The Acute Antihyperalgesic Action of Nonsteroidal, Anti-Inflammatory Drugs and Release of Spinal Prostaglandin E_2 Is Mediated by the Inhibition of Constitutive Spinal Cyclooxygenase-2 (COX-2) but not COX-1

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Western blots show the constitutive expression of COX-1 and COX-2 in the rat spinal dorsal and ventral horns and in the dorsal root ganglia. Using selective inhibitors of cyclooxygenase (COX) isozymes, we show that in rats with chronic indwelling intrathecal catheters the acute thermal hyperalgesia evoked by the spinal delivery of substance P (SP; 20 nmol) or NMDA (2 nmol) and the thermal hyperalgesia induced by the injection of carrageenan into the paw are suppressed by intrathecal and systemic COX-2 inhibitors. The intrathecal effects are dose-dependent and stereospecific. In contrast, a COX-1 inhibitor given systemically, but not spinally, reduced carrageenan-evoked thermal hyperalgesia but had no effect by any route with spinal SP hyperalgesia. Using intrathecal loop dialysis catheters, we showed that intrathecal SP would enhance the release of prostaglandin E_2 (PGE_2). This intrathecally evoked release of spinal PGE_2 was diminished by systemic delivery of nonspecific COX and COX-2-selective inhibitors, but not a COX-1-selective inhibitor. Given at systemic doses that block SP- and carrageenan-evoked hyperalgesia, COX-2, but not COX-1, inhibitors reduced spinal SP-evoked PGE_2 release. Thus, constitutive spinal COX-2, but not COX-1, is an important contributor to the acute antihyperalgesic effects of spinal as well as systemic COX-2 inhibitors.

Key words: cyclooxygenase inhibitor; intrathecal injection; thermal hyperalgesia; NK-1; substance P; NMDA; ibuprofen; SC-58125; SC-560

Tissue injury results in a heightened sensitivity to subsequent noxious input (hyperalgesia). In behavioral models of injury-induced hyperalgesia, nonsteroidal, anti-inflammatory drugs (NSAIDs) normalize the otherwise sensitized pain thresholds (Yaksh et al., 1998). Early work showed that systemically delivered NSAIDs were effective inhibitors of cyclooxygenase (COX) (Smith and Willis, 1971; Vane, 1971) and that peripheral prostanoids could sensitize the peripheral terminal. This suggested that hyperalgesia arose from a peripheral afferent sensitization.

Tissue injury also evokes persistent afferent traffic that initiates a spinal sensitization. Studies on the pharmacology of this sensitization using intrathecal drug delivery indicate that the hyperalgesia results in part from a complex cascade starting with the activation of spinal neurokinin-1 (NK-1) and NMDA receptors secondary to the spinal release of substance P (SP) and glutamate. Among several elements, this cascade activates spinal phospholipases and generates prostanoids by spinal COX (Yaksh et al., 1999), leading to spinal prostanoid release (Yang et al., 1996a,b; Willingale et al., 1997; Ebersberger et al., 1999). The hyperalgesic effects (Yaksh, 1982; Malmberg and Yaksh, 1992a,b) and the spinal release of prostaglandins (Malmberg and Yaksh, 1995a,b) are diminished by spinal COX inhibitors at doses that have no systemic action. Consistent with the hypothesized spinal organization, intrathecal SP and NMDA, in the absence of tissue injury, induce a transient thermal hyperalgesia (Malmberg and Yaksh, 1992b; Dirig and Yaksh, 1996) and an increase in the spinal prostaglandin E_2 (PGE_2) release (Dirig and Yaksh, 1999; Hua et al., 1999).

Two COX enzymes (COX-1 and COX-2) catalyze the conversion of arachidonic acid to PGE_2 (Kujubu et al., 1991; O'Banion et al., 1992). Originally based on work with inflammatory cells, COX-1 was thought to be constitutive, whereas COX-2 was upregulated as an immediate-early gene in response to injury (Kujubu et al., 1991; Tomlinson et al., 1994; Katori et al., 1995). However, in the spinal cord, both COX-1 and COX-2 are expressed constitutively (Beiche et al., 1996; Willingale et al., 1997; Ebersberger et al., 1999). Because classic NSAIDs exhibit nonpreferential inhibition of both COX isozymes (Meade et al., 1993; Gierse et al., 1995), we wanted to determine the contribution of each spinal isozyme. We showed that intrathecally delivered COX-2 inhibitors reduce paw carrageenan-evoked hyperalgesia to the same degree as nonspecific NSAIDS (Dirig et al., 1998). This observation, nevertheless, does not exclude the role that spinal COX-1 may play in isozyme involvement. Moreover, the use of a carrageenan inflammatory model permits the possibility that the interval necessary for the inflammatory reaction to develop results in an upregulation of COX-2 in either the periphery or the spinal cord. We therefore sought to use COX-1 and COX-2 inhibitors to define the contribution of COX-1 and

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COX-2 isozymes to (1) the immediate hyperalgesia induced by intrathecal SP and NMDA, (2) the hyperalgesia induced by peripheral inflammation, and (3) intrathecal SP-evoked PGE_2 release. We present evidence that constitutive spinal COX-2 is uniquely important for initiating a centrally mediated, behaviorally defined hyperalgesia.

MATERIALS AND METHODS

All studies were performed under protocols approved by the University of California, San Diego, Institutional Animal Care and Use Committee.

Intrathecal catheter implantation. Adult male Holtzman Sprague Dawley rats (Indianapolis, IN) were implanted with chronic lumbar intrathecal injection or microdialysis catheters by a modified procedure of Yaksh and Rudy (1976). Briefly, an incision was made through the atlantooccipital membrane, and an 8.5 cm probe (injection or injection/microdialysis) was inserted into the intrathecal space such that the caudal end of the probe localized to the lumbar enlargement. At a minimum of 3 d after surgery the rats were allocated randomly to different experimental groups receiving the intrathecal vehicle (100% dimethyl sulfoxide), NK-1 antagonist, or COX inhibitor. Each animal was used a maximum of two times with at least 4 d between intrathecal treatments.

Thermal nociception and intrathecal SP-induced hyperalgesia. To assess the thermally evoked paw withdrawal response, we used a commercially available device. Specific information on the device construction and operation have been published previously (Dirig et al., 1997). Briefly, this device consisted of a 30°C glass surface on which the rats were placed. The thermal nociceptive stimulus originated from a focused projection bulb below the glass surface, and the stimulus was delivered separately to either hind paw. Basal paw withdrawal latencies (PWL) were assessed at time (t) = −15 min. At t = −10 min the animals received intrathecal vehicle or drug in 10 μl, followed by a 10 μl vehicle flush. At t = 0 the animals received intrathecal SP (30 nmol), followed by a 10 μl flush. PWL were assessed every 15 min afterward for 1 hr and expressed as the mean PWL of the left and right paws at each time point.

Paw carrageenan-induced thermal hyperalgesia. To induce a state of local inflammation, we injected 2 mg of λ-carrageenan (2 mg in 100 μl of physiological saline; Sigma, St. Louis, MO) subcutaneously into the plantar surface of the left hind paw at time 0 (t = 0). Thermally evoked paw withdrawal latencies were assessed 120 min after injury as described above. Drugs were administered intrathecally or intraperitoneally 10 min before paw carrageenan injection. Intrathecal SC-560 and SC-58125 doses were 280 and 50 nmol, respectively. The intraperitoneal dose for both drugs was 30 mg/kg.

Spinal PGE_2 release. Loop microdialysis/injection catheters (Marsala et al., 1995) were implanted intrathecally in male Holtzman Sprague Dawley rats as described above. At 5 d after implantation, spinal microdialysate (10 μl/min) samples were collected from anesthetized rats (O_2 + 1% halothane). COX inhibitor or vehicle (0.5% methylcellulose + 0.025% Tween 20; Sigma) was administered by oral gavage or intraperitoneally (ibuprofen) in a dose of 30 mg/kg. Then the animal was anesthetized lightly. After a 30 min washout and two 10 min baseline collections, SP (30 nmol) was injected intrathecally, and an additional 10 min sample was collected. The PGE_2 content of microdialysate samples was assessed by using a competitive radioimmunoassay. Specifics of this methodology have been published previously (Dirig and Yaksh, 1999).

Western blot. To examine the spinal expression of COX-1 and COX-2, we extracted freshly harvested spinal cord and dorsal root ganglion (DRG) tissues in 50 mM Tris buffer, pH 8.0, containing 0.5% Triton X-100, 150 mM NaCl, 1 mM EDTA, and protease inhibitors, subjected them to NuPAGE Bis-Tris (10%) gel electrophoresis, and then transferred them to nitrocellulose membrane (Osmonics, Westborough, MA) electrophoretically. Nonspecific binding sites were blocked with 10% low-fat milk in PBS containing 0.1% Tween 20 (PBS-T) for 2 hr in room temperature. Membranes then were incubated with polyclonal COX-1 or COX-2 antisera in PBS-T buffer overnight at 4°C. After the nitrocellulose membrane was washed twice with the same buffer and once with a buffer containing 150 mM NaCl and 50 mM Tris-Cl, pH 7.5, the antibody–protein complexes were blotted for 1 hr at room temperature with secondary antibodies labeled with horseradish peroxidase. After extensive washing, the protein–antibody complexes were detected with chemiluminescent reagents. Bis-Tris gels (NuPAGE) and buffers were from Novex (San Diego, CA). Polyclonal antibodies against COX-1 and COX-2 were from Cayman Chemical (Ann Arbor, MI). Secondary anti-rabbit antibody labeled with horseradish peroxidase was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Chemiluminescence substrate and enhancer were from Pierce (Rockford, IL).

To determine the role of glycosylation, we deglycosylated purified ovine COX-2 (0.05 μg of protein/20 μl) with an enzymatic deglycosylation reaction. N-linked oligosaccharides were cleaved by peptide-*N*-glycosidase F (PNGaseF), 500 U, at 37°C for 1 hr in 50 mM sodium phosphate, 1% Nonidet P-40 buffer, pH 7.5. Anti-proteases were added to prevent protein degradation. The protein was denatured by treatment with 0.1% SDS and 1% β-mercaptoethanol at 95°C for 10 min before digestion. The glycolytic digestion was analyzed by Western transfer blotting. The PNGaseF kit was purchased from New England Biolabs (Beverly, MA).

Drugs. The following drugs were used in these studies: substance P (SP; Peninsula, Belmont, CA); λ-carrageenan (Sigma); NMDA (Sigma); RP67580 (NK-1 antagonist, Rhône-Polenc Rorer, Collegeville, PA), 2-[1-imino 2-(methoxyphenyl) ethyl] 7,7-diphenyl 4-perhydroisoindole (3aR–7aR); RP68651 (inactive enantiomer of RP67580, Rhône-Polenc Rorer), 2-[1-imino 2-(methoxyphenyl) ethyl] 7,7-diphenyl 4-perhydroisoindole (3aS–7aS); SC-58125 (COX-2 inhibitor, Pharmacia, St. Louis, MO), 1-[(4-methysufonyl)phenyl]-3-tri-fluoromethyl-5-(4-fluorophenyl)pyrazole; SC-236 (COX-2 inhibitor, Pharmacia), 4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1*H*-pyrazol-1-yl]benzenesulfonamide; SC-384 (COX-2 inhibitor, Pharmacia), 4-(4-fluorophenyl)-3-[(4-methylsulfonyl)phenyl]-1-(-2-propenyl)-5-(trifluoromethyl)-1*H*-pyrazole; SC-385 (inactive isomer of SC-384), 4-(4-fluorophenyl)-5-[(4-methylsulfonyl)phenyl]-1-(-2-propenyl)-3-(trifluoromethyl)-1*H*-pyrazole; SC-560 (COX-1 inhibitor, Pharmacia), 5-(4-chlorophenyl)-1-(4-methoxy phenyl)- 3-(trifluoromethyl)pyrazole.

RESULTS

Constitutive expression of COX-1 and COX-2 in spinal cord

In untreated rats, COX-1 and COX-2 protein are expressed constitutively in lumbar and cervical dorsal and ventral horns and DRG (Fig. 1). Examination of the COX-2 immunoreactivity typically reveals two bands at or around the molecular weight corresponding to a slightly larger form of COX-2. Pretreatment of the sample with glycosidases abolishes these bands, indicating they represent glycosylated enzyme.

Intrathecal SP-induced thermal hyperalgesia

Intrathecal SP (30 nmol) produced a potent thermal hyperalgesia (decreased paw withdrawal latencies relative to baseline; Fig. 2*A*) that persisted for 30 min in vehicle-pretreated animals. Pretreatment with the NK-1 receptor antagonist RP67580 blocked this thermal hyperalgesia, whereas an equimolar dose of the inactive enantiomer RP68651 was without effect (Fig. 2*A*). Spinal pretreatment with *S*(+)-ibuprofen (a nonselective COX inhibitor), but not its inactive isomer *R*(−)-ibuprofen, dose-dependently reduced SP-induced thermal hyperalgesia, indicating the role of COX in the SP-evoked hyperalgesia (Fig. 2*B*). Selective inhibitors (Seibert et al., 1994; Gierse et al., 1996) of COX-2 (SC-384, SC-58125, or SC-236) or COX-1 (Smith et al., 1998) (SC-560) were injected intrathecally 10 min before intrathecal SP. Pretreatment with each COX-2 inhibitor dose-dependently reduced SP-induced thermal hyperalgesia: SC-384 (Fig. 2*C*), SC-58125 (Fig. 2*D*), and SC-236 (data not shown). Spinal pretreatment with an isomer of SC-58125 that inhibits neither COX-1 nor COX-2 (SC-385; see Table 1) did not change the hyperalgesia observed at 15 min in vehicle-treated controls (Fig. 2*C*). Thus, both the NSAID and the selective COX-2 inhibitors blocked thermal hyperalgesia, whereas isomers that did not inhibit COX were without significant effect. In contrast to the efficacy of the COX-2 inhibitors, spinal COX-1 inhibition with doses of SC-560 that were 10 times greater than the highest dose used for the COX-2 inhibitors did not alter SP-induced thermal hyperalgesia (Fig. 2*F*).

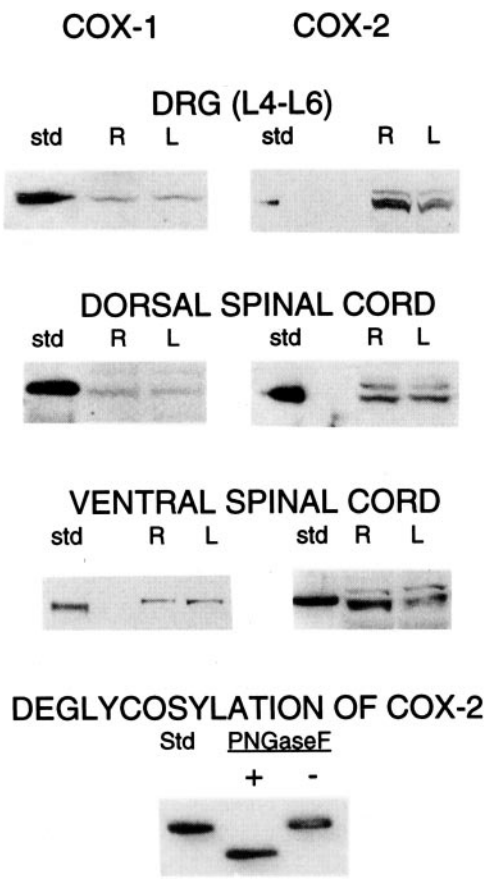

Figure 1. Immunoblots showing that COX-1 and COX-2 are present in protein extractions from DRG (L4–L6) and dorsal and ventral spinal cord (segments L1–L6) of untreated rats. COX-1 and COX-2 isoforms ran to ~72 kDa in 10% Bis-Tris gels. A second band was present at 74 kDa in spinal cord and DRG samples that were labeled with the COX-2 antibody. After deglycosidation by PNGaseF treatment, COX-2 displayed an electrophoretic mobility shift to 65 kDa, suggesting that N-linked carbohydrates had been removed.

Intrathecal NMDA-induced thermal hyperalgesia

It could be argued that COX-2 inhibitors actually interfered with the interaction of SP with its receptor (i.e., acting as an NK-1 antagonist). To rule out this possibility, we examined the effects of the above COX manipulations on the thermal hyperalgesia induced by the intrathecal agent NMDA. This thermal hyperalgesia induced by intrathecal NMDA (2 nmol) is blocked by an intrathecal NMDA antagonist (MK-801; 5 nmol), but not by RP67580, an NK-1 antagonist (data not shown). Rats were pretreated intrathecally with the COX-2 inhibitor SC-58125 or the COX-1 inhibitor SC-560. The NMDA-evoked thermal hyperalgesia was reduced significantly by spinal COX-2 inhibition (Fig. 2*E*), but not by SC-560 (data not shown), emphasizing that the hyperalgesia induced by either spinal NK-1 or NMDA receptor activation was mediated by a COX-2 isozyme, but not a COX-1 isozyme.

Systemic versus intrathecal delivery of COX inhibitors

To compare further the effects of COX-1 and -2 inhibitors on central versus peripheral inflammation-induced thermal hyperalgesia, we examined the efficacy of COX-1 and COX-2 inhibitors given orally in blocking the hyperalgesia induced by intraplantar carrageenan. At 2 hr after carrageenan was injected into the plantar surface of the hind paw, a pronounced thermal hyperalgesia was observed as a significant decrease in paw withdrawal latencies in vehicle-pretreated animals (see vehicle in Fig. 3, *bottom*). As shown in Figure 3, intrathecal pretreatment (10 min before paw carrageenan) with ibuprofen or the COX-2 inhibitor SC-58125 reduced carrageenan-induced hyperalgesia, but spinal COX-1 inhibition (SC-560) did not. In contrast, when these same drugs were delivered systemically (PO), the COX-1 and the COX-2 inhibitors reduced the thermal hyperalgesia. For comparison, additional studies were performed with the same doses against the thermal hyperalgesia induced by intrathecal SP. As indicated, both oral ibuprofen and the COX-2 inhibitor SC-58125 reduced intrathecal SP-induced hyperalgesia, but oral COX-1 inhibition (SC-560) did not.

Intrathecal SP-induced spinal PGE_2 release

Given the efficacy of intrathecal COX-2 inhibitors against SP-induced hyperalgesia, we hypothesized that systemic antihyperalgesic doses of a COX-2 inhibitor (Dirig et al., 1998) would suppress SP-evoked spinal PGE_2 release. Consistent with previous work from our lab (Hua et al., 1999), intrathecal SP increased spinal microdialysate PGE_2 concentration in vehicle-pretreated rats (Figs. 4, 5). Oral (+/−) ibuprofen (COX-1/COX-2 inhibitor, 30 mg/kg), SC-58125 (COX-2 inhibitor, 30 mg/kg), or SC-560 (COX-1 inhibitor, 30 mg/kg) was given 30 min before the intrathecal delivery of SP (30 nmol). These doses were chosen on the basis of their ability to attenuate the thermal hyperalgesia induced by intrathecal SP and/or intraplantar carrageenan (see Fig. 3).

There was no significant difference in SP-evoked PGE_2 release after the systemic COX-1 inhibitor or vehicle pretreatments (Figs. 4, 5). In contrast, ibuprofen and SC-58125 both produced a comparable and highly significant reduction in the SP-evoked PGE_2 release in comparison with either vehicle or SC-560 ($p < 0.05$ vs vehicle; Figs. 4, 5).

DISCUSSION

Repetitive activity generated in primary afferents by peripheral inflammation milieu can release primary afferent transmitters and can initiate, by the activation of at least glutamate and SP receptors, a spinal cascade that leads to the spinal release of prostanoids. It has become certain that, in contrast to the periphery, COX-2 as well as COX-1 is expressed constitutively in the spinal cord. The present studies, aimed at defining the contribution of the two isozymes in mediating the hyperalgesia and the synthesis of spinal prostanoids, make several assertions.

COX-1 and COX-2 are expressed constitutively in spinal parenchyma

COX-1 and 2 are expressed constitutively in the spinal cord and DRG. In normal rats, COX-1 mRNA and protein are expressed constitutively in dorsal horn neurons and DRG and in the ventral horns of the spinal cord, as shown by *in situ* hybridization (Chopra et al., 2000), Northern blotting (Beiche et al., 1998a,b; Hay and de Belleroche, 1998), immunohistochemistry (Willingale et al., 1997; Beiche et al., 1998b), and Western blotting techniques (Willingale et al., 1997; Beiche et al., 1998b; Ebersberger et al., 1999; present studies). In DRG primary cell cultures we have observed COX-1 and COX-2 immunoreactivity in SP and calcitonin gene-related peptide-expressing cells (I. Khan, C. Svensson, and T. L. Yaksh, unpublished observations). It is noteworthy that interleukin-1β induces SP release from primary afferent neurons and that this effect is blocked by COX-2 inhibition (Inoue et al.,

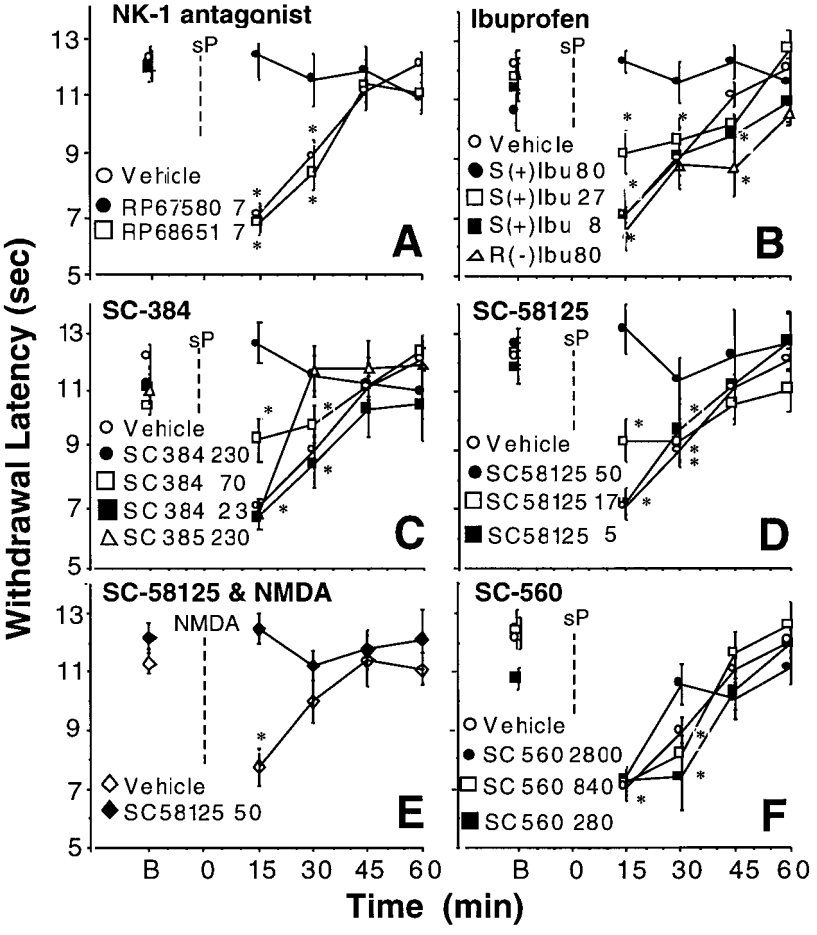

Figure 2. Spinal COX-2-dependent hyperalgesia. Intrathecal injection of substance P (SP) produced a thermal hyperalgesia that was blocked by intrathecal COX-2 inhibition, but not by COX-1 inhibition. *A*, Paw withdrawal latency is presented as a function of time (relative to intrathecal SP; 30 nmol) and intrathecal drug pretreatment. Two-way repeated measures ANOVA indicated a significant drug-by-time interaction for the results in *A–E* (smallest $F = 2.99$; $p < 0.01$). Dunnett's test revealed that intrathecal SP significantly decreased paw withdrawal latencies for up to 30 min in vehicle-treated animals (*open circles*) compared with baseline. This decrease was blocked by intrathecal injection of an NK-1 antagonist (RP67580, *filled circles*), but not by the inactive enantiomer (RP68651, *filled squares*). *B–D*, Intrathecal pretreatment with the NSAID *S*(+)-ibuprofen (*B*) or the COX-2 inhibitors SC-384 (*C*) and SC-58125 (*D*) dose-dependently blocked the intrathecal SP-induced hyperalgesia (*open circles*), as shown by the absence of a difference from vehicle baseline seen at 15 and 30 min with the higher doses (*filled circles*). *R*(−)-ibuprofen (*B*, *open triangles*) or the isomer SC-385 that is inactive against either COX isozyme (*C*, *open triangles*) did not alter significantly the hyperalgesia induced by intrathecal SP. *E*, Spinal COX-2 inhibition (SC-58125, *filled diamonds*) blocked the thermal hyperalgesia evoked by intrathecal NMDA (2 nmol; *open diamonds*). *F*, Intrathecal COX-1 inhibition did not change significantly the thermal hyperalgesia induced by intrathecal SP. There was no significant interaction or main effects as indicated by two-way repeated measures ANOVA ($F = 0.43$; $p > 0.73$). In *A–F* the drug doses are indicated in nanomoles administered intrathecally, and paw withdrawal latencies are expressed as mean ± SEM of four to six rats per dose group. $^*p < 0.01$ denotes significant hyperalgesia compared with vehicle baseline.

Table 1. Relative drug 50% inhibitory concentration (IC_{50}) against recombinant human COX-1 and COX-2

Inhibitor	hCOX-1 (μM)	hCOX-2 (μM)
S(+)-ibuprofen	3.3	37.5
SC-58125	>1000	0.1
SC-384	>500	0.075
SC-385	>500	>500
SC-560	0.0053	160

Enzyme activity methodology (Gierse et al., 1996) and structural information on selective COX inhibitors have been published previously (Seibert et al., 1994; Penning et al., 1997; Smith et al., 1998).

1999). In addition to neuronal structures, there is little doubt that at least a portion of the COX-2 isozymes is found within activated microglia and astrocytes (Bauer et al., 1997; Levi et al., 1998; Petrova et al., 1999).

Western blots indicate that spinal COX-2 shows additional higher molecular weight forms that are shifted by glycosidase treatment. Both COX isoforms are glycosylated (N-linked) at three sites, with the distinction that COX-2 in ~50% of the molecules is glycosylated at an additional fourth site, resulting in two peptide bands on gel electrophoresis. Glycosylation of COX is necessary for the expression of active enzyme, but glycosylation of COX-2 at the fourth site does not affect activity (Otto et al., 1993). Studies *in vitro* suggest that COX-2 levels in mouse neuronal cells are modulated via the ubiquitin/proteasome pathway (Rockwell et al., 2000).

Spinal COX-2, but not COX-1, inhibition mediates a potent antihyperalgesic action after peripheral inflammation

The acute thermal hyperalgesia induced after the direct activation of spinal NK-1 or NMDA receptors is reduced immediately by intrathecal delivery of nonspecific (e.g., COX1/2 inhibitors) or COX-2-specific inhibitors. Based on these and previous studies, this action has several characteristics. (1) This COX inhibitory effect is produced in a dose-dependent manner by both intrathecal and systemic delivery, but the doses given intrathecally are up to several hundred times lower than the doses delivered systemically. (2) Both families of COX inhibitors show comparable efficacy after systemic and intrathecal delivery. (3) The relative potency of COX1/2 and COX-selective inhibitors after intrathecal delivery is comparable whether defined in models of either inflammatory (carrageenan) or noninflammatory-dependent (intrathecal SP/NMDA) hyperalgesia. (4) In contrast, the COX-1-selective inhibitor was effective only against the carrageenan-evoked thermal hyperalgesia after systemic delivery in a model of peripheral inflammation and had no effect on the SP-evoked hyperalgesia. Thus, it was ineffective after intrathecal delivery against the carrageenan-evoked hyperalgesia and ineffective after the systemic delivery against the spinal SP-evoked hyperalgesia. A similar blockade has been reported on paw carrageenan-induced hyperalgesia by the oral COX-2 inhibitor celecoxib, but not with the COX-1 inhibitor SC-560 (Smith et al., 1998). In contrast, in the present study, systemic SC-560 was effective. This disparity may reflect on several methodological details, including route of administration

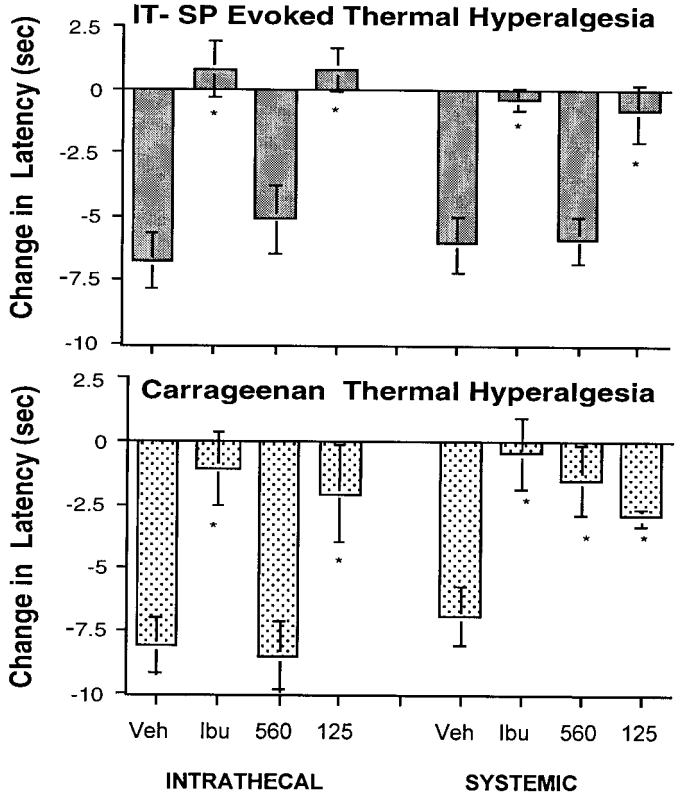

Figure 3. Effects of intrathecal and systemic (*PO*) delivery of vehicle (*Veh*), nonspecific COX inhibitor (ibuprofen, *Ibu*; 80 nmol intrathecally/30 mg/kg, i.p.), COX-1 inhibitor (SC-560, *560*; 280 nmol intrathecally/30 mg/kg, PO), or COX-2 inhibitor (SC-58125, *125*; 50 nmol intrathecally/30 mg/kg, PO) on the thermal hyperalgesia induced by the intrathecal delivery of SP (30 nmol; *top*) or the intraplantar injection of carrageenan (*bottom*). Each *bar* represents the mean ± SEM of four to eight animals. $^{*}p < 0.05$ compared with vehicle.

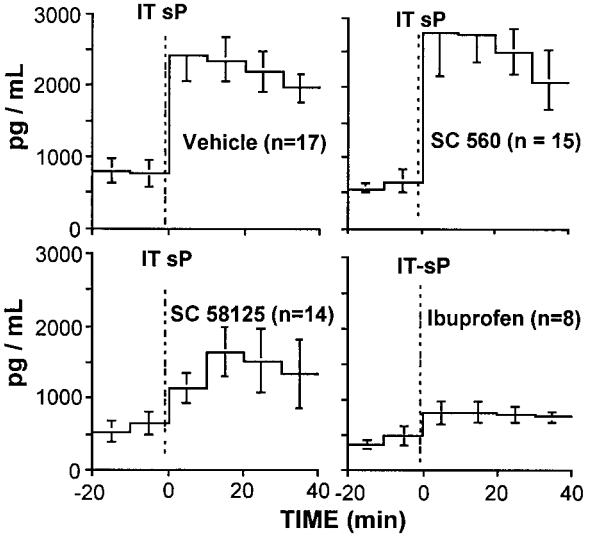

Figure 4. Effects of vehicle (0.5% methyl cellulose, PO; $n = 17$), COX-1 (*SC-560*; 30 mg/kg, PO; $n = 15$), COX-2 (*SC-58125*; 30 mg/kg, PO; $n = 17$), or nonspecific COX inhibitor [(+/−) *ibuprofen*; 30 mg/kg, PO; $n = 8$] on the time-dependent release by intrathecal SP (20 nmol, given at $t = 0$) on the release of PGE_2 into the intrathecal dialysate. Drugs were given at −25 min. Data are presented as the mean ± SEM of the concentrations of PGE_2 in the dialysate (pg/ml). Spinal dialysis probes were perfused with artificial CSF at 10 μl/min.

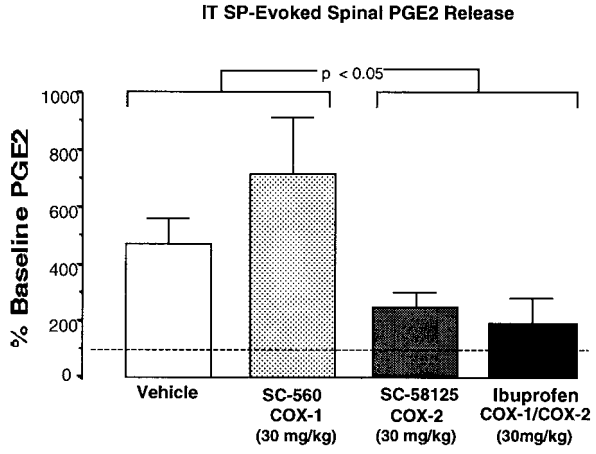

Figure 5. Histogram presents the peak release expressed at the percentage of the concentrations of PGE_2 in the spinal dialysate obtained immediately before intrathecal SP and in the 10 min immediately after intrathecal SP, as shown in Figure 4 in animals pretreated with vehicle (0.5% methyl cellulose, PO), COX-1 (*SC-560*; 30 mg/kg, PO; $n = 15$), COX-2 (*SC-58125*; 30 mg/kg, PO), or nonspecific COX inhibitor [(+/−) ibuprofen; 30 mg/kg, PO]. As indicated, systemic (+/−) ibuprofen and COX-2, but not COX-1, inhibition suppresses intrathecally SP-evoked spinal PGE_2 release. PGE_2 in prestimulation dialysate outflow did not differ across groups and is presented as a percentage of basal levels. Oral pretreatment with (+/−) ibuprofen (*black-filled bar*) and the COX-2 inhibitor SC-58125 (*gray-filled bar*) significantly suppressed the intrathecally SP-induced spinal PGE_2 release compared with vehicle and COX-1 inhibition ($p < 0.05$). *Horizontal dashed line* indicates the control value (100%).

(oral vs intraperitoneal), stimulus intensity (dose of paw carrageenan), and dosing interval. These observations jointly argue for the importance of a spinal COX-2 mechanism, a spinal action of systemically delivered drugs even in the face of peripheral inflammation.

COX-2 mediates spinal hyperalgesic actions of spinal NK-1/NMDA receptor activation

In the absence of any peripheral inflammation, the activation of spinal NK-1 or NMDA receptors will induce a well defined, short-lasting thermal hyperalgesia. This spinally initiated hyperalgesia is mediated by an isozyme with a COX-2, but not a COX-1, pharmacology. This assertion rests on the relative selectivity of the antagonists, the demonstration of dose dependency, and the stereoselective properties of the active agents. The failure of intrathecal SC-560, the COX-1 antagonist, to block the SP/NMDA-evoked hyperalgesia or to alter the carrageenan-evoked thermal hyperalgesia might arise from a problem of kinetics or metabolism. We discount this likelihood in view of the fact that (1) the drug was given intrathecally in doses up to 50 times that of the COX-2 inhibitor SC-58125 and that (2) SC-560 demonstrates some antihyperalgesic efficacy after systemic delivery in carrageenan inflammation.

Systemic doses of nonspecific and COX-2, but not COX-1, inhibitors, which were antihyperalgesic, blocked the SP-evoked release of PGE_2

Previous studies demonstrated that intrathecal SP and peripheral inflammatory stimuli, such as carrageenan, evoke spinal release of PGE_2, as measured *in vivo* in the unanesthetized rat by intrathecal loop microdialysis (Marsala et al., 1995; Yang et al., 1996a; Hua et al., 1999). The association of spinal COX-2 with spinal

PGE_2 secretion and hyperalgesia is emphasized by the results in Figures 4 and 5 in which oral administration of nonspecific COX or COX-2 specific inhibitors at doses that were effective in blocking the SP-evoked thermal hyperalgesia reduced the SP-evoked spinal PGE_2 release. In contrast, at an oral dose of a COX-1 inhibitor that diminished the carrageenan-evoked hyperalgesia, spinal PGE_2 release was not different from vehicle controls. These results, emphasizing a direct spinal action, are in accord with those of Smith and colleagues (Smith et al., 1998), who reported an increased CSF content of PGE_2 after paw carrageenan inflammation that was reversed by systemic delivery of the COX-2 inhibitor celecoxib, but not the COX-1 inhibitor SC-560. Those results may be ambiguous, because the systemic agents would affect peripheral and central COX-2, both of which may have been upregulated by the inflammation.

Origin of spinal PGE_2

An important question relates to the cells of origin from which the COX-2-dependent PGE_2 release arises. SP receptors are found on spinal neuronal and non-neuronal cells (e.g., microglia and astrocytes). In microglia and astrocyte cultures, SP has been shown to release PGE_2 (Giulian et al., 1996; Palma et al., 1997). We note that, after peripheral injury and inflammation, the activation of spinal microglia and astrocytes is observed routinely (Watkins and Maier, 1999). In models of osteosarcoma, marked activation of spinal astrocytes has been reported (Schwei et al., 1999). Such astrocyte activation often is associated with an enhanced expression of COX-2 (Koyama et al., 1999). This role of non-neuronal cells that display an aggressive reactive response to peripheral injury and inflammation thus provides an added dimension to the complexity of the COX-2–prostaglandin systems that contribute to the chemical milieu underlying hyperalgesia. These observations point to non-neuronal substrates that may contribute to the apparent efficacy of COX-2 inhibitors in various clinical states, including those induced by tissue injury and cancer (Yaksh et al., 1998).

Role of spinal COX-1

The apparent lack of a contribution by COX-1 to the observed spinally mediated hyperalgesia or release is unexpected, given its constitutive presence in the spinal cord. We recognize that this important assertion hinges in part on a very limited pharmacological assessment with a single drug (SC-560). The differences between COX-1 and COX-2 inhibitors on pain behavior and prostanoid release presented in this paper indicate that these isozymes do not play equivalent roles. Two possibilities for the lack of contribution by COX-1 may be that COX-1 requires (1) higher arachidonic acid concentrations than COX-2 (Versteeg et al., 1999) or (2) differential coupling to cytosolic, secretory, and noncalcium-dependent phospholipases (Leslie, 1997; Murakami et al., 1999). It will be of considerable interest to determine the functional role played by COX-1 in future work.

In conclusion, this study demonstrates a constitutive role for spinal COX-2 synthesis of PGE_2 in spinally mediated as well as paw carrageenan-induced thermal hyperalgesia. Moreover, a peripheral action is apparent from the occurrence of local edema and erythema after carrageenan is injected into the paw. This may be attributable to the local release of inflammatory mediators, including bradykinin, cytokines, and prostaglandins (Uda et al., 1990; Dirig and Yaksh, 1998). This peripheral edema is suppressed by the systemic administration of nonselective COX inhibitors (Ferreira, 1972) and selective COX-2 inhibitors (Zhang et al., 1997; Dirig et al., 1998). Nevertheless, the acute effects reported here after systemic and intrathecal delivery, in the absence of injury and inflammation, argue for a functionally significant role of a constitutive COX-2 in injury-induced hyperalgesia.

The clinical relevancy of these observations is emphasized by trial studies that showed COX-2-selective inhibitors and nonselective NSAIDs are equally analgesic (Simon, 1998) when delivered systemically. Together with the present results, this suggests that the clinical efficacy of NSAIDs with mixed COX-1 and COX-2 actions as well as COX-2 inhibitors in treating acute pain may be mediated by the inhibition of a constitutively expressed COX-2. Given the constitutive localization of COX-2 in the CNS and not at the peripheral injury site, the present data argue consistently that a prominent, if not unique, component of the antihyperalgesic actions of NSAIDs in general and COX-2 inhibitors in particular is attributable to the inhibition of COX-2 within the CNS, likely at the level of the spinal dorsal horn.

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