampal NK-1 receptor and BDNF mRNA levels (Fig. 7), as predicted by previous reports (Nibuya et al., 1995). Furthermore, the effects of CFA-induced changes in NK-1 receptor and BDNF gene expression were also fully inhibited by imipramine pretreatment (Fig. 7).

Levels of β-actin mRNA were determined to serve as gel loading controls and to further ensure that the detected changes in NK-1 receptor and BDNF mRNA levels are not due to a pain- or stress-related global modulation of gene expression within the CNS. β-Actin mRNA levels were unaffected by peripheral inflammatory nociception, immobilization, or drug treatments alone in either the spinal cord or hippocampus.

**Effects of Indomethacin on Pain-Related Behaviors and Thermal Sensitivity during the First 24 h after CFA.** Since the results of the first two experiments demonstrated that indomethacin inhibited hyperalgesia, peripheral inflammation and gene expression up-regulation in the spinal cord after CFA administration without prevention of its effects on the hippocampus, an additional experiment assessed the onset of indomethacin-produced analgesia. The quantification of pain-related behaviors during...
ing the first 26 min after CFA is shown in Fig. 8A. Baseline behaviors were measured before the nociceptive stimulation. The animals received an average rating of 0.1, mainly due to routine grooming and rearing behaviors. In rats that did not receive indomethacin, CFA injection evoked robust withdrawal, elevation, and frequent licking and biting of the ipsilateral hind paw that peaked at 6 min postinoculation (Fig. 8A) as well as redness and edema (Fig. 2). In animals pretreated with indomethacin (20 mg/kg), initial redness and swelling was also observed, but the pain-related behaviors were considerably attenuated (Fig. 8A). Pain-related behaviors were quantified by measuring the area under the curve in Fig. 8A; the CFA-only group had an AUC value of 17.0 ± 1.5, whereas CFA + indomethacin produced an AUC value of 6.5 ± 1.6 (mean ± S.E.M.). Nevertheless, the rats still appeared agitated; the CFA-inflamed paws generally bore little to no weight or were slightly elevated with occasional licking and biting despite the presence of indomethacin (i.e., the AUC of pain-related behaviors of CFA + indomethacin rats was greater than 0).

Thermal sensitivity of the CFA-inflamed paws is shown in Fig. 8B. At 30 min postinjection, animals injected with CFA alone displayed a robust hyperalgesia that persisted for at least 24 h (Fig. 8B). It is noteworthy that the indomethacin-treated group also showed significant hyperalgesia during the initial 60 min; however, by 90 min after CFA, the drug’s analgesic effect was clearly present as the withdrawal latencies returned to baseline levels (Fig. 8B).
Drug Effects on Spinal and Hippocampal NK-1 and BDNF Levels

Discussion

The use of NSAIDs and antidepressants in the management of various chronic pain conditions has been well documented in clinical settings (Walker, 1995; Richeimer et al., 1997; Korzeniewska-Rybicka and Plaznik, 1998; Minotti et al., 1998; Fishbain, 2000; Fishbain et al., 2000). Their analgesic properties have been associated with alterations of pain signaling in the sensory pathways within the CNS, primarily the spinal cord and brain stem. However, pain may also have an affect on the emotion- and cognition-processing components of the CNS. The possibility of stress-like modulation in higher brain centers has been previously supported by numerous clinical observations that people suffering from chronic pain are often also depressed (Bair et al., 2003). The effects of NSAIDs and antidepressants on mood-regulating brain regions such as the hippocampal formation are poorly characterized in animal models of persistent pain. The experiments in this study were designed to assess the effects of either indomethacin or imipramine pretreatment on gene expression within sensory and affective pain processing regions of the CNS during peripheral inflammatory nociception or immobilization stress.

Initially, the effects of analgesic and antidepressant drugs on inflammation-evoked hyperalgesia were determined. As reported by other studies, animals treated with CFA developed robust swelling and inflammation of the injected limb, as well as significant thermal and mechanical hyperalgesia (Fig. 2) measured at the plantar surface of the paw (Ma and Woolf, 1996). Three different doses (10, 20, and 30 mg/kg) of indomethacin were used in this experiment that were comparable with previously suggested doses (Nagakura et al., 2003). The results indicated that, at 24 h post-CFA administration, pretreatment with indomethacin induced a complete reversal of both thermal and mechanical hyperalgesia in the injected paw (Fig. 2). The observed analgesia was fully attributable to the effects of the drug alone since the vehicle (ethanol) alone did not produce a similar outcome. Interestingly, the highest dose of the NSAID indomethacin (30 mg/kg) evoked only partial inhibition of mechanical hypersensitivity, probably due to the gastrointestinal side effects of indomethacin and its overall toxicity at high doses (Baueroa et al., 2004). Indomethacin given at a dose of 20 mg/kg emerged as the most efficacious analgesic treatment, so this dose was used in subsequent experiments addressing peripheral inflammation and gene expression within the CNS. Moreover, the CFA-evoked redness and edema observed in the injected paws at 24 h were completely inhibited by indomethacin (20 mg/kg) pretreatment (Fig. 2A), clearly confirming the anti-inflammatory properties of this drug.

Other reports have suggested that tricyclic and various other classes of antidepressant drugs produce both antinociceptive and anti-inflammatory effects in rats exposed to painful stimuli (Abdel-Salam et al., 2003). However, the administration of imipramine for 21 days at 15 mg/kg/day produced no considerable thermal or mechanical hyperalgesia (Fig. 2) and no effect on the CFA-evoked edema (Fig. 2B). This treatment paradigm was chosen for this study since it has been shown to induce antidepressant effects in the hippocampus, as manifested by increased neurogenesis and expression of growth factors needed for neuronal survival (Nibuya et al., 1995; Malberg et al., 2000). In these experiments, the imipramine administration was long-term, with the analgesic and anti-inflammatory properties measured 24 h after inflammatory nociception challenge. In previous studies, a single dose of antidepressants was usually applied 30 to 90 min before painful stimulation, and the effects were observed soon after (Yokogawa et al., 2002; Zarrindast and Sahebgharani, 2002; Abdel-Salam et al., 2003; Nagakura et al., 2003). Thus, the differences between the antidepressant and analgesic actions of antidepressant drugs may be a function of both the time of initiation and the duration of the drug treatment.
more, the relevant sites of action within the CNS may be different with regard to antidepressant versus analgesic activity. The antidepressants effects are thought to be related to activity in the hippocampus and other mood-regulating regions of the limbic system, whereas the analgesic properties are thought to involve modulation of endogenous pain pathways projecting from the brain stem to the spinal cord. This is supported by clinical studies showing that analgesic effects of antidepressant drugs may occur without the antidepressant effects, occurring with a much more rapid onset and at lower doses (McQuay et al., 1996; Lynch, 2001; Raja et al., 2002).

The main goal of this study was to investigate the effects of analgesic and antidepressant drugs on changes in NK-1 receptor and BDNF gene expression evoked by pain or stress. Results confirm that CFA produced significant increases in NK-1 receptor and BDNF mRNA levels in the ipsilateral dorsal horn, consistent with previous results (McCarson and Krause, 1994) and with the role of these neuromodulators in the generation of hyperalgesia and amplification of nociceptive signaling during persistent pain (Woolf and Salter, 2000). Treatment with the NSAID analgesic drug indomethacin (Fig. 4) or the antidepressant drug imipramine (Fig. 5) did not alter spinal gene expression when the drugs were administered alone. However, at the level of the spinal cord, both indomethacin and imipramine provided full protection against CFA-induced increases in NK-1 receptor and BDNF gene expression. Indomethacin-evoked inhibition of spinal gene activation may mechanistically support the analgesic effects initially observed in pain-related behaviors and hyperalgesia. Even though imipramine did not attenuate thermal or mechanical hyperalgesia (Fig. 2), the gene expression results suggest that trycyclic antidepressants (Fig. 5), like NSAIDs (Fig. 4), may exhibit analgesic-like properties at the level of gene expression within nociceptive sensory neuronal pathways of the CNS. Of course, nociception induces a myriad of cellular and molecular changes within the spinal cord, and these data do not imply that nociception-induced changes in expression of NK-1 receptor and BDNF genes are the sole factors important in spinal modulation of inflammation and hyperalgesia.

Activation of peripheral and central nociceptive pathways may have an affect on the limbic system through initiation of cellular transduction mechanisms similar to those characterized as important in stress and depression. Contrary to the pain-evoked increases in gene expression previously characterized in the spinal cord (see above), significant decreases in hippocampal NK-1 receptor and BDNF mRNA levels were observed after either peripheral inflammation or immobilization stress. These data further support our previous findings (Duric and McCarson, 2005, 2006) and provide additional evidence that both pain and stress activate the same “nonsensory” brain regions and induce potentially detrimental modulatory effects. Despite its anti-inflammatory (Fig. 3) and analgesic (Fig. 2) effects in the periphery and inhibitory effects in the spinal cord (Fig. 4), indomethacin had no significant effect on nociceptive activation of the brain, as shown by a lack of inhibition of pain-evoked decreases in hippocampal NK-1 receptor or BDNF gene expression. Thus, under these conditions, analgesic therapy that effectively blocked the development of inflammation and hyperalgesia was insufficient to prevent the initiation of stress-like effects in the limbic system.

Therefore, an additional experiment was performed to address CFA-induced behaviors and thermal hypersensitivity at early time points to clarify the interpretation of findings from the gene expression experiments. Since all measurements of behavior and gene expression to this point were conducted only at the 24-h time point, the final experiment showed the onset of indomethacin’s analgesic activity during the first 24 h after CFA. As indicated in Fig. 8, pretreatment with indomethacin only partially inhibited pain-related behaviors during the first 30 min after CFA injection, and analgesic effects on thermal hyperalgesia were not apparent until 90 min after CFA injection. This behavioral result clearly shows that there is an initial window of approximately 90 min immediately after CFA administration when pain-related behaviors are apparent before the analgesic effects of indomethacin begin. Therefore, even brief, temporary nociceptive stimulation may be sufficient to induce delayed and long-lasting hippocampal plasticity. This finding is similar to recent observations that early life exposures to short-term stress can lead to delayed onset of cellular mechanisms causing profound, progressive hippocampal impairment (Brunson et al., 2005). Taken together, the current results suggest that analgesic drugs such as indomethacin are very effective in diminishing nociception in the periphery and spinal cord but may have little effect on the negative emotional and cognitive impact of pain.

Repetitive administration of antidepressants have been shown to enhance synaptic levels of norepinephrine and serotonin in the hippocampus, with concomitant increases in BDNF and tyrosine kinase B mRNAs and protein levels (Duman et al., 1997). Regulation of hippocampal BDNF has been linked to increased expression and activation of cAMP response element binding protein and is thought to be associated with the reversal of stress-related limbic pathophysiology (Nibuya et al., 1995). In the current study, prolonged antidepressant treatments alone did not affect NK-1 receptor and BDNF mRNA levels in the spinal cord or hippocampus (Figs. 5 and 7), which is consistent with previous observations (Coppell et al., 2003; Sartori et al., 2004). However, in rats that were exposed to pain or stress, imipramine completely inhibited the pain- or stress-evoked down-regulation of hippocampal NK-1 receptor and BDNF gene expression (Fig. 7). Thus, antidepressants apparently block the changes in expression of genes that contribute to long-term nociceptive sensory plasticity not only in the spinal cord but also in limbic regions involved in the modulation of affect. Furthermore, these findings suggest that early antidepressant drug administration as part of persistent pain therapy may prevent predisposition toward development of pain-related depression.

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