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# c-Jun N-terminal kinase activation in dorsal root ganglion contributes to pain hypersensitivity

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**Abstract**

Inflammatory pain, characterized by a decrease in the nociceptive threshold, arises through the actions of inflammatory mediators. Mitogen-activated protein kinase cascades participate in peripheral nociceptive sensitization. We examined the involvement of c-Jun N-terminal kinase (JNK) in the dorsal root ganglion (DRG) in the early phase of inflammation-induced hyperalgesia. An intra-plantar (i.pl.) injection of complete Freund's adjuvant induced the activation of JNK in DRG neurons within 30 min. Pre-treatment as well as post-treatment of rats with a JNK inhibitor, SP600125, significantly attenuated thermal hyperalgesia, as assessed by paw-withdrawal latency, and the upregulation of c-fos immunoreactivity in dorsal horn neurons. An i.pl. injection of nerve growth factor (NGF) also induced the phosphorylation of JNK as well as thermal hyperalgesia, and SP600125 improved hyperalgesia. Inhibitor experiments suggest that JNK and extracellular signal-regulated protein kinase act on primary nociceptive neurons synergistically. These findings demonstrate that JNK is a therapeutic target for treating inflammation-induced pain hypersensitivity.


*Keywords:* DRG; Inflammation; JNK; ERK

Intense noxious stimuli and tissue inflammation produce a pain hypersensitivity that results from both peripheral and central sensitization [1,2]. Peripheral inflammation results in changes in neuropeptides, ion channels, and receptor levels in the cell bodies of dorsal root ganglion (DRG) neurons [3–6]. Nerve growth factor (NGF) is upregulated in inflamed tissues [6] and plays an important role in inflammatory pain by driving peripheral sensitization, acting directly on the peripheral terminal to produce, within a short period, heat hyperalgesia [7]. In addition, NGF is retrograde-transported to the cell bodies of DRG neurons, where it increases the gene expression [6,8].

Mitogen-activated protein kinase (MAPK) cascades are involved in inflammation and tissue destruction, and have been shown to participate in peripheral nociceptive sensitization. Three major MAPK families that differ in their substrate specificity and responses to stress have been identified in vertebrates; extracellular signal-regulated kinase (ERK), p38 MAPK, and c-Jun N-terminal kinase (JNK). ERK activation in the peripheral terminals of DRG neurons contributes to pain hypersensitivity by increasing gene transcription as well as by post-translational modifications of target proteins [9–13]. P38 MAPK activation in DRG neurons has also been implicated in exaggerated pain states [14–16]. Peripheral inflammation induces p38 MAPK activation in the soma of C fibers of the DRG, and this activation is necessary for the upregulation of heat-gated ion

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channel TRPV1 in these cells for slow-onset NGF-dependent hyperalgesia. It is noted that phospho-p38 MAPK-positive DRG neurons are not increased in the early period (2 or 6 h) after the onset of inflammation [14–16]. Thus, the activation of ERK as well as p38 MAPK in nociceptors could provide an important mechanism for inflammation-induced pain. However, whether JNK is involved in peripheral inflammation-induced nociceptive sensitization remains to be determined.

JNK can be induced by inflammatory cytokines, bacterial endotoxin, osmotic shock, UV radiation, and hypoxia. Several JNK isoforms, encoded by three genes, phosphorylate-specific sites (serine 63 and serine 73) on the amino-terminal transactivation domain of c-Jun after exposure to ultraviolet irradiation, growth factors, or cytokines [17]. In this study, we focused on the early phase of inflammation-induced hyperalgesia and examined whether JNK activation is involved in thermal hyperalgesia induced by NGF or inflammation in vivo. We report that an intra-plantar (i.pl.) injection of complete Freund's adjuvant (CFA) or NGF elicited thermal hyperalgesia and activated JNK, and that inhibiting this pathway results in the elimination of thermal hyperalgesia.

## Materials and methods

*Animals.* Adult male Sprague–Dawley rats (200–250 g) were used according to Chiba University Animal Care Institutional Guidelines following the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (1996 revision).

*Surgical procedures.* All procedures were performed under 3% halothane anesthesia in 50% $O_2$. Complete Freund's adjuvant (50 μ; CFA; Calbiochem, La Jolla, CA) was i.pl. injected into the ipsilateral hind paw 60 min after SP600125 (5 mg/kg) ($n = 7$) or DMSO (100 μl) ($n = 7$) s.c. administration. In further experiments, SP600125 (500 μg) ($n = 8$) was i.pl. injected to the ipsilateral hind paw 10 min after CFA (50 μl) i.pl. injection. Another group of rats received DMSO (50 μl) i.pl. at 10 min after the CFA (50 μl) i.pl. injection ($n = 6$) as a control. In another set of experiments, nerve growth factor-β (NGF-β, 0.5 μg in 50 μl normal saline; Sigma, Saint Louis, MO) was injected into the plantar surface (i.pl.) of the left hind paw. Treatments were administered as follows: the JNK inhibitor anthra[1,9-*cd*]pyrazol-6(2*H*)-one SP600125 (5 mg/kg; Biomol, Plymouth Meeting, PA) in dimethyl sulfoxide (10 μg/μl; DMSO; Wako, Osaka, Japan), the mitogen-activated protein kinase kinase (MAPKK) inhibitor 2-(2-amino-3-methoxyphenyl)-4*H*-1-benzopyran-4-one PD98059 (500 μg/kg; Sigma, Saint Louis, MO) in DMSO (1 μg/μl), and a mixture of SP600125 (5 mg/kg) and PD98059 (500 μg/kg). These drugs (100 μl) were systemically administered subcutaneously (s.c.) 60 min before NGF i.pl. injection ($n = 8$ per treatment group).

*Behavioral test.* Thermal nociceptive thresholds in rat hind paws were evaluated using a Hargreaves device (Ugo Basile, Varese, Italy). The animals were placed in a Plexiglas testing chamber (17 × 22 cm) with a floor maintained at 27–29 °C. A heat stimulus (150 mcal/s/cm$^2$) was delivered using a 0.5-cm-diameter radiant heat source positioned under the plantar surface of the paw to be tested. The heat source was placed alternately under each hind paw, the first stimulated paw being randomly selected to avoid anticipation by the animal. A cut-off time of 20 s was used after it was ascertained that no injury was produced within this time. Three trials were performed in each session, and the latency of paw withdrawal (s) from all trials was averaged. Nociceptive thresholds were measured before (for basal control) and at 1, 2, 4, and 6 h after NGF-β i.pl. injection, and in a separate series of experiments, before and at 2, 6, 12, and 24 h after CFA i.pl. injection. The extent of hyperalgesia is expressed as the percentage of ipsilateral paw-withdrawal latency before stimulus.

*Western blots.* At 30, 60 or 120 min after CFA (50 μl i.pl.) injection, or at 10, 30 or 60 min after NGF-β (0.5 μg in 50 μl saline; Sigma) i.pl. injection, rats were anesthetized with halothane. The ipsilateral L5 DRGs were quickly removed after decapitation ($n = 4$ for each time point) and homogenized in buffer containing a cocktail of proteinase and phosphatase inhibitors. Protein samples were separated on a SDS–PAGE gradient gel (10%; Bio-Rad) and transferred to PVDF filters. The blots were blocked with 5% bovine serum albumin (BSA) for 1 h and incubated with either phospho-JNK antibody (1:1000; Cell Signaling Technology, Beverly, MA) or total JNK antibody (1:1000; Cell Signaling Technology) overnight at 4 °C. These antibodies detected all three isoforms of JNK. The blots were then incubated in HRP-conjugated secondary antibody (1:2000) for 1 h at room temperature, developed in ECL solution (NEN) for 1 min, and exposed onto X-ray films (Superfilm; Amersham) for 2–10 min. Each experiment was repeated at least three times.

*Immunohistochemistry.* Rats with CFA treatment for 120 min (50 μl, i.pl.; $n = 6$) or naïve control rats ($n = 6$) were perfused transcardially with 500 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer solution (PBS), and ipsilateral L5 DRGs were dissected out. The specimens were immersed in the same fixative overnight at 4 °C after which they were transferred into 20% sucrose and incubated at 4 °C overnight. Serial sections (12 μm thick) were prepared from DRG using a cryostat and mounted on poly-L-lysine-coated slides. The sections were incubated with rabbit polyclonal anti-p-JNK antibody (1:400; Cell Signaling Technology) overnight at 4 °C. After washing with PBS, the sections were incubated with Alexa Fluor 488-conjugated donkey anti-rabbit IgG antibody (5 μg/ml; Molecular Probes, Eugene, OR) for 2 h, followed by washing. All the slides were then coverslipped using Permafluor (Shandon, Pittsburgh, PA).

In some sets of experiments, SP600125 (5 mg/kg of 10 mg/ml in DMSO) or 100 μl DMSO was injected subcutaneously 60 min before the CFA i.pl. injection ($n = 6$ per each treatment group). At 120 min after CFA i.pl. injection, rats were perfused with PFA, and the L5 segment of the spinal cord was dissected out. The specimens were processed for immunohistochemistry in the same manner using the anti-c-Fos antibody (1:5000; Calbiochem-Oncogene, La Jolla, CA) as the primary antibody.

*Statistical analysis.* The images of immunostained sections were captured with a CCD camera, and the number of immunoreactive cell profiles was counted in a blinded fashion. Control and treated sections were mounted on the same slides and processed under the same conditions. Every fifth section was selected from a series of consecutive sections, and four sections were counted. For Western blot, the films were scanned, and the density of specific bands was measured and normalized with the control band. All data were analyzed by unpaired *t* tests with $p$ values $<0.05$ considered statistically significant and are shown as means ± SEM.

## Results

### *Localized peripheral inflammation activates JNK*

Experimental inflammation produced by an i.pl. injection of CFA results in local sensory hypersensitivity. To test if localized peripheral inflammation activates

JNK in the DRG, CFA was injected into the plantar surface of the left hind paw. This injection induces localized inflammation that develops over minutes, lasts more than a week, and is associated with swelling and erythema, as well as thermal and mechanical pain hypersensitivity [18]. CFA-induced inflammation produced an increase in p-JNK protein levels at 60 and 120 min after CFA stimulation in the ipsilateral L5 DRG, as detected by Western blots using the anti-phospho-JNK (p-JNK) antibody (Fig. 1A). Total JNK levels did not increase after inflammation (Fig. 1A), indicating that the increase was caused by JNK phosphorylation. Immunohistochemistry confirmed that there were low levels of p-JNK in DRG neurons under naïve conditions using a phospho-specific JNK antibody. We found that p-JNK was present in the nucleus of approximately 25% of neurons in the DRG in naïve rats (Fig. 1B). p-JNK was also located in some surrounding non-neuronal cells, presumably satellite cells. Importantly, there was a significant increase in the number of p-JNK-positive neurons at 120 min after CFA (50 μl, i.pl.) injection ($59.2 \pm 11.2\%$, $p < 0.01$ unpaired $t$ tests, compared with the naïve group, $24.7 \pm 12.0\%$) (Fig. 1B). Size frequency analysis demonstrates that p-JNK was present mainly in small-to-medium diameter DRG neurons in both control and inflamed rats, although some of the large cells are also immunoreactive for p-JNK. C fiber sensory neurons are divided into two groups [19]. NGF-responsive/tropomyosin receptor kinase (TrkA)-expressing neurons are positive for substance P and calcitonin gene-related peptide (CGRP), and glial cell line-derived neurotrophic factor (GDNF)-responsive/c-ret-expressing neurons bind to isolectin IB4 [10]. However, we found no specificity in the localization of p-JNK in C fiber sensory neurons (data not shown).

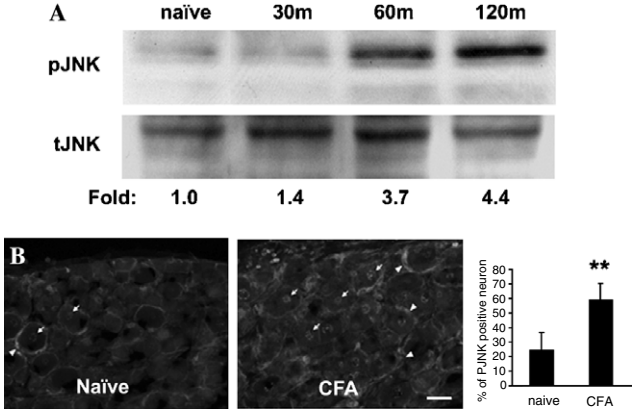


Fig. 1. CFA induces JNK activation in DRG. (A) The representative figure for the Western blot showed increased JNK phosphorylation in the ipsilateral L5 DRG at 60 and 120 min after CFA (50 μl i.pl.) injection. The bottom panel indicates the levels of total JNK (tJNK) ($n = 4$ per time point). (B) Immunohistochemistry showing that JNK phosphorylation is increased in ipsilateral L5 DRG 120 min after CFA (50 μl i.pl.) injection. Arrows and arrowheads indicate neuronal nucleus and non-neuronal cells, presumably satellite cells, respectively. Scale bar, 50 μl. $^{**}p < 0.01$, compared with the naïve group ($n = 6$ per group).

### *Inhibition of JNK blocks CFA-induced thermal hyperalgesia*

To examine if JNK activation in DRG neurons is involved in the production of inflammatory pain hypersensitivity, we administered a specific JNK inhibitor, SP600125 (5 mg/kg s.c.), 60 min before the CFA (50 μl, i.pl.) injection. SP600125 does not block the activity of ERK or p38 MAPK [20], but blocks all three isoforms of JNK [21]. No heat basal sensitivity was affected by SP00125 administration in non-treated rats (data not shown). The pain behavior was tested 2, 6, 12, and 24 h after CFA stimulation in the ipsilateral hind paw. SP600125 pre-treatment did not significantly change the earliest phase (2 h) of inflammatory pain (Fig. 2A). The inhibitor, however, reduced inflammation-induced heat hyperalgesia at 6 h ($54.6 \pm 7.4\%$, $p < 0.01$ unpaired $t$ tests, compared with the DMSO s.c. group, $38.6 \pm 7.4\%$), 12 h ($64.6 \pm 9.1\%$, $p < 0.01$ unpaired $t$ tests, compared with the DMSO s.c. group, $48.3 \pm 5.4\%$), and 24 h ($74.2 \pm 9.7\%$, $p < 0.01$ unpaired $t$ tests, compared with the DMSO s.c. group, $57.5 \pm 12.3\%$) after CFA injection (Fig. 2A). To investigate whether the timing of the JNK inhibitor influenced the behavioral response, a bolus of SP600125 (500 μg in 50 μl DMSO) was injected into the inflammatory site (plantar surface) at 10 min after CFA (50 μl, i.pl.) injection. The post-treatment of rats with SP600125 decreased thermal hyperalgesia at all time points examined; 2 h ($73.7 \pm 14.5\%$, $p < 0.05$ unpaired $t$ tests, compared with the DMSO i.pl. group, $57.8 \pm 14.3\%$), 6 h ($61.4 \pm 11.8\%$, $p < 0.01$ unpaired $t$ tests, compared with the DMSO i.pl. group, $38.8 \pm 9.1\%$), and 12 h ($70.2 \pm 13.9\%$, $p < 0.01$ unpaired $t$ tests, compared with the DMSO i.pl. group, $47.3 \pm 10.6\%$). Notably, rats receiving the inhibitor treatment showed little hypersensitivity at 24 h ($86.1 \pm 12.8\%$, $p < 0.001$ unpaired $t$ tests, compared with the DMSO i.pl. group, $57.0 \pm 10.0\%$) after CFA i.pl. injection (Fig. 2B). We next assessed whether c-Fos expression in the spinal cord of rats with the CFA injection was dependent on JNK activation (50 μl, i.pl.). The expression of c-fos was upregulated 2 h after the CFA injection in laminae I–II of the ipsilateral lumbar spinal dorsal horn of L5. However, SP600125 significantly suppressed the increase in the number of c-Fos-positive cells following the CFA injection ($4.7 \pm 3.0\%$, $p < 0.01$ unpaired $t$ tests, compared with the vehicle-treatment group, $14.1 \pm 4.7\%$) (Fig. 2C). The behavioral tests as well as the c-Fos immunohistochemistry strongly suggested that JNK activation is involved in pain hypersensitivity induced by CFA.

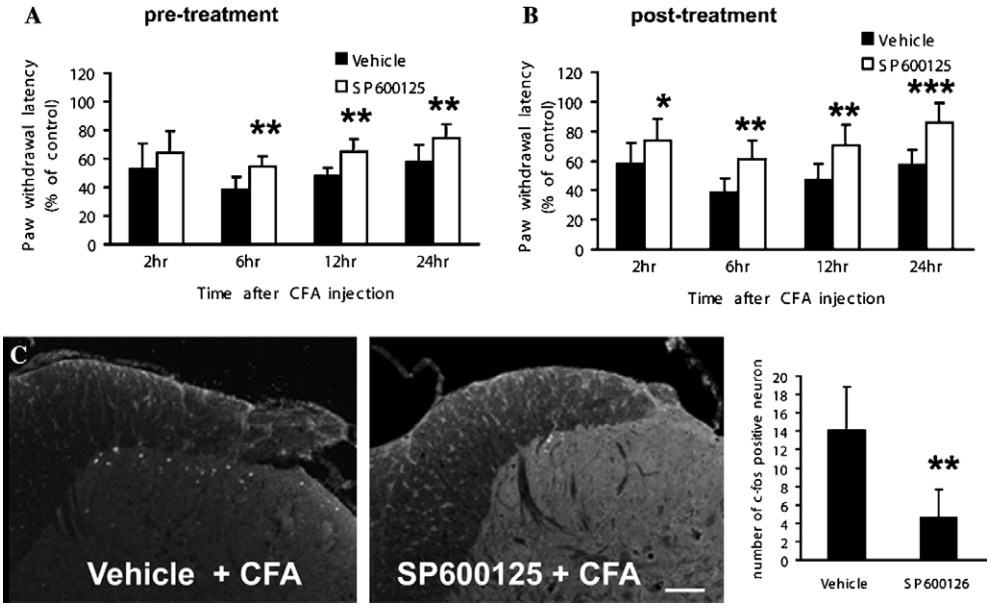


Fig. 2. Inhibition of JNK blocks CFA-induced thermal hyperalgesia. (A) Prior systemic administration of SP600125 (5 mg/kg s.c.) reduces inflammatory heat hyperalgesia at 6, 12, and 24 h after CFA (50 μl i.pl.) injection. $^{**}p < 0.01$, compared to the vehicle-treated (DMSO, 50 μl s.c.) group ($n = 7$ per group). (B) Local injection of SP600125 (500 μg i.pl.) 10 min after CFA (50 μl i.pl.) injection produces an inhibition of inflammatory heat hyperalgesia at all time points ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, compared to the vehicle treatment (saline, 50 μl i.pl.) group ($n = 6$ for vehicle group and $n = 8$ for SP600125 group)). (C) Effects of the JNK inhibitor on c-Fos immunoreactivity in the ipsilateral dorsal horn of the spinal cord at 2 h after CFA injection (50 μl i.pl.). The number of c-Fos-positive cells following CFA stimulation is decreased by systemic pre-treatment with SP600125 (5 mg/kg s.c) compared to the vehicle treatment (DMSO, 100 μl s.c.) group. Scale bar, 100 μl. $^{**}p < 0.01$, compared with the vehicle group ($n = 6$ per group).

### *Intra-plantar injection of NGF induces the activation of JNK in DRG neurons*

NGF levels increase in inflamed tissues, and this neurotrophin is suggested to be a major contributor to the production of inflammatory hyperalgesia [6]. The i.pl. injection of nerve growth factor-β (NGF-β; 0.5 μg in 50 μl normal saline) into the left hind paw led to thermal hyperalgesia, as shown by the reduction in the paw-withdrawal time (Fig. 3A). The paw-withdrawal latency was not significantly influenced at 1 or 6 h after NGF i.pl. injection, but was reduced at both 2 h ($55.0 \pm 9.7\%$, $p < 0.01$ unpaired $t$ tests, compared with the contralateral paw, $91.3 \pm 12.2\%$) and 4 h ($68.4.0 \pm 11.0\%$, $p < 0.01$ unpaired $t$ tests, compared with the contralateral paw, $95.3 \pm 12.8\%$), in comparison with the uninjected contralateral paw (Fig. 3A). Thus, NGF-induced thermal hyperalgesia can be evaluated during this period. To test whether NGF-induced thermal hyperalgesia induces JNK activation in DRG neurons, we assessed the activity of JNK using phospho-specific antibody. JNK was significantly phosphorylated at 10, 30, and 60 min after NGF-β i.pl. injection in the ipsilateral L5 DRG, although the total amount of JNK in the DRG did not change after NGF injection (Fig. 3B). This result is consistent with the data obtained in the rat model of CFA-induced hyperalgesia.

### *Inhibition of JNK activation reduces NGF-induced thermal hyperalgesia*

To test whether JNK activation in DRG is involved in the production of NGF-induced thermal hyperalgesia, we administered SP600125 in order to target JNK activity in the DRG. SP600125 was administered subcutaneously (s.c.) at a concentration of 5 mg/kg 60 min before NGF-β i.pl. injection. Decreased heat hyperalgesia was observed at both 2 h ($70.2 \pm 8.3\%$, $p < 0.05$ unpaired $t$ tests, compared with the DMSO s.c. group, $55.0 \pm 9.7\%$) and 4 h ($76.1 \pm 8.2\%$, $p < 0.05$ unpaired $t$ tests, compared with the DMSO s.c. group, $68.4.0 \pm 11.0\%$) in the ipsilateral paw (Fig. 4A). Similarly, the MAPKK inhibitor PD98059 (1 μg/μl in DMSO), which was systemically administered at a concentration of 500 μg/kg s.c., decreased heat hyperalgesia at 2 h ($74.2 \pm 11.9\%$, $p < 0.05$ unpaired $t$ tests, compared with the DMSO s.c. group, $55.0 \pm 9.7\%$) after NGF injection in the ipsilateral paw (Fig. 4B). Interestingly, a mixture of SP600125 (5 mg/kg) and PD98059 (500 μg/kg) almost completely attenuated thermal hyperalgesia at 2 h ($83.1 \pm 9.7\%$, $p < 0.01$ unpaired $t$ tests, compared with the DMSO s.c. group, $55.0 \pm 9.7\%$) as well as 4 h ($81.7 \pm 12.9\%$, $p < 0.05$ unpaired $t$ tests, compared with the DMSO s.c. group, $68.4.0 \pm 11.0\%$) after

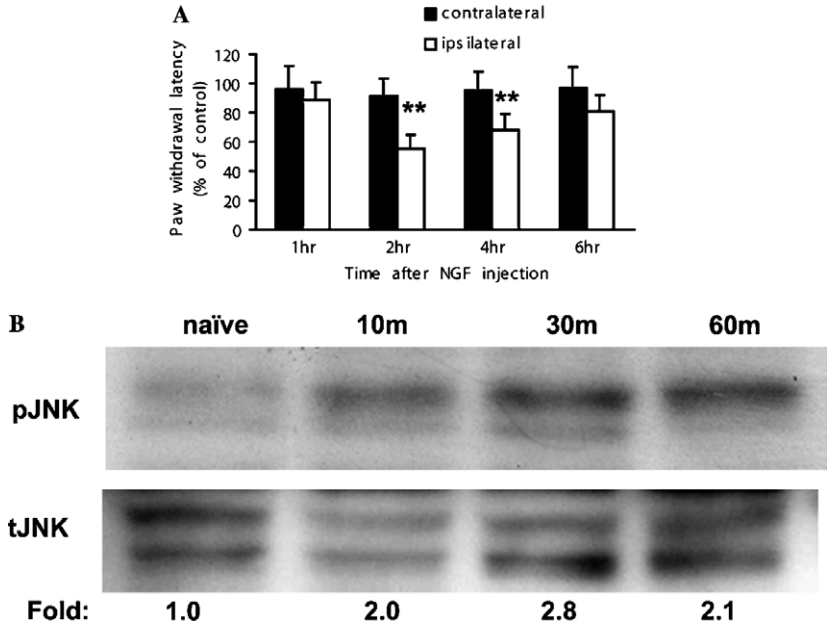


Fig. 3. NGF induces heat hyperalgesia and JNK activation in DRG. (A) The paw-withdrawal latency was significantly reduced at 2 and 4 h after NGF-β (0.5 μg in 50 μl normal saline, i.pl.) injection in comparison with the contralateral paw. Nociceptive thresholds were measured before (for basal control) and at 1, 2, 4, and 6 h after NGF-β i.pl. injection (**$p < 0.01$ compared with the contralateral paw) ($n = 8$ per group). (B) Representative figure showing the phosphorylation state of JNK. NGF-β (0.5 μg i.pl.) injection induces JNK phosphorylation in ipsilateral L5 DRG at 10, 30, and 60 min after NGF injection. The bottom panel indicates the levels of total JNK (tJNK) ($n = 4$ per time point).

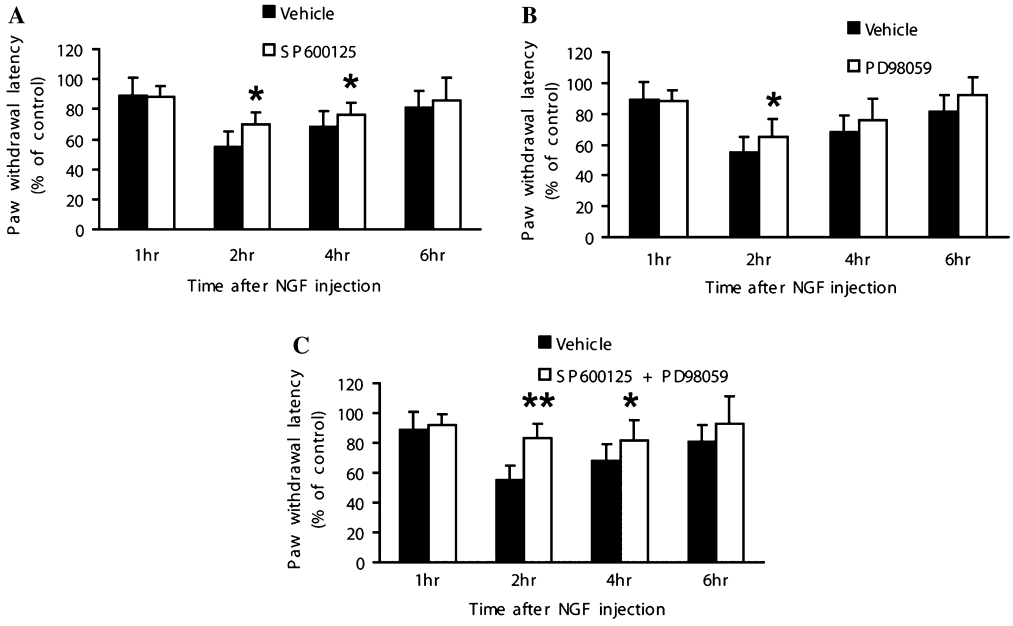


Fig. 4. Inhibition of JNK activation reduces NGF-induced thermal hyperalgesia. (A) Treatment with SP600125 (5 mg/kg s.c.) attenuates heat hyperalgesia in the ipsilateral hind paw at 2 and 4 h after NGF-β i.pl. injection. (B) Treatment with the MAPKK inhibitor PD98059 (500 μg/kg s.c.) attenuated heat hyperalgesia in the ipsilateral paw at 2 h after NGF-β i.pl. injection. (C) Treatment with a mixture of SP600125 and PD98059 (5 mg/kg and 500 μg/kg, s.c., respectively) dramatically attenuates heat hyperalgesia in the ipsilateral paw at 2 and 4 h after NGF-β i.pl. injection. Nociceptive thresholds are measured before (for basal control) and at 1, 2, 4, and 6 h after NGF-β i.pl. injection (A–C) (*$p < 0.05$, **$p < 0.01$ compared with vehicle-treated rats (A–C)) ($n = 8$ per group (A–C)).

NGF injection in the ipsilateral paw (Fig. 4C). These results suggest that JNK and ERK contribute synergically to eliciting thermal hyperalgesia induced by NGF.

## Discussion

In this study, we employed a rat model of hyperalgesia induced by CFA or NGF to address whether JNK is

involved in inflammation-induced nociceptor sensitization, and demonstrated that an i.pl. injection of CFA or NGF-activated JNK in the DRG. Some signals for p-JNK were found in small-to-medium-diameter neurons in the DRG, whereas some large neurons were also immunopositive for p-JNK. JNK activation is involved in CFA- or NGF-induced hyperalgesia, as the specific inhibitor of JNK was effective in attenuating it.

Members of the MAPK family act as regulators of pain sensitivity. ERK members of the MAPK family act both in the nociceptor peripheral terminal and the dorsal horn to produce pain hypersensitivity within 20 min of their activation by epinephrine and nociceptive synaptic input, respectively, an effect that is likely due to post-translational processing [9,22,23]. Inflammation induces a sustained activation of ERK in second order dorsal horn neurons, and this activation contributes to the gene expression and maintenance of inflammatory pain [14]. In addition, increased p38 MAPK immunoreactivity was seen in small DRG neurons following chronic constriction injury, peripheral inflammation as well as axotomy [14–16,24,25]. In the case of chronic constriction injury, the induction of p38 MAPK was found in adjacent uninjured as well as injured DRG neurons [26]. Inhibiting the p38 MAPK cascade partially attenuates pain-related behavior, suggesting that p38 MAPK is involved in the production or maintenance of pain hypersensitivity.

The third member of MAPK, JNK, is a serine threonine protein kinase that phosphorylates c-Jun, a component of the transcriptional factor activator protein 1 [17]. In a complex with other DNA-binding proteins, transcriptional factor activator protein 1 regulates the transcription of numerous genes, including cytokines, immunoglobulins, and matrix metalloproteinases. JNK can be induced by inflammatory cytokines, bacterial endotoxin, osmotic shock, UV radiation, and hypoxia. Peripheral axotomy has been shown to induce JNK activation in DRG neurons [27]. Recently, it was reported that p-JNK immunoreactivity was detected in injured DRG neurons after L5 spinal nerve ligation in rats [28]. JNK activation is involved in injury-induced mechanical allodynia. In these animal models, JNK activation is presumably induced by neuronal injury, and this JNK activation seems to be involved in pain-related behavior. By peripheral inflammation, JNK phosphorylation is not increased in the dorsal horn [29]. In this study, we focused on peripheral inflammation-induced hyperalgesia and examined the phosphorylation of JNK and pain-related behavior in the early phase. Our new findings demonstrate peripheral inflammation as well as the NGF-induced activation of JNK in DRG and that this activation is, at least partly, necessary for thermal hyperalgesia. This effect might involve the regulation of neuronal excitability with changes in transcription, as various proteins serve as JNK targets such as the activator protein-1 transcriptional factor c-Jun, activating transcription factor-2 or Elk-1. Considering that the ERK inhibitor had an additive effect, ERK and JNK seem to act on neurons by independent and non-converging molecular pathways. The precise molecular mechanism underlining pain hypersensitivity may be elucidated by exploring the downstream molecules of JNK. In addition, p-JNK was present in large as well as small DRG neurons. It would also be interesting to assess the functional implication of JNK activation in large neurons in the DRG.

The level of NGF rises substantially in inflamed tissues [30,31], and NGF is retrograde-transported to the primary afferent neurons. NGF acts on neurons by using a two-receptor system, which consists of TrkA tyrosine kinase and the p75 neurotrophin receptor [32]. Trk-receptor tyrosine kinases undergo rapid transphosphorylation following ligand binding, leading to a cascade of protein phosphorylation in the cell. One representative example is MAPK. Therefore, we hypothesized that NGF synthesized and released in the inflamed tissue after peripheral inflammation induces the activation of not only ERK but also other members of MAPK. It remains to be determined, however, which NGF receptor is responsible for the activation of JNK, as the p75 receptor also plays a role in activating the MAPK pathway. Indeed, NGF binding to the p75 receptor has been shown to activate JNK, whose role in oligodendrocytes and some neurons is apoptosis [32]. Targeting the JNK pathway might become a good therapeutic potential to treat peripheral inflammation-induced pain.

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