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Differential effects of naproxen and rofecoxib on the development of hypersensitivity following nerve injury in rats

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Abstract

The present study was undertaken to determine the effects of cyclooxygenase (COX) inhibitors on the development of neuropathic pain in rats following chronic constriction injury (CCI). A single intraperitoneal administration of naproxen, a nonselective COX inhibitor (10 or 30 mg/kg), or rofecoxib, a selective COX-2 inhibitor (3 or 10 mg/kg) 2 h before nerve injury did not attenuate the development of neuropathic state for 28 days. However, the administration of naproxen [10 or 30 mg/kg, intraperitonelly (i.p.)], but not rofecoxib (3 or 10 mg/kg, i.p.), on day 7 attenuated hypersensitivity but did not alter its development for 28 days. Furthermore, naproxen significantly reduced hyperalgesia and allodynia for 4 h, but the efficacy was not observed 24 h after the treatment, whereas rofecoxib failed to modify the hypersensitivity following perineural (p.n.) or intrathecal (i.t.) administration on day 7. Chronic administration of naproxen (3, 10 or 30 mg/kg), but not rofecoxib (1, 3 or 10 mg/kg), 2 h before, daily for 7 days, after nerve injury significantly attenuated and further delayed the development of hypersensitivity for 21 days following nerve injury. These results suggest that the development of hypersensitivity in the CCI model is not COX-2 dependent and that the chronic administration of naproxen started early before peripheral nerve injury could attenuate the development of hypersensitivity.

Keywords: Allodynia; Cyclooxygenase; Hyperalgesia; Hypersensitivity; Nerve injury; Neuropathic pain

1. Introduction

Neuropathic pain is a common and chronically debilitating condition characterized by persistent pain, dysthesia, hyperalgesia, and allodynia (Zimmerman, 2001). It is generally agreed that both peripheral and central mechanisms have been involved in the pathogenesis of neuropathic pain. Peripheral nerve injury is associated with Wallerian degeneration and significant neuroplastic changes in the spinal cord that include changes in the expression and up-regulation of mRNA for neurotransmitters, neuromodulators, and neuroimmune activation in the dorsal root ganglia and spinal cord and subsequent increase in excitability of primary afferent neurons and alterations of the afferent signals to the spinal cord (peripheral sensitization; DeLeo and Yezierski, 2001; Taylor, 2001; Zimmerman, 2001; Watkins et al., 2001). The central mechanisms include central sensitization, reorganization of neuronal circuits in the dorsa horn, and changes in the descending pain facilitation and pain inhibition (Taylor, 2001; Zimmerman, 2001).

Prostaglandins (PGs), potent inflammatory and pronociceptive mediators, are thought to play an important role in peripheral and central sensitization at peripheral sites and in the spinal cord (Willingale et al., 1997; Beiche et al., 1998). They are synthesized in tissues by cyclooxygenase (COX), which is the rate-limiting enzyme that catalyzes the conversion of arachidonic acid to generate PGs. There are two isoforms of COX, namely, COX-1 and COX-2. COX-1 is constitutively expressed in most cells for housekeeping functions, while COX-2 is present in low levels in physiological conditions but is rapidly induced by inflammatory stimuli (Willingale et al., 1997; Kulkarni et al.,

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2000). Recently, COX-3 has been identified, but its physiological and pathological roles are yet to be characterized (Chandrasekharan et al., 2002).

It has been reported that the intrathecal (i.c.) administration of PGE_2 and $PGF_{2\alpha}$ results in spontaneous agitation, hyperalgesia, and tactile allodynia in mice and rats (Minami et al., 1994; Turnbach et al., 2002). Furthermore, COX inhibitors significantly attenuate the PGE_2- and $PGF_{2\alpha}$-induced hyperalgesia and allodynia (Taiwo and Levine, 1988; Park et al., 2000). Recently, Zhu and Eisenach (2003) have shown that a greater number of COX-1 immunoreactivity profile in the spinal cord is up-regulated 4 days and 2 weeks after spinal nerve ligation. Accumulating evidence indicates that COX-2 plays an important role in the maintenance of neuropathic pain, as increased COX-2 expression without any change in COX-1 expression in the spinal cord is observed from 2 weeks onwards following peripheral nerve injury (Ma and Eisenach, 2002, 2003a). Despite an understanding of the role of COX isoforms in the maintenance of hypersensitivity, little is known about the relative role of COX-1 and COX-2 in the development of neuropathic pain following peripheral nerve injury. Furthermore, the effect of COX inhibitors during the development of hypersensitivity following peripheral nerve injury is not fully understood.

The present study was undertaken to determine the effects of acute and chronic systemic treatment with a nonselective COX-1/COX-2 or selective COX-2 inhibitor on the development of neuropathic pain following peripheral nerve injury in rats. Furthermore, intrathecal (i.t.) and perineural (p.n.) administrations of COX inhibitors were also employed to differentiate the site-specific effects of COX inhibitors on the development of hypersensitivity to nerve injury in rats.

2. Methods

2.1. Animals

The protocol was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the guidelines of the Indian National Science Academy. Male Wistar rats (Central Animal House of Panacea Biotec, India) weighing 150–180 g at the start of the surgery were used. Following surgery, the animals were kept under standard conditions of light and dark cycle, with food and water ad libitum in groups of three in plastic cages with soft bedding.

2.2. Chronic constriction nerve injury (CCI)

The unilateral mononeuropathy was produced according to the method described by Bennett and Xie (1988). Briefly, the rats were anesthetized using 40 mg/kg sodium pentobarbital intraperitoneally (i.p.), and the common sciatic nerve of the left hind paw was exposed at the level of the middle of the thigh by blunt dissection through the biceps femoris muscle. Proximal to the sciatic trifurcation, approximately 7 mm of nerve was freed, and four ligatures of 4-0 chromic gut were placed around the sciatic nerve, with 1-mm intervals. Great care was taken not to interrupt epineural blood flow during tying the ligatures. In sham-operated rats, the same surgical procedure was followed, the connective tissue was freed, and no ligatures were applied. After surgery, all animals received gentamicin (5 mg/kg, i.p.) to prevent sepsis.

2.3. Assessment of neuropathic pain

Allodynia (heightened response to normally nonnoxious stimuli) and hyperalgesia (decreased threshold to noxious stimuli) were evaluated in sham and sciatic nerve-injured rats. Cold allodynia was evaluated as the withdrawal latency to thermal, nonnoxious stimuli of the left and right hind paws (ipsilateral and contralateral to nerve injury, respectively) when dipped in water bath maintained at 10 ± 0.5 °C (Attal et al., 1990). Baseline latency of paw withdrawal to thermal stimulation was established thrice, 5 min apart, and averaged. A cut-off time of 15 s was imposed.

Mechanical nociceptive thresholds were evaluated using an analgesymeter (Ugo Basile, Italy) by applying noxious pressure to the ipsilateral and contralateral paws. In brief, by pressing a pedal that activated a motor, the force is increased at a constant rate on a linear scale. When the animal displayed pain by withdrawal of the paw, the pedal was immediately released, and the nociceptive pain threshold was read on the scale. The paw withdrawal threshold was expressed in grams, and a cut-off of threshold of 500 g was used to avoid potential tissue injury.

2.4. Drugs and drug administration

The drugs used in the study, naproxen and rofecoxib, were procured from Panacea Biotec. All the drug solutions for intraperitoneal (i.p.) administration were freshly prepared by suspending them in one or two drops of Tween 80 in normal saline and administered 1 ml/100 g body weight. Because COX inhibitors are lipophilic, poorly soluble in aqueous vehicles, and the volume of injection for intrathecal (i.t.) and perineural (p.n.) administration is low, the drugs were dissolved in a vehicle containing 70% dimethyl sulfoxide (DMSO) and 30% normal saline. Drugs for spinal administration were mixed such that all doses were delivered in a total volume of 10 μl, intrathecally, via a lumbar puncture at the L4/5 level in restrained animals. The intrathecal administration of 10 μl of 1% solution of methylene blue in 70% DMSO, followed by dissection in a separate set of three control animals, confirmed the correct position of the injection and the spread of the dye in the intrathecal space. Perineural injection was performed as previously described (Thalhammer et al., 1999). In brief, the

rat was restrained and held in lateral recumbency with the limb to be injected forming a right angle with the longitudinal axis of the trunk. On an imaginary line from the greater trochanter to the ischial tuberosity, about one third of the distance caudal to the greater trochanter, a 26-gauge injection needle was advanced from a dorsolateral direction at a 45° angle until the tip encountered the ischium. Drugs for perineural administration were mixed such that all doses were delivered in a maximum volume of 0.2 ml at the site of nerve injury.

2.5. Study design

All animals were acclimatised to laboratory environment for at least 2 h before testing. All the rats were subjected to these two pain tests on day 0 before performing surgery. To evaluate the effect of COX inhibition at the time of nerve injury, rats were administered with a single dose of saline/Tween mixture, naproxen, a nonselective COX inhibitor (10 or 30 mg/kg, i.p.), or rofecoxib, a selective COX-2 inhibitor (3 or 10 mg/kg, i.p.), 2 h before surgery, and the response to behavioural tests was tested on day 1 and, thereafter, once a week for 4 weeks following nerve injury. Similarly, to determine the effect of COX inhibitors during the development of hypersensitivity, a single dose of saline/Tween mixture, naproxen (10 or 30 mg/kg, i.p.), or rofecoxib (3 or 10 mg/kg, i.p.) was administered on day 7 to separate the groups of rats, and the development of allodynia and hyperalgesia was tested on day 7, 2 h before and after treatment, and once a week thereafter for 4 weeks after nerve injury. Furthermore, separate groups of rats were intrathecally or perineurally administered with 70% DMSO, naproxen (100 or 300 μg i.t. or 3 or 10 mg/kg, p.n.), or rofecoxib (30 or 100 μg i.t. or 1 or 3 mg/kg, p.n.) and were tested for behavioural response 2 h before and 0.5, 1, 2, 4, and 24 h after the treatment on day 7 following peripheral nerve injury in rats. Motor function

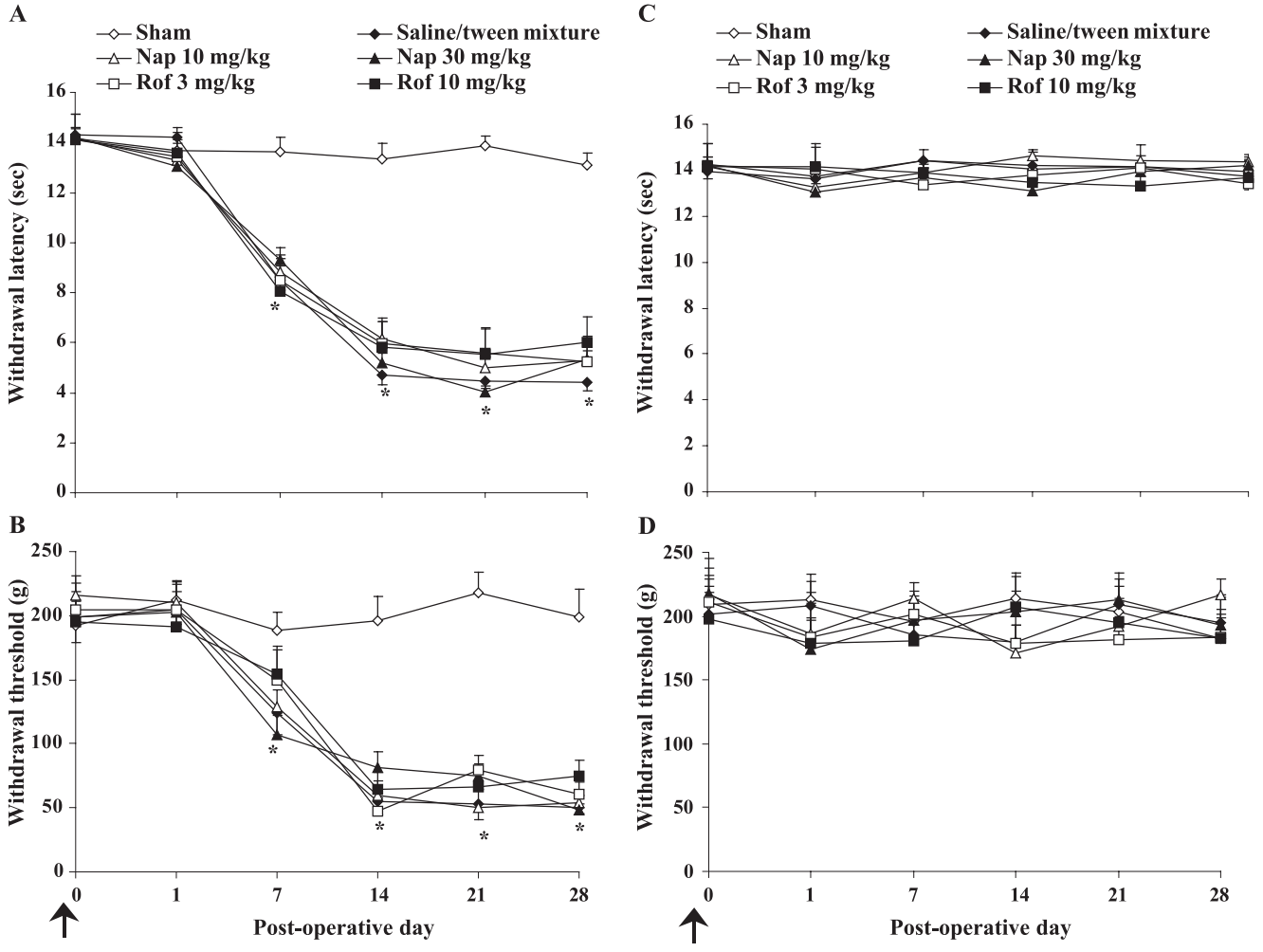

Fig. 1. Effect of intraperitoneally administered naproxen (Nap; 10 and 30 mg/kg) or rofecoxib (Rof; 3 and 10 mg/kg) at 2 h before nerve ligation (on day 0) on (A, C) cold allodynia and (B, D) mechanical hyperalgesia in nerve-injured rats. Ipsilateral (A, B) and contralateral (C, D) paw withdrawal responses to thermal (seconds) and mechanical (grams) stimulation were assessed on day 1 and, thereafter, once a week for 4 weeks following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean±S.E.M. *P<0.05 vs. sham-operated (one-way ANOVA followed by Dunnett's test; n=6–8 in each group).

was evaluated by the placing/stepping reflex 15 min after intrathecal or perineural administration of 70% DMSO in rats. To evaluate the effect of chronic administration of COX inhibitors on the development of neuropathic pain, saline/Tween mixture, naproxen (3, 10 or 30 mg/kg, i.p.), or rofecoxib (1, 3 or 10 mg/kg, i.p.) was administered 2 h before surgery and continued once daily for 7 days post nerve injury. The paw withdrawal response to thermal and mechanical stimulations was tested on days 1 and 7, 2 h after treatment, and once a week thereafter for 4 weeks after nerve injury.

2.6. Data analysis

All the values were expressed as mean±S.E.M. for the 6–8 animals per group. The significance of the difference in the mean values of paw withdrawal latency to thermal and withdrawal threshold to mechanical stimulation from all groups were analysed by one-way analysis of variance with Dunnett's test for multiple comparisons. $P<0.05$ was considered statistically significant.

3. Results

In the present series of experiments, the baseline paw withdrawal response in each test obtained on day 0 for each rat was relatively stable and showed no significant variation. The mean paw withdrawal latency to thermal stimulation was 14.18±0.77 s on the left and 14.09±0.51 s on the right, and the withdrawal threshold to pressure was 201.5±18.77 and 207.44±20.73 g, respectively, on day 0 before performing surgery. Following surgery, the animals kept their nerve-injured paw elevated above the cage floor, but otherwise appeared healthy, exhibited normal grooming and feeding behaviour, and gained weight normally. The paw with-

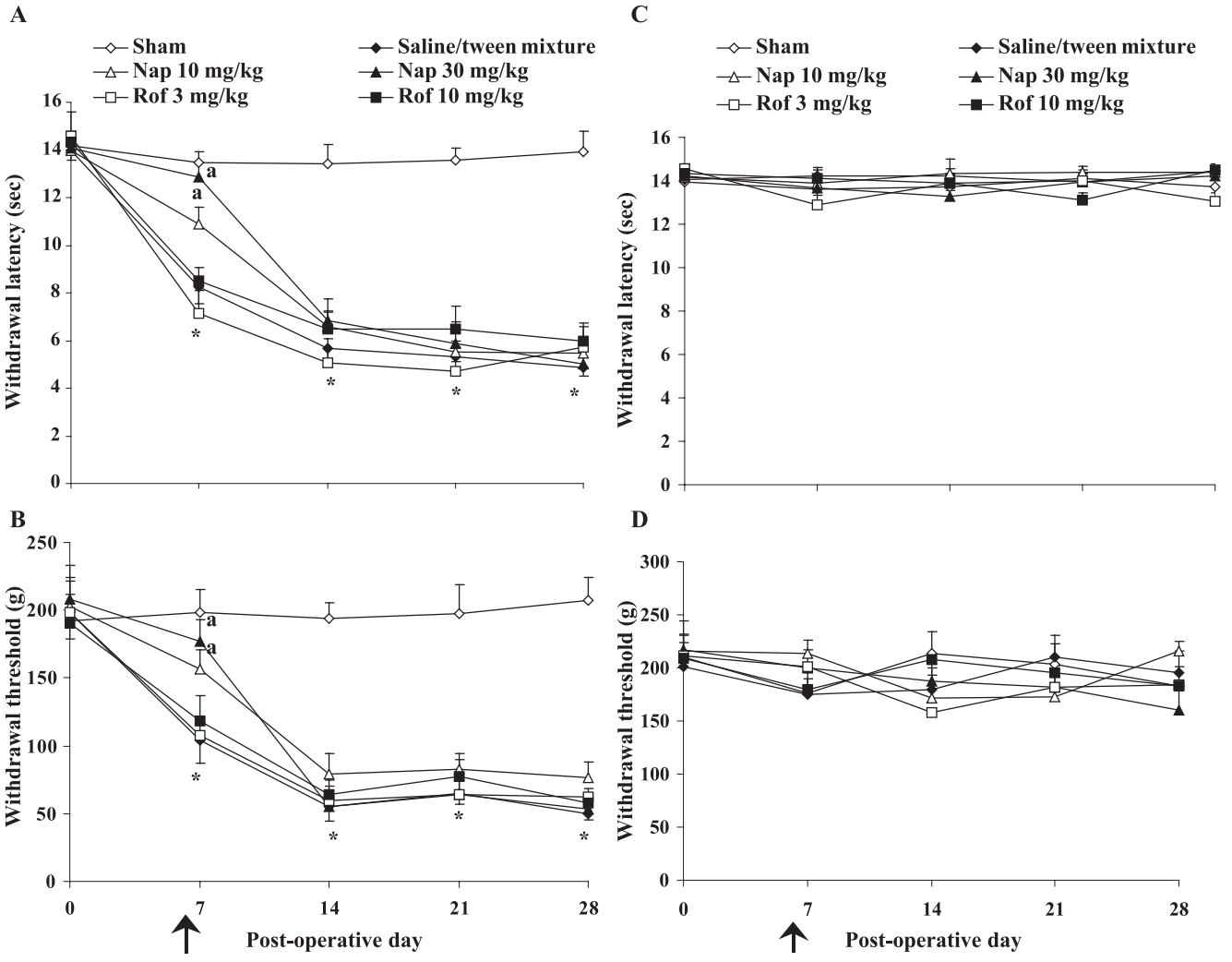

Fig. 2. Effect of intraperitoneally administered naproxen (Nap; 10 and 30 mg/kg) or rofecoxib (Rof; 3 and 10 mg/kg) on day 7 following nerve injury on (A, C) cold allodynia and (B, D) mechanical hyperalgesia in nerve-injured rats. Ipsilateral (A, B) and contralateral (C, D) paw withdrawal responses to thermal (seconds) and mechanical (grams) stimulation were assessed on day 7 (2 h after treatment) and once a week, thereafter, for 4 weeks following nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean±S.E.M. *$P<0.05$ vs. sham-operated and $^{\dagger}P<0.05$ vs. saline/Tween-mixture-treated nerve-injured animals (one-way ANOVA followed by Dunnett's test; n=6–8 in each group).

drawal responses to thermal and mechanical stimulations in sham-operated rats remained unchanged from baseline values throughout the entire observation period. The ipsilateral paw withdrawal responses of all the vehicle-treated nerve-injured rats were significantly less than that of the sham-operated rats on day 7 onwards and reached steady state between days 14 and 28 after surgery, indicating the development of allodynia and hyperalgesia in a time-dependent manner (Fig. 1A and B).

Acute systemic administration of single dose of naproxen (10 or 30 mg/kg, i.p.) or rofecoxib (3 or 10 mg/kg, i.p.) 2 h before nerve ligation on day 0 had no effect on the development of hypersensitivity as compared with saline/Tween mixture treatment throughout the observation period (Fig. 1A and B). There was no significant difference in ipsilateral paw withdrawal responses between various groups of animals, except in the sham-operated animals on day 7, before drug administration. An acute systemic administration of a single dose of naproxen (10 or 30 mg/kg, i.p.) to rats on day 7 following nerve injury significantly altered the decrease in ipsilateral paw withdrawal responses to thermal and mechanical stimulations 2 h after the treatment as compared with the saline/Tween mixture treatment (Fig. 2A and B). However, it did not alter the development of hypersensitivity at later time points throughout the study period. Rats treated with rofecoxib (3 or 10 mg/kg, i.p.) showed paw withdrawal responses similar to saline/Tween-mixture-treated nerve-injured rats (Fig. 2A and B). Because the systemic administration of naproxen showed antiallodynic and antihyperalgesic effects, intrathecal or perineural administration of COX inhibitors was employed to differentiate the possible site-specific effects of COX

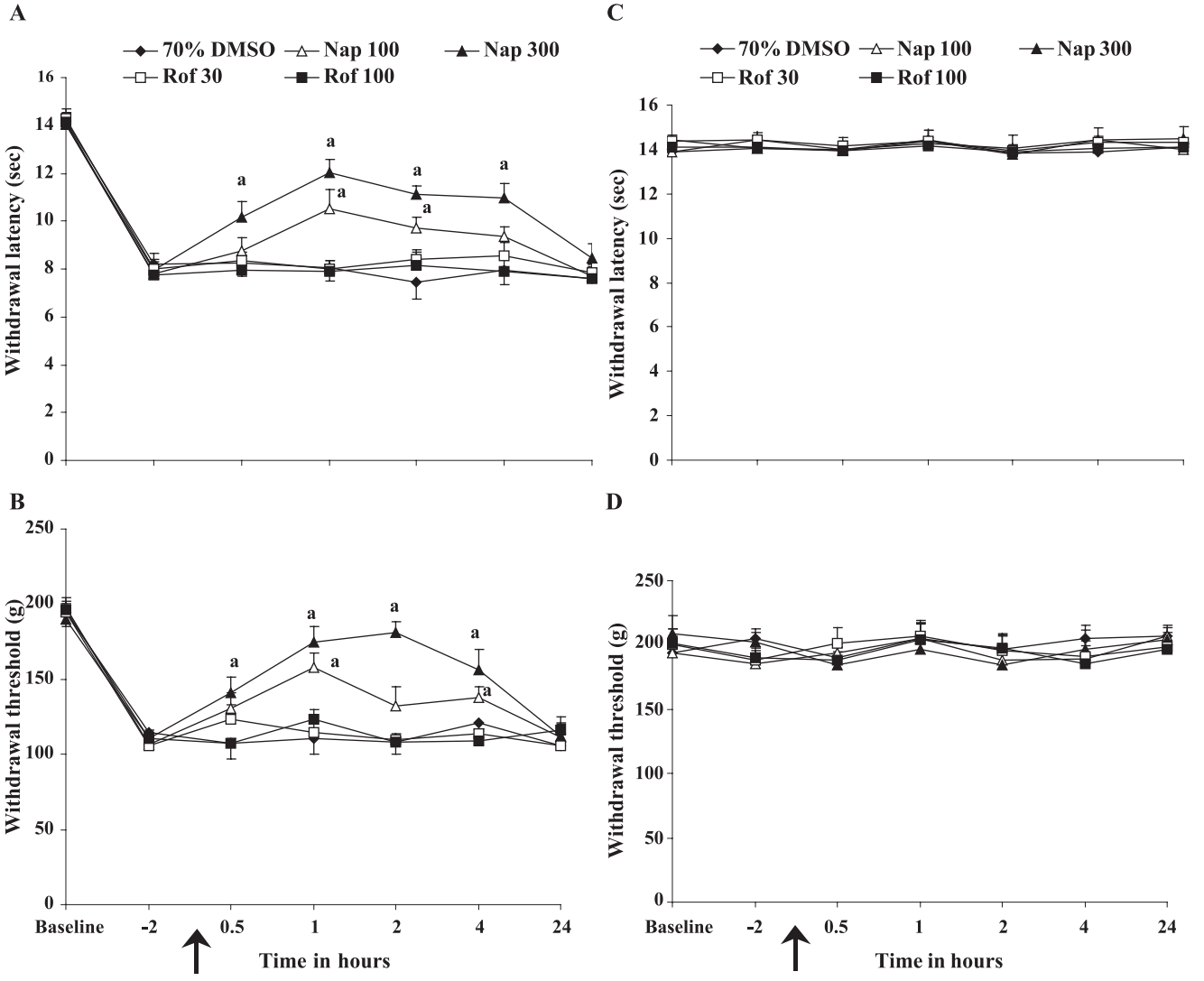

Fig. 3. Effect of intrathecally administered naproxen (Nap; 100 and 300 μg/rat) or rofecoxib (Rof; 30 and 100 μg/rat) on day 7 following nerve injury on (A, C) cold allodynia and (B, D) mechanical hyperalgesia in nerve-injured rats. Ipsilateral (A, B) and contralateral (C, D) paw withdrawal responses to thermal (seconds) and mechanical (grams) stimulation were assessed 2 h before and 0.5, 1, 2, 4, and 24 h after treatment on day 7 following peripheral nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean±S.E.M. $^{\dagger}P<0.05$ vs. vehicle (70% DMSO)-treated nerve-injured animals (one-way ANOVA followed by Dunnett's test; n=6–8 in each group).

inhibitors on the attenuation of hypersensitivity on day 7 following peripheral nerve injury in rats. Intrathecal or perineural administration of 70% DMSO caused no detectable motor weakness, as judged by placing/stepping reflexes. Intrathecally (100 or 300 μg/rat) or perineurally (3 or 10 mg/kg) administered naproxen was found to attenuate hypersensitivity in the ipsilateral paw for 4 h, but the effect was not observed 24 h after the drug treatment on day 7 (Figs. 3A and B, 4A and B). The peak antiallodynic and antihyperalgesic effect of naproxen was observed 1 or 2 h after intrathecal or perineural administration, respectively; however, perineural administration showed slow onset of action (Fig. 3A and B). On the contrary, intrathecal (30 or 100 μg/rat) or perineural (1 or 3 mg/kg) treatment of rofecoxib did not show any significant difference in the cold allodynia and mechanical hyperalgesia compared with vehicle (70% DMSO)-treated nerve-injured rats (Figs. 3A and B, 4A and B).

Chronic treatment with naproxen (3, 10, or 30 mg/kg, i.p., 2 h before and once daily for 7 days post nerve injury) in nerve-injured rats significantly attenuated and further delayed the development of hypersensitivity in the ipsilateral paw on days 7, 14, and 21, but not on day 28, as compared with saline/Tween-mixture-treated nerve-injured rats (Fig. 5A and B). In contrast, rats administered rofecoxib (1, 3, or 10 mg/kg, i.p., 2 h before and once daily for 7 days post nerve injury) did not show any significant difference in the cold allodynia and mechanical hyperalgesia in ipsilateral paw compared with the saline/Tween-mixture-treated nerve-

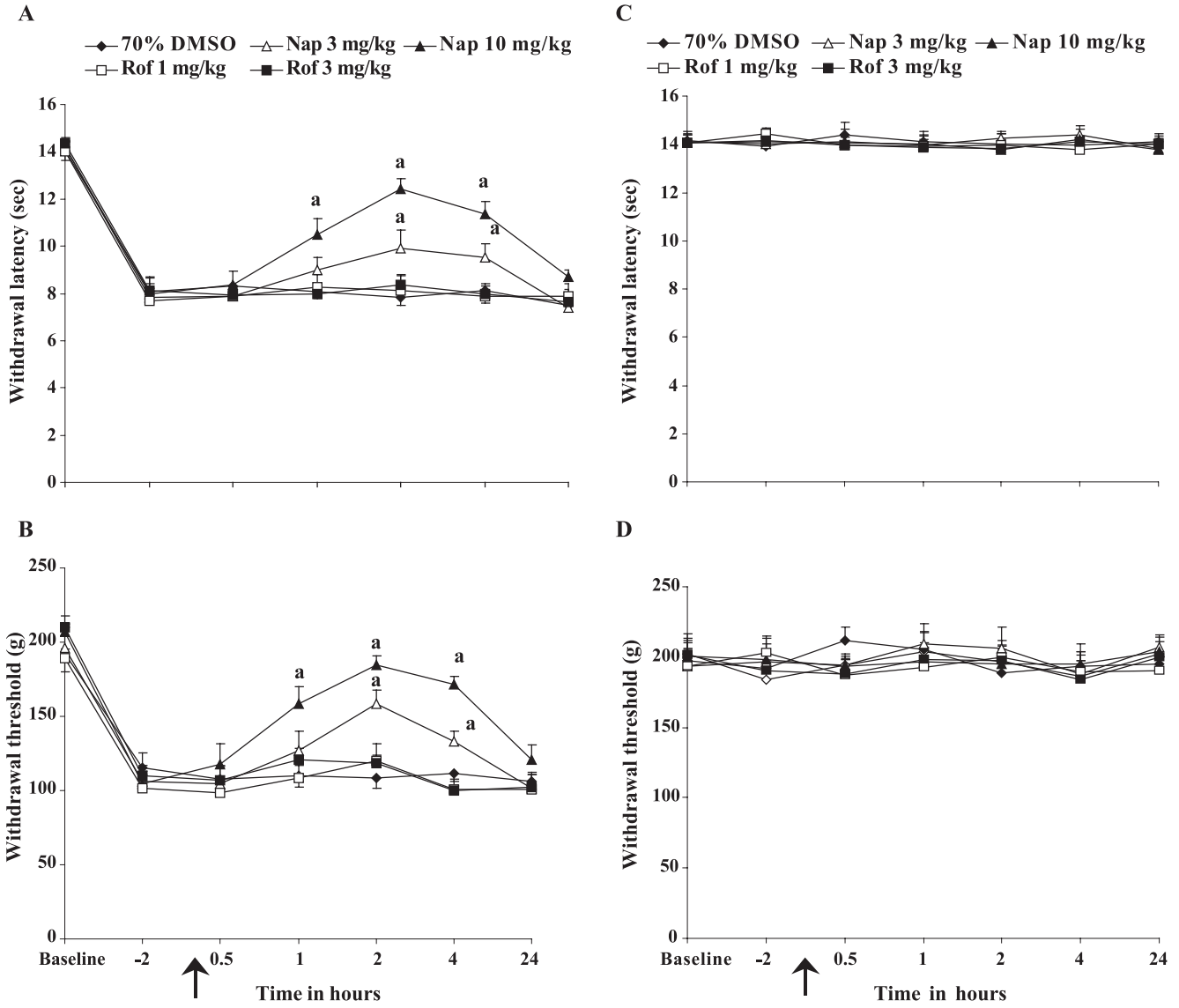

Fig. 4. Effect of perineurally administered naproxen (Nap; 3 and 10 mg/kg) or rofecoxib (Rof; 1 and 3 mg/kg) on day 7 following nerve injury on (A, C) cold allodynia and (B, D) mechanical hyperalgesia in nerve-injured rats. Ipsilateral (A, B) and contralateral (C, D) paw withdrawal responses to thermal (seconds) and mechanical (grams) stimulation were assessed 2 h before and 0.5, 1, 2, 4, and 24 h after treatment on day 7 following peripheral nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Arrow indicates the time of drug administration. Values are mean±S.E.M. $^{\dagger}P<0.05$ vs. vehicle (70% DMSO)-treated nerve-injured animals (one-way ANOVA followed by Dunnett's test; n=6–8 in each group).

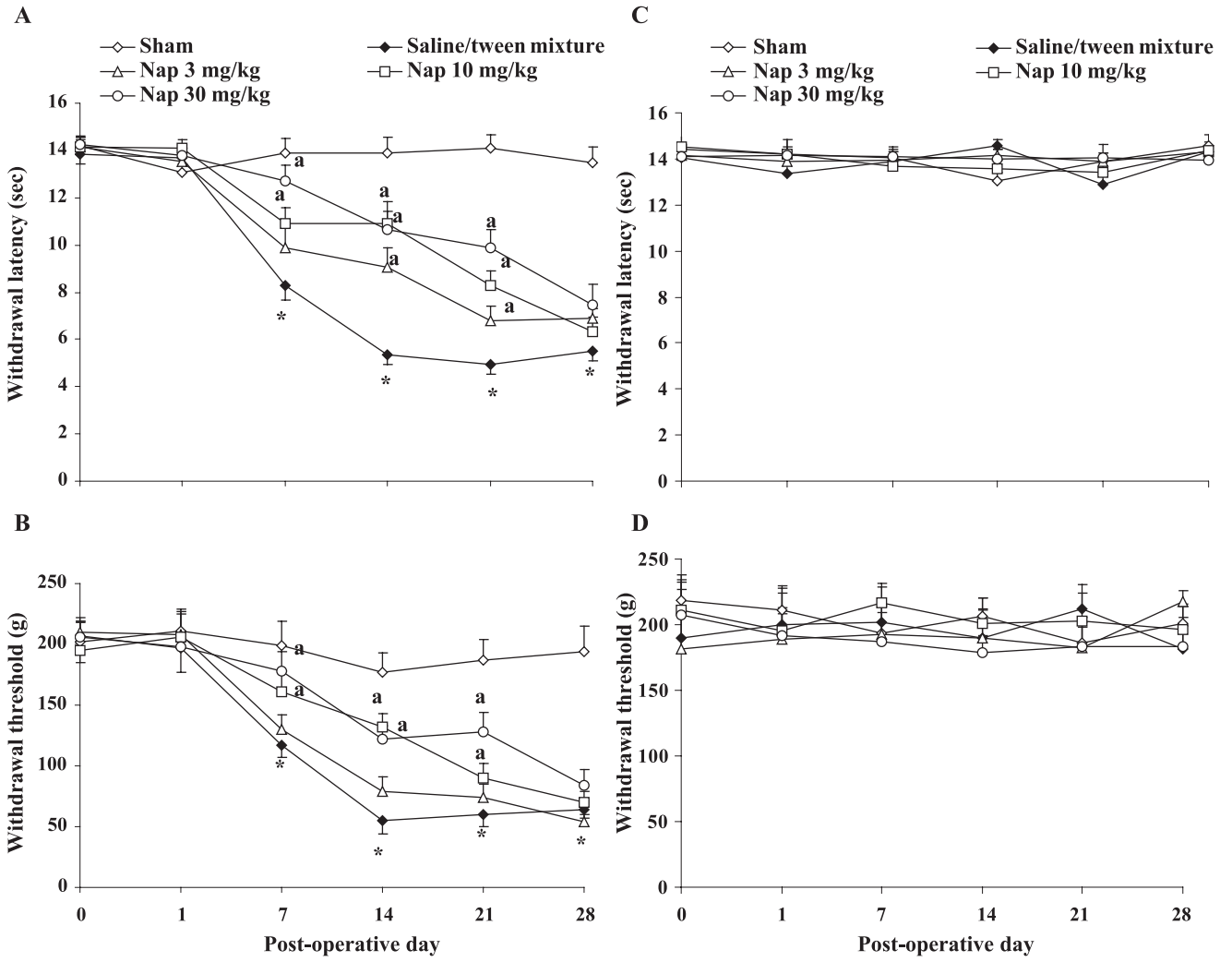

Fig. 5. Effect of intraperitoneally administered naproxen (Nap; 3, 10, and 30 mg/kg) on (A, C) cold allodynia and (B, D) mechanical hyperalgesia in nerve-injured rats. Treatment was initiated 2 h before surgery and continued once daily for 7 days following nerve injury. Ipsilateral (A, B) and contralateral (C, D) paw withdrawal responses to thermal (seconds) and mechanical (grams) stimulation were tested on days 1 and 7 following 2 h after treatment and once a week, thereafter, for 4 weeks after nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean±S.E.M. *P<0.05 vs. sham-operated and $^{\dagger}P$<0.05 vs. saline/Tween-mixture-treated nerve-injured animals (one-way ANOVA followed by Dunnett's test; n=6–8 in each group).

injured group (Fig. 6A and B). In all these experiments, systemic, intrathecal, or perineural administration of COX inhibitors on days 0 and 7, for 7 days, had no effect on the contralateral paw withdrawal responses in these tests as compared with respective vehicle treatment (Figs. 1C and D, 2C and D, 3C and D, 4C and D, 5C and D, 6C and D).

4. Discussion

In the present study, when naproxen, a nonselective COX inhibitor, or rofecoxib, a selective COX-2 inhibitor, was administered at the time of nerve injury, they did not affect the development of hypersensitivity, indicating that there was no involvement of either COX isoforms in the initiation of hypersensitivity. In contrast, naproxen, but not rofecoxib, significantly altered hypersensitivity when administered on day 7 (during the development of hypersensitivity) following nerve injury. However, such effect was observed on that day only, but did not persist. Previous studies have shown that the administration of COX inhibitors attenuate behavioural and neurochemical indices of tactile allodynia following spinal strychnine or bicuculline, which has been reported as PG dependent (Hall et al., 1999; Zhang et al., 2001). Furthermore, COX inhibitors significantly attenuated PGE_2- and $PGF_{2\alpha}$-induced hyperalgesia and allodynia (Taiwo and Levine, 1988; Park et al., 2000). Because PGs are produced upon the metabolism of arachidonic acid by COX, the rate-limiting enzyme that consists of two isoforms, COX-1 and COX-2, it seems likely that the alteration of hypersensitivity by naproxen could be due to the preferential inhibition of COX-1, but not COX-2.

The most striking findings of the present study were those revealing that the systemic administration of naproxen, but not rofecoxib, for 7 days following nerve injury significantly attenuated cold allodynia and mechanical

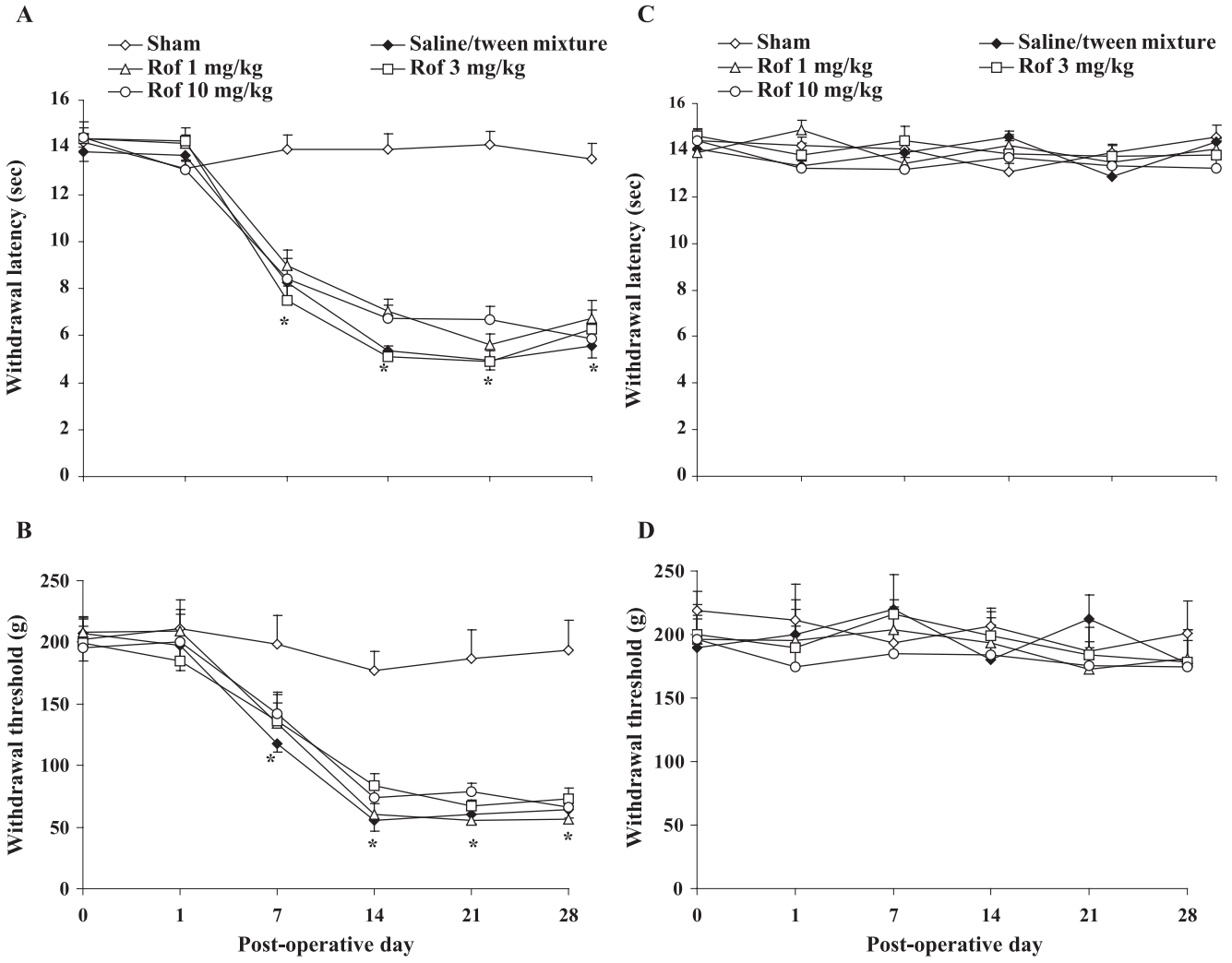

Fig. 6. Effect of intraperitoneally administered rofecoxib (Rof; 1, 3, and 10 mg/kg) on (A, C) cold allodynia and (B, D) mechanical hyperalgesia in nerve-injured rats. Treatment was initiated 2 h before surgery and continued once daily for 7 days following nerve injury. Ipsilateral (A, B) and contralateral (C, D) paw withdrawal response to thermal (seconds) and mechanical (grams) stimulation were tested on days 1 and 7 following 2 h after treatment and once a week, thereafter, for 4 weeks after nerve injury. Responses on day 0 represent baseline paw withdrawal responses. Values are mean±S.E.M. *P<0.05 vs. sham-operated and $^{\dagger}P$<0.05 vs. saline/Tween-mixture-treated nerve-injured animals (one-way ANOVA followed by Dunnett's test; n=6–8 in each group).

hyperalgesia. The effects of naproxen do not reflect hypoalgesic activity because contralateral paw withdrawal responses were not affected by systemic administration. It is unlikely that rofecoxib failed to reach threshold levels in the spinal cord to alleviate sufficient nociceptive response because rofecoxib is known to readily cross the blood–brain barrier and sufficient levels to inhibit COX-2 in the spinal cord are achieved after systemic administration (Schwarz et al., 1999; Broom et al., 2004). It has been reported that COX-2 expression is increased without any change in COX-1 expression in the spinal cord, at the nerve injury site, and in an adjacent region from 2 weeks onwards after nerve injury, indicating the lack of COX-2 involvement during the early stages of development of neuropathic pain (Ma and Eisenach, 2002, 2003a,b). Recently, in the spared nerve injury model of neuropathic pain, rofecoxib failed to modify the development of allodynia and hyperalgesia, and COX-2 expression in the dorsal horn of the spinal cord was not markedly changed up to day 7 following nerve injury (Broom et al., 2004).

The antinociceptive activity of locally, perineurally, or systemically administered COX inhibitors in alleviating established neuropathic pain has been well documented; however, greater efficacy was observed when administered locally or perineurally than when systemically (Syriatowicz et al., 1999; Ma and Eisenach, 2002, 2003a,b). In the present study, both the drugs were also administered via intrathecal and perineural routes to nerve-injured rats to distinguish the potential site-specific effects of COX inhibitors in attenuating the development of neuropathic pain because systemically administered drug is distributed throughout the body. Interestingly, intrathecally or perineurally administered naproxen, but not rofecoxib, attenuated hypersensitivity on that day, indicating that PGs produced by COX-1 might be involved in the development of hypersensitivity following nerve injury. Furthermore, in one of the reported studies, a

greater number of COX-1 immunoreactivity profiles was observed in the epidermis of the ipsilateral footpad of nerve-injured rats, suggesting the involvement of COX-1-generated peripheral PGs (Ma and Eisenach, 2002). Recently, perineural administration of indomethacin early after nerve injury attenuated the development of allodynia (Takahashi et al., 2004). In addition, spinal administration of ketorolac, a COX-1-preferring inhibitor, on day 7 was effective in attenuating cold allodynia and thermal hyperalgesia in sciatic nerve-injured rats, indicating that spinal PGs were also involved (Parris et al., 1996). Thus, it is plausible that COX-1, rather than COX-2, is involved in generating PGs, in both the spinal cord and sciatic nerve, and contributes to the development of neuropathic pain state.

After chronic constriction injury, Aβ fibers, which normally terminate in deep laminae III and IV of the dorsal horn, have been shown to sprout into the superficial laminae I and II of the dorsal horn, where afferent Aδ and C fibers terminate and form a novel physiological synapse with transmission neurons (Chung et al., 1997). This presynaptic interaction between low-threshold mechanoceptors and C fibers through these second-order neurons, which normally code for nociceptive input, now appears to receive non-noxious input. Recently, it has been shown that a greater number of COX-1 immunoreactivity cells with glial morphology in the superficial laminae of the ipsilateral spinal dorsal horn L4–L6 of the spinal cord increased 4 days and 2 weeks after spinal nerve ligation (Zhu and Eisenach, 2003). Furthermore, the time course of the activation and up-regulation of COX-1 in those studies parallels the development of hypersensitivity in the present study. Indeed, the neuroanatomical sites for the increased COX-1 expression and the termination of nociceptive afferent Aδ and C fibers and second-order neurons due to nerve sprouting are in the superficial laminae I and II, which further supports that PGs produced by COX-1, but not COX-2, play an important role in the development of hypersensitivity following peripheral nerve injury.

Furthermore, chronic systemic treatment with naproxen, but not rofecoxib, markedly delayed the development of hypersensitivity in nerve-injured rats, suggesting that there is persistent generation of PGs following nerve injury. It has been previously reported that continuous administration of systemic or epidural NSAIDs relieved neuropathic pain in cancer patients, indicating that PGs are continuously produced for longer periods in neuropathic pain (Ripamonti et al., 1996; Lauretti et al., 1998). Moreover, daily intraplantar injections of PGE_2 in rats for 14 days, causing the development of persistent mechanical nociceptor hypersensitivity state for more than 30 days, was significantly attenuated by chronic treatment with indomethacin (Ferreira et al., 1990). Recent evidence suggests that COX-1 expression is not static but changes in a time-dependent manner after peripheral nerve injury (Zhu and Eisenach, 2003). Together, the present findings suggest that the development of neuropathic pain is PG dependent and that a gradual and persistent increase in the production of PGs by COX-1 might be expected to last for several weeks in the periphery, injured sciatic nerve, and spinal cord and continuously contribute to the development of neuropathic pain. Although a more long-term administration naproxen was not done, it cannot be excluded that long-term administration of naproxen could result in a longer-lasting decrease in sensitivity after nerve injury. Our findings are in general agreement with this, as chronic treatment with naproxen, which preferentially inhibits COX-1, but not rofecoxib, which inhibits COX-2, started early before nerve injury for 7 days, significantly attenuated the development of the neuropathic pain state following nerve injury.

In summary, the results suggest that there is no role for COX-2 in the development of neuropathic pain in this model of nerve injury. We conclude that chronic treatment with naproxen started early before nerve injury might prevent or at least delay the development of hypersensitivity.

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