Characterization of the Analgesic and Anti-Inflammatory Activities of Ketorolac and Its Enantiomers in the Rat

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ABSTRACT

The marked analgesic efficacy of ketorolac in humans, relative to other nonsteroidal anti-inflammatory drugs (NSAIDs), has lead to speculation as to whether additional non-NSAID mechanism(s) contribute to its analgesic actions. To evaluate this possibility, we characterized (R,S)-ketorolac’s pharmacological properties in vivo and in vitro using the nonselective cyclooxygenase (COX) inhibitors [indomethacin (INDO) and diclofenac sodium (DS)] as well as the selective COX-2 inhibitor, celecoxib, as references. The potency of racemic (R,S)-ketorolac was similar in tests of acetic acid-induced writhing, carrageenan-induced paw hyperalgesia, and carrageenan-induced edema formation in rats; ID₅₀ values = 0.24, 0.29, and 0.08 mg/kg, respectively. (R,S)-ketorolac’s actions were stereospecific, with (S)-ketorolac possessing the biological activity of the racemate in the above tests. The analgesic potencies for (R,S)-, (S)-, and (R)-ketorolac, INDO, and DS were highly correlated with their anti-inflammatory potencies, suggesting a common mechanism. (R,S)-ketorolac was significantly more potent than INDO or DS in vivo. Neither difference in relative potency of COX inhibition for (R,S)-ketorolac over INDO and DS nor activity of (S)-ketorolac at a number of other enzymes, channels, or receptors could account for the differences in observed potency. The distribution coefficient for (R,S)-ketorolac was approximately 30-fold less than for DS or INDO, indicating that (R,S)-ketorolac is much less lipophilic than these NSAIDs. Therefore, the physicochemical and pharmacokinetics properties of (R,S)-ketorolac may optimize the concentrations of (S)-ketorolac at its biological target(s), resulting in greater efficacy and potency in vivo.

Racemic ketorolac (Toradol) is a non-steroidal anti-inflammatory drug (NSAID) that is effective in the clinic as an analgesic in the treatment of postsurgical pain (Yee et al., 1986; O’Hara et al., 1987; Stanski et al., 1990). The marked efficacy of (R,S)-ketorolac as an analgesic, relative to other NSAIDs, has lead to speculation regarding the mechanism underlying its analgesic actions. Initially, it was suggested that (R,S)-ketorolac was a highly potent cyclooxygenase (COX) inhibitor (Rooks et al., 1982). Moreover, it was thought that it was this activity alone that accounted for (R,S)-ketorolac’s analgesic potency in vivo, consistent with the mechanism by which NSAIDs were proposed to act (Vane, 1971; Higgs et al., 1976). Subsequently, it was reported that (R,S)-ketorolac was no more potent than indomethacin (INDO) (Parnham, 1993) or diclofenac sodium (DS; Pallapies et al., 1995) as inhibitors of COX-1 or COX-2. Collectively, these results suggest that additional unknown mechanism(s) might contribute to the analgesic actions of (R,S)-ketorolac.

After the introduction of Toradol, evidence accumulated suggesting that the analgesic and anti-inflammatory activities of certain NSAIDs (Brune et al., 1991), specifically (R,S)-ketorolac (McCormack and Ughart, 1995), could be discriminated. Consequently, several different COX-independent activities of (R,S)-ketorolac were examined in an effort to explain its marked, clinical analgesic efficacy relative to other NSAIDs. These mechanisms included facilitation of extracellular calcium entry (Chavez et al., 1993), indirect activation of κ opioid receptors (Upshouse et al., 1993; Tripathi and Welch, 1995), and modulation of nitric oxide (NO) synthase (Granados-Soto et al., 1995). Currently, none of these activities have been conclusively shown to account for the analgesic efficacy or potency of (R,S)-ketorolac in vivo.

Although several studies have shown that peripherally administered (R,S)-ketorolac does not readily cross the blood-

ABBREVIATIONS: COX, cyclooxygenase; 5-HT, hydroxytryptamine (serotonin); NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug; NMDA, N-methyl-D-aspartate; INDO, indomethacin; DS, diclofenac sodium; i.t., intrathecal; CNS, central nervous system; i.c.v., intracerebroventricular; DMSO, dimethyl sulfoxide.
brain barrier in either rats or humans (Mroszczak et al., 1987), evidence that (R,S)-ketorolac acts at sites in the central as well as the peripheral nervous system to produce analgesia has accumulated. For example, after intrathecal (i.t.) administration, (R,S)-ketorolac blocks pain states associated with central sensitization: formalin-induced hyperalgesia in rats (Malmberg and Yaksh, 1992, 1993) and thermal hyperalgesia in a neuropathic rat model (Parris et al., 1996). Taken together, it appears that (R,S)-ketorolac produces analgesia in several rodent models when administered centrally as well as the peripheral nervous system to produce analgesia as well.

The purpose of this work was to evaluate the pharmacology of (R,S)-, (S)-, and (R)-ketorolac in vivo and in vitro to elucidate possible mechanism(s) underlying its analgesic efficacy.

Materials and Methods

Compounds Used

(R,S)-ketorolac, (S)-ketorolac, (R)-ketorolac, and celecoxib (Pennyng et al., 1997) were synthesized in the Institute of Organic Chemistry, Roche Bioscience (Palo Alto, CA). Gabapentin was purified from a commercial source of Neurontin. [3H](R,S)-ketorolac (49.5 Ci/mmol) was prepared by the Radiochemistry Group, Roche Bioscience and dissolved in dimethyl sulfoxide (DMSO) to a final specific activity of 0.495 Ci/mmol. DS, INDO, morphine sulfate, and Type IV carrageenan (λ) were obtained from Sigma Chemical Co., St. Louis, MO. Carprofen was purchased from Cayman Chemical Co., Inc. (Ann Arbor, MI). (R,S)-ketorolac, INDO, and DS were dissolved in a vehicle containing 40% propylene glycol, 10% ethanol, 5% sodium benzoate/benzoic acid buffer, and 1% benzyl alcohol (pH 6.8). Celecoxib was dissolved in a vehicle containing 85% propylene glycol and 5% sodium benzoate/benzoic acid buffer (pH 6.8). Drug doses were calculated from the free base weight and the drugs were administered in a dose volume of 2 ml/kg.

Animals and Surgical Preparation

Animals. Male Sprague-Dawley rats were purchased from Harlan Sprague-Dawley Inc. (San Diego, CA) and housed at 22°C with a 12-h light/dark cycle for 7 days before the onset of experimentation. All procedures were reviewed and approved by the Roche Bioscience Institutional Animal Care and Use Committee.

Intracerebroventricular (i.c.v.) Cannulation. Rats (120 g) were anesthetized with halothane (5%) using a vaporizer (Foregger, Smithtown, NY). The shaved dome of the head was then swabbed with alcohol and a small incision (1 cm) was made in the skin. A 27-gauge needle (Infusion set no. 4995; Abbott Laboratories, Chicago, IL) was then inserted through the cranium a total distance of 4 mm at a point 1 mm lateral and 1 mm caudal to the bregma and 4 mm caudal. To determine the latency (s) to withdrawal of the neuropathic hindpaw from the ice water bath was measured.

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i.t. Cannulation. Rats were anesthetized with ketamine (100 mg/ml) and xylazine (20 mg/ml) in a 3:1 ratio. Cannulation was performed essentially as described by LoPachin et al. (1981). After cannulation, the rats were individually housed for 1 week before testing.

Unilateral Mononeuropathy. Rats were rendered neuropathic by chronic constriction of the common sciatic nerve as described by Bennett and Xie (1988). The rats were assessed for cold allodynia 4 to 6 days after surgery.

In Vivo Testing

Writing. The writhing tests were carried as described previously (Arrigoni-Martelli, 1978; Akarsu et al., 1989). Rats (~120 g) were randomly assigned to treatment groups and administered vehicle or drug. At the times indicated, acetic acid (20 mg/kg, 2 ml/kg) in deionized water or carbaprostacyclin (30 μg/kg, 2 ml/kg) in deionized water containing <1% of DMSO was injected into the peritoneum (i.p.). The number of writhes (i.e., abdominal constriction followed by dorsiflexion and extension) occurring during a 15 min period beginning 15 or 5 min after acetic acid or carbaprostacyclin administration, respectively, was measured. The results are expressed as the number of writhes per 15-min period.

Carrageenan-Induced Paw Hyperalgesia. Rats (~120 g) were randomly assigned to treatment groups, anesthetized with halothane (5%), and administered 100 μl of vehicle or carrageenan (1% in saline) s.c. on the plantar surface of the left hindpaw (Vinage et al., 1976). The rats received vehicle or drug 2 h after carrageenan administration and were evaluated for paw hyperalgesia 1 h later. Hindpaw hyperalgesia was measured as described previously (Randall and Selitto, 1957) using an Ugo Basile Analgesy-meter (Stoeling Co., Wood Dale, IL). The force at which a rat withdrew its hindpaw, vocalized, or struggled was multiplied by 10, as recommended by the manufacturer, and recorded as the withdrawal force (g).

Carrageenan-Induced Edema Formation. Rats (~130–140 g) were assigned to treatment groups so that each group was weight-balanced and administered vehicle or drug. Immediately thereafter, the rats were anesthetized with halothane (5%) and administered 50 μl of vehicle or carrageenan (0.5% in saline) s.c. on plantar surface of the left hindpaw, essentially, as described earlier (Vingar et al., 1976). Three hours later, the rats were euthanized and the difference in the weight (g) of the treated and untreated hindpaws was recorded as an expression of edema formation (g).

Neuropathy-Induced Cold Allodynia. Rats (~220–260 g) were randomly assigned to treatment groups and evaluated for allodynia as described previously (Gogas et al., 1997). The rats were then administered vehicle or drug and evaluated again 1 h later. In each case, the latency (s) to withdrawal of the neuropathic hindpaw from the ice water bath was measured.

Pharmacokinetics Analysis of (R,S)-Ketorolac

Rats were anesthetized with 5% halothane and administered [3H](R,S)-ketorolac (100 nmol; 0.495 mCi/mmol) by i.c.v. injection. At 0, 5, 20, 35, 60, and 180 min after injection, the rats were anesthetized with CO2/O2 (60:40%); blood was withdrawn into a heparinized syringe and a plasma fraction was obtained by centrifugation of the blood at 2600g for 5 min in a clinical centrifuge. To determine the total [3H](R,S)-ketorolac in each sample, aliquots of plasma were subjected to scintillation spectroscopy and HPLC. Protein was removed from the samples by precipitation with 1 volume of acetonitrile and centrifugation (500g, 15 min). The resulting supernatant was applied to a BDS-Hypersil-C18 (4.6 x 250 mm) reversed phase analytical column run isocratically at a flow rate of 1.0 ml/min with a solvent system consisting of 34% acetonitrile and 66% phosphate buffer (20 mM, pH 7.4). The concentration of (R,S)-ketorolac in the HPLC effluent was determined by comparison with the internal standard and by UV absorption at 317 nm. The [3H](R,S)-ketorolac in the effluent was determined radiometrically using a Radiomatic Flo-One/Beta A-500 radioactive flow detector (Packard Instruments Co., Inc., Meriden, CT) equipped with a 1-ml cell.

In Vitro Assays

Inhibition of Prostaglandin Formation. Recombinant COX-1 and COX-2 from rat (rCOX) and human (hCOX) were expressed in a baculovirus system and purified as described previously (Barnett et al., 1994). The specific activity of the final enzyme preparations used was between 20,000 and 35,000 units (1 unit = 1 nmol of oxygen consumed/mg protein/min) with a purity of > 80% as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining.
The purified COX enzymes were reconstituted with 2 mM phenol and 1 μM hematin and the cyclooxygenase activity was measured using a radiometric assay (Barnett et al., 1994). Putative inhibitors was then applied to a 1 ml C18 Sep-pak column that had previously been equilibrated with 0.2 N HCl and 750 μl of water. The total reaction volume was then applied to a 1 ml C18 Sep-pak column that had previously been washed with 2 ml of methanol followed by 5 ml of deionized water. Oxygenated products were eluted with 3 ml of a mixture of acetonitrile/water/acetic acid (50:50:0.1, v/v/v) and quantified by liquid scintillation spectroscopy. All inhibitors were assayed in triplicate using at least three independent samples.

**Ligand Binding and Enzyme Assays.** Membrane preparations enriched with the target receptor or channel (Table 3) were isolated and incubated with selective radioligands (Table 3) in the absence or presence of (S)-ketorolac (10 μM) or an appropriate positive control (Panlabs Inc. Pharmacology Services, Bothell, WA). Nonspecific binding was estimated using an excess of unlabeled, receptor-selective ligands. Details of the specific binding assays are described in the following sections: adenosine A1 (Lohe et al., 1987), A2 (Jarvis et al., 1989); α-adrenergic (Greengrass and Bremner, 1979); α2-adrenergic (Boyaajan and Leslie, 1987); β-adrenergic (UPrichard et al., 1978); calctionin gene-related peptide (Yoshizaki et al., 1987); γ-amino butyric acidα, benzodiazepine site (Damm et al., 1978); galanin (Sernv et al., 1987); glutamate-α,β-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (Olsen et al., 1987); glutamate-kainate (London and Coyle, 1979); glutamate-N-methyl-D-aspartate (NMDA), agonist site (Jones et al., 1989); glutamate-NMDA, glycine site (Snell et al., 1985); glycine (Young and Snyder, 1974); histamine (H1, Hill, 1978); H2 (Korte et al., 1990); muscarinic (Luthin and Wolfe, 1984); neurokinin NK1 (Lee et al., 1983); neuropeptide Y2 (Shelkh et al., 1989); opiate (Pasternak et al., 1975); hydroxytryptamine (serotonin; 5-HT1), (Middlemess, 1984); 5-HT1A (Hall et al., 1985); 5-HT2 (Leyisen et al., 1982); 5-HT3 (Pinkus et al., 1989); sigma (Weber et al., 1986) receptors as well as L type calcium channel, benzothiazepine site (Schoemaker and Langer, 1985); L type calcium channel, dihydro-pyridine site (Gould et al., 1982); N type calcium channel (Moreno et al., 1990); and sodium channel, site 2 (Catterall et al., 1981).

The effects of (S)-ketorolac on the activity of selected enzymes was also evaluated (Panlabs Inc. Pharmacology Services). (S)-ketorolac (10 μM) was tested with the constitutive isof orm of NO synthase from rat cerebellum (Nathan, 1992) and with the inducible isof orm of NO synthase from mouse macrophages (Nathan, 1992). (S)-ketorolac (300 μM) was also evaluated for its effects on porcine pancreatic phospholipase A2 (Katsumata et al., 1986), and rat brain protein kinase C (Hannu et al., 1985).

**Estimation of the Distribution Coefficient.** Partition coefficients for (R,S)-ketorolac, DS, and INDO were determined experimentally in a 1-octanol/water system at 25°C and an ionic strength of 0.2 N HCl and 750 μl of distilled water. The total reaction volume was then applied to a 1 ml C18 Sep-pak column that had previously been washed with 2 ml of methanol followed by 5 ml of deionized water. Oxygenated products were eluted with 3 ml of a mixture of acetonitrile/water/acetic acid (50:50:0.1, v/v/v) and quantified by liquid scintillation spectroscopy. All inhibitors were assayed in triplicate using at least three independent samples.

**Data Analysis**

All treatment groups were compared using a one-way ANOVA. Pairwise comparisons for the drug-treated groups to the vehicle group were then performed using Fisher’s least significant difference test. Bonferroni’s adjustment for multiple comparisons was made if the overall difference was not significant. In the writhing and paw edema tests, the analysis was carried out with ranked data. The following sigmoidal model was used: % Inhibition = MAX/1 + (dose/ID50)^n, where ID50 is the dose of the compound needed to achieve half of the maximum response in the dose-response curve; N is the curvature parameter; MAX is the maximum response and is assumed to be 100% in the writhing and paw hyperalgesia tests. All analyses were performed using SAS/STAT (SAS Institute Inc., 1989).

**Results**

**Tests of Nociception, Hyperalgesia, and Inflammation**

To determine the relationship between (R,S)-ketorolac’s analgesic and anti-inflammatory actions, (R,S), (S)-, and (R)-ketorolac as well as selected reference compounds were evaluated in tests of nociception (i.e., acetic acid-induced writhing), hyperalgesia (i.e., carrageenan-induced paw hyperalgesia), and inflammation (i.e., carrageenan-induced paw edema formation). The tests were optimized for use with NSAIDs (Figs. 1 and 2), such that the responses produced were completely blocked with INDO (10 mg/kg).

To characterize the COX involvement in the acute writhing response elicited by i.p. administration of acetic acid, the effects of INDO (0.3–5.0 mg/kg p.o.), a nonselective COX-1 inhibitor (Mitchell et al., 1994; Barnett et al., 1994), celecoxib (3–30 mg/kg p.o.), a selective COX-2 inhibitor (Penning et al., 1997), and (S)-ketorolac (0.01–1 mg/kg p.o.) on the writhing response were assessed (Fig. 1). Both (S)-ketorolac and INDO completely inhibited writhing in a dose-dependent manner with ID50 values of 0.05 ± 0.002 and 1.56 ± 0.12 mg/kg, respectively. Celecoxib did not significantly affect the writhing response at the doses tested (3–30 mg/kg p.o.). Under the same conditions, however, celecoxib effectively blocked carrageenan-induced paw hyperalgesia: 75% inhibition at 30 mg/kg p.o. with an ID50 value of 7.9 ± 1.2 mg/kg (Table 1).

These values were then used to calculate the distribution coefficient (D) at pH 7.4, using the following equation:

\[
D = \frac{(P[H^+] + P[K_a^+] - P)}{[H^+] + K_a}
\]

**Fig. 1.** Effects of (S)-ketorolac (0.01–1 mg/kg p.o.; ○), INDO (0.3–5 mg/kg p.o.; ▲), and celecoxib (1–30 mg/kg p.o.; ▼) in the acid-induced abdominal constriction in rats. The results are expressed as a percentage of inhibition from control values, where the control writhing responses were 9.13 ± 0.85, 12.57 ± 1.34, and 14 ± 0.62 writhes/15 min for (S)-ketorolac, INDO, and celecoxib, respectively. Each point represents the mean ± S.E.; n = 5 to 8 per group.
Injection of carrageenan into the rat hindpaw elicits a persistent inflammatory response, as reflected by mechanical hyperalgesia and edema formation (Vinegar et al., 1976), which are mediated by COX-2 (Seibert et al., 1994). To characterize the mechanical hyperalgesia, (S)-ketorolac (0.01–1 mg/kg s.c.), or INDO (0.3–6 mg/kg s.c.) were administered 2 h after carrageenan treatment and 1 h before testing (therapeutic regimen). To characterize edema formation elicited by carrageenan treatment, the same drugs were administered immediately before carrageenan treatment and 3 h before testing (prophylactic regimen). Under these conditions, (S)-ketorolac or INDO completely blocked mechanical hyperalgesia (Fig. 2A), but reduced edema formation by only 50 to 60% (Fig. 2B), as has been reported previously (Higgs et al., 1976). Therefore, carrageenan-induced mechanical hyperalgesia is completely dependent on COX activity and prostaglandin production. Carrageenan-induced edema formation, on the other hand, involves both COX-dependent and COX-independent mechanisms. In the present work, ketorolac and selected reference compounds were evaluated for their ability to inhibit COX-dependent edema formation only.

### Analgesic and Anti-Inflammatory Actions of Ketorolac

Under the conditions used, (R,S)-ketorolac was marginally, but significantly (p < .05), more potent as an antinociceptive than as an antihyperalgesic or an antinflammatory agent; ID$_{50}$ values = 0.08 (0.05, 0.11)$^b$, 0.24 (0.20, 0.27), or 0.29 (0.19, 0.39), respectively (Table 1). The (S)- and (R)-enantiomers of ketorolac as well as DS, a nonselective COX-1/COX-2 inhibitor (Mitchell et al., 1994; Pallapies et al., 1995), and INDO showed a similar potency pattern (Table 1). To determine whether the analgesic and anti-inflammatory actions of (R,S)-ketorolac, (S)-ketorolac, (R)-ketorolac, DS, and INDO were related, the potencies of these compounds in the tests of nociception or hyperalgesia were compared with the potencies obtained in the test of edema formation. The potencies of these compounds as inhibitors of acute nociception and edema formation (inflammation) or hyperalgesia and edema formation were highly correlated with Pearson correlation coefficients of 0.968 (Fig. 3A) and 0.994 (Fig. 3B), respectively.

In each test, (S)-ketorolac was the most potent compound evaluated, being 200- to 378-fold more potent than (R)-ketorolac and ~4-fold more potent than the racemate (Table 1). (R,S)-ketorolac was also significantly (p < .05) more potent than DS, INDO, or celecoxib in the tests of nociception, hyperalgesia, and edema formation (Table 1).

### Mechanism(s) Underlying the Actions of Ketorolac

**Inhibition of COX-1 and COX-2.** (R,S)-, (S)-, and (R)-ketorolac, as well as DS, INDO, and celecoxib were assessed for their ability to inhibit both isoforms of COX in recombinant rat and human enzyme systems (Table 2). The compounds were similar as inhibitors of rat COX (rCOX) and human COX (hCOX) under the conditions used. Each compound also exhibited a similar pattern of activity in the two enzyme systems. (R,S)-ketorolac inhibited rCOX-1 with an IC$_{50}$ of 0.27 ± 0.06 µM, a value not significantly different from that exhibited by DS or INDO (i.e., 0.20 ± 0.11 and 0.22 ± 0.14 µM, respectively). (R,S)-ketorolac also inhibited rCOX-1 in a stereoselective manner. The (S) enantiomer of ketorolac with an IC$_{50}$ value of 0.10 ± 0.08 µM was approx-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Test of Nociception ID$_{50}$</th>
<th>Test of Hyperalgesia ID$_{50}$</th>
<th>Test of Edema Formation$^a$ ID$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R,S)-ketorolac</td>
<td>s.c.</td>
<td>0.24 (0.20, 0.27)</td>
<td>0.29 (0.19, 0.39)</td>
<td>0.08 (0.05, 0.11)$^b$</td>
</tr>
<tr>
<td>(S)-ketorolac</td>
<td>s.c.</td>
<td>0.06 (0.04, 0.07)$^c$</td>
<td>0.07 (0.04, 0.09)$^c$</td>
<td>0.02 (0.01, 0.03)$^b$</td>
</tr>
<tr>
<td>(R)-ketorolac</td>
<td>s.c.</td>
<td>20.8 (14.1, 26.5)$^d$</td>
<td>26.5 (16.9, 36.1)$^d$</td>
<td>4.0 (1.4, 6.6)$^{c,e}$</td>
</tr>
<tr>
<td>DS</td>
<td>s.c.</td>
<td>5.89 (4.46, 7.33)$^d$</td>
<td>4.40 (1.62, 7.17)$^d$</td>
<td>0.27 (0.17, 0.37)$^{c,e}$</td>
</tr>
<tr>
<td>INDO</td>
<td>s.c.</td>
<td>1.10 (0.60, 1.60)$^d$</td>
<td>2.6 (2.1, 3.1)$^d$</td>
<td>0.46 (0.16, 0.76)$^{d,e}$</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>p.o.</td>
<td>&gt;30</td>
<td>7.9 (5.5, 10.3)$^d$</td>
<td>8.4 (4.9, 11.9)$^e$</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>2.6</td>
<td>2.8 (1.7, 3.8)$^d$</td>
<td>7.5 (4.0, 11.0)$^d$</td>
</tr>
</tbody>
</table>

$^a$ Estimation of ID$_{50}$ values assumes a maximum response of 50 to 60% inhibition.

$^b$ Significantly different from values in the test of nociception (p < .05).

$^c$ Significantly different from (R,S)-ketorolac (p < .05).

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**Fig. 2.** Effect of (S)-ketorolac (0.01–1 mg/kg s.c.) and INDO (0.3–6 mg/kg s.c.) on carrageenan-induced paw hyperalgesia (A) and carrageenan-induced paw edema formation (B). Each point represents the mean ± S.E. percentage of inhibition; n = 10 to 18 rats/group.
The COX-1/COX-2 activity ratio has been used as a measure of selectivity for COX-1 or COX-2 (Mitchell et al., 1994). (S)-ketorolac with activity ratios of 0.13 and 0.35 for the rat and human enzyme systems, respectively, was intermediate between the COX-1-selective agent INDO (activity ratios, 0.93 and 0.64, respectively) and the nonselective COX inhibitor diclofenac (activity ratios, 0.33 and 0.60, respectively).

**COX-Independent Actions of Ketorolac.** To determine whether peripherally administered ketorolac produces COX-independent analgesic actions, the effects of (S)-ketorolac on carbachol-stimulated platelet aggregation (Fig. 4), a response that is insensitive to COX inhibitors but reversible by opiates (Doherty et al., 1987; Akarsu et al., 1989) were assessed. In this test, the stable analog of prostacyclin, the most abundant prostaglandin produced in response to peritoneal irritation (Doherty et al., 1987), acts at IP receptors [i.e., the receptors at which prostacyclin (PGI₂) binds selectively] on visceral afferent fibers stimulating a nociceptive response. After induction of the mononeuropathy, the rats remain in a cold bath (0–4°C) for 20 s without signs of discomfort. After induction of the mononeuropathy, the rats develop cold allodynia, as reflected by rapid withdrawal of the affected hindpaw from the cold bath in less than 20 s (Fig. 6). Under the conditions used, (S)-ketorolac (1, 3, and 10 mg/kg p.o.) did not significantly affect cold allodynia in rats rendered neuropathic, although in a parallel study, the centrally acting antiepileptic drug gabapentin (30, 100, and 300 mg/kg p.o.) effectively reversed the allodynia.

**Distribution Coefficient of (R,S)-Ketorolac.** The distribution coefficient is a measure of the extent to which a compound partitions into an organic medium at pH 7.4 and

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**Fig. 3.** Relationship between the potency of (R,S)-ketorolac (RSK), (S)-ketorolac (SK), (R)-ketorolac (RK), DS, and INDO (I) in the tests of nociception and edema formation (A) and in the tests of nociception and hyperalgesia (B; see Table 1 for the ID₅₀ values). A Pearson correlation coefficient was calculated for each comparison.
predictions the ability of a compound to penetrate lipophilic, biological membranes (Avdeef, 1996). To compare the partition coefficient of (R,S)-ketorolac with those of INDO and DS, the three compounds were evaluated in a 1-octanol/water system (pH 7.4) at 25°C with an ionic strength of 0.15 M KCl. Under these conditions, the respective distribution coefficients for INDO and diclofenac were 30- and 35-fold greater than that for (R,S)-ketorolac (Table 4).

Discussion

Mechanism(s) Underlying the Actions of (R,S)-Ketorolac

During the initial pharmacological evaluation of (R,S)-ketorolac, it was suggested that the drug’s potency in vivo relative to other NSAIDs resulted from its relative potency as a COX inhibitor (Roos et al., 1982). In the present work, (R,S)-ketorolac was a potent inhibitor of COX-1 and COX-2 from rat or human in vitro. It was, however, no more potent than INDO or DS as an inhibitor of COX-1. Neither was (R,S)-ketorolac highly selective for COX-1 over COX-2 (i.e., COX-1/COX-2 activity ratios were 0.13 and ~0.33, respectively), consistent with earlier reports (Parnham, 1993; Pallapies et al., 1995). Therefore, (R,S)-ketorolac is a potent, nonselective COX inhibitor, like other NSAIDs.

To what extent, then, do the analgesic and anti-inflammatory actions of (R,S)-ketorolac result from inhibition of COX-1 or COX-2 in vivo? First, in the acute abdominal con-
This hypothesis is supported by previous reports indicating that \( (R,S) \)-ketorolac does not distribute well beyond the vascular compartment (Mroszczak et al., 1996) and by our observation that \( (R,S) \)-ketorolac exhibits a smaller volume of distribution relative to the other reference compounds (Table 5). Therefore, the marked potency of \( (R,S) \)-ketorolac in vivo may well depend on its pharmacokinetics.

**Table 3**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Selective Ligand</th>
<th>Tissue</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine ( A_1 )</td>
<td>( ^{3}H )DPCPX</td>
<td>Brain</td>
<td>-11</td>
</tr>
<tr>
<td>Adenosine ( A_{2A} )</td>
<td>( ^{3}H )CGS-21680</td>
<td>Striatum</td>
<td>4</td>
</tr>
<tr>
<td>Adrenergic ( \alpha_1 ) (nonselective)</td>
<td>( ^{3}H )Prazosin</td>
<td>Brain(^a)</td>
<td>-17</td>
</tr>
<tr>
<td>Adrenergic ( \beta ) (nonselective)</td>
<td>( ^{3}H )Rauwolscine</td>
<td>Cortex(^d)</td>
<td>-7</td>
</tr>
<tr>
<td>Cgrp</td>
<td>( ^{3}H )DHIA</td>
<td>Brain</td>
<td>2</td>
</tr>
<tr>
<td>( \gamma )-amino butyric acid ( \gamma )-aminobutyric acid (benzodiazepine site)</td>
<td>( ^{125}I )CGRP</td>
<td>Brain(^e)</td>
<td>6</td>
</tr>
<tr>
<td>Type L calcium channel (benzothiazepine site)</td>
<td>( ^{3}H )Diltiazem</td>
<td>Cortex(^d)</td>
<td>16</td>
</tr>
<tr>
<td>Type L calcium channel (dihydropyridine site)</td>
<td>( ^{3}H )Nifedipine</td>
<td>Cortex(^d)</td>
<td>27</td>
</tr>
<tr>
<td>Type ( N ) calcium channel</td>
<td>( ^{125}I )Camotoxin</td>
<td>Frontal lobe(^d)</td>
<td>6</td>
</tr>
<tr>
<td>( \gamma )-aminobutyric acid ( \gamma )-aminobutyric acid (benzodiazepine site)</td>
<td>( ^{3}H )Flunitrazepam</td>
<td>Brain(^d)</td>
<td>6</td>
</tr>
<tr>
<td>Galanin</td>
<td>( ^{3}H )AMPA</td>
<td>Cortex(^d)</td>
<td>2</td>
</tr>
<tr>
<td>Glutamate-AMPA</td>
<td>( ^{3}H )Kainate</td>
<td>Brain(^d)</td>
<td>13</td>
</tr>
<tr>
<td>Glutamate-NMDA (agonist site)</td>
<td>( ^{3}H )CGS-19755</td>
<td>Cortex(^d)</td>
<td>11</td>
</tr>
<tr>
<td>Glutamate-NMDA (glycine site)</td>
<td>( ^{3}H )Glycine</td>
<td>Cortex(^d)</td>
<td>2</td>
</tr>
<tr>
<td>Glutamate-NMDA (phencyclidine site)</td>
<td>( ^{3}H )TCP</td>
<td>Cortex(^d)</td>
<td>22</td>
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<tr>
<td>Glycine</td>
<td>( ^{3}H )Strychnine</td>
<td>Spinal cord</td>
<td>19</td>
</tr>
<tr>
<td>Histamine ( H_1 )</td>
<td>( ^{3}H )Pyrilamine</td>
<td>Brain(^d)</td>
<td>-2</td>
</tr>
<tr>
<td>Histamine ( H_3 )</td>
<td>( ^{3}H )NAMH</td>
<td>Brain</td>
<td>21</td>
</tr>
<tr>
<td>Muscarinic (nonselective)</td>
<td>QNB</td>
<td>Cortex(^d)</td>
<td>2</td>
</tr>
<tr>
<td>Neurokinin ( NK_1 )</td>
<td>( ^{3}H )Substance P</td>
<td>Submaxillary glands(^e)</td>
<td>-16</td>
</tr>
<tr>
<td>Neuropeptide ( Y_1 )</td>
<td>( ^{125}I )FFY</td>
<td>SK-N-MC cells(^f)</td>
<td>8</td>
</tr>
<tr>
<td>Opiate (nonselective)</td>
<td>Naloxone</td>
<td>Brain(^d)</td>
<td>6</td>
</tr>
<tr>
<td>5-HT(_1)</td>
<td>( ^{3}H )5-HT</td>
<td>Cortex(^d)</td>
<td>-12</td>
</tr>
<tr>
<td>5-HT(_1A)</td>
<td>( ^{3}H )8-OH-DPAT</td>
<td>Cortex(^d)</td>
<td>-7</td>
</tr>
<tr>
<td>5-HT(_2)</td>
<td>( ^{3}H )Retanserin</td>
<td>Brain(^d)</td>
<td>16</td>
</tr>
<tr>
<td>Sigma (nonselective)</td>
<td>( ^{3}H )5-HT(_3)</td>
<td>5-HT(_3)</td>
<td>5</td>
</tr>
<tr>
<td>Sodium channel (site 2)</td>
<td>( ^{3}H )BDG</td>
<td>Brain(^d)</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>( ^{3}H )IBTX</td>
<td>Brain(^d)</td>
<td>3</td>
</tr>
</tbody>
</table>

AMP \( A \) is \( \pm \)-\( \delta \)-2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
\(^a\) Average of two determinations.
\(^b\) Except cerebellum
\(^c\) From brain
\(^d\) Guinea pig
\(^e\) Human
\(^f\) Rabbit

**Fig. 5.** Appearance of \( (R,S) \)-ketorolac (100 nmol) administered i.e.v. in the plasma at measured by radiometric \( \blacksquare \) or HPLC \( \blacktriangle \) methods. Each point represents the mean \( \pm \) S.E.; \( n = 4 \) per time point.

Fat and thereby exhibit better biological activity and potency. This hypothesis is supported by previous reports indicating that \( (R,S) \)-ketorolac does not distribute well beyond the vascular compartment (Mroszczak et al., 1996) and by our observation that \( (R,S) \)-ketorolac exhibits a smaller volume of distribution relative to the other reference compounds (Table 5). Therefore, the marked potency of \( (R,S) \)-ketorolac in vivo may well depend on its pharmacokinetics.

**Analgesic versus Anti-Inflammatory Activities of \( (R,S) \)-Ketorolac**

Previously it was reported that \( (R,S) \)-ketorolac’s overall pharmacological profile favored its analgesic over its anti-inflammatory activity (Rooks et al., 1985; Young and Yee, 1994). This was based on observations that \( (R,S) \)-ketorolac was less effective than other NSAIDs, such as INDO or DS, at reducing paw inflammation in a rat model of adjuvant-induced arthritis involving the therapeutic administration of drugs (Rooks et al., 1985; Young and Yee, 1994). The results presented here suggest that the association or dissociation of the analgesic and anti-inflammatory activities of \( (R,S) \)-ketorolac depends on whether the drug is administered prophylactically or therapeutically.

When \( (R,S) \)-ketorolac was administered prophylactically, its anti-inflammatory and analgesic potencies were only marginally different. This difference was not unique to \( (R,S) \)-ketorolac, because INDO and DS were also somewhat more potent as anti-inflammatory than analgesic drugs when administered prophylactically. Second, the analgesic potencies of \( (R,S) \)-ketorolac, \( (R,S) \)-ketorolac, \( (R,S) \)-ketorolac, INDO, and DS were highly correlated with their anti-inflammatory potencies, suggesting that these compounds share a common mechanism. Third, the \( (S) \) enantiomer of \( (R,S) \)-ketorolac pro-duced the biological effects of the racemate in tests of nociception and hyperalgesia as well as in tests of inflammation, consistent with previous reports (Guzman et al., 1986). Together these results suggest that there is no dissociation of the anti-inflammatory and analgesic activities of \( (R,S) \)-ketorolac when the drug is administered prophylactically.

There remains, however, clear evidence for dissociation of these activities when \( (R,S) \)-ketorolac is administered therapeutically (Rooks et al., 1985; Young and Yee, 1994; McCor-
inflammation, then its analgesic and anti-inflammatory properties of ketorolac exerted its analgesic actions only at the site of injury, R,S-NSAIDs, as has been shown previously (Young and Yee, 1994). If INDO or DS, this would effectively reduce the inflammatory process into inflamed joints and tissues (Avdeef, 1996) as well as vasoconstriction of the microvascular bed (Mroszczak et al., 1996) and may not partition through the “walled off” by edema formation and swelling. (R,S)-ketorolac, being less lipophilic, does not distribute well beyond the site of injury, since it remains a potent analgesic in chronic inflammatory conditions (Rooks et al., 1985), but loses its potency as an anti-inflammatory agent relative to other NSAIDs (Rooks et al., 1985; Young and Yee, 1994) suggests that (R,S)-ketorolac can act elsewhere to produce analgesia. One possibility is that it acts peripherally to block COX activity in the dorsal root ganglia, as has been proposed recently for other COX inhibitors (Willingdale et al., 1997).

### Central Effects of (R,S)-Ketorolac

The lipophilicity of small compounds determines in large part their ability to cross the blood-brain barrier and exert central effects (Pardridge, 1991; Avdeef, 1996). (R,S)-ketorolac is less lipophilic than either INDO or DS and does not readily cross the blood-brain barrier in rodents (Mroszczak et al., 1987) or humans (Physicians’ Desk Reference, 1995b). In fact, the levels of (R,S)-ketorolac in cerebrospinal fluid are 0.002 times less than those in the plasma of humans (Physicians’ Desk Reference, 1995b), suggesting that plasma levels would have to be raised 500-fold to obtain therapeutic levels of (R,S)-ketorolac in the cerebrospinal fluid. Taken together, these data suggest that it is unlikely that peripherally administered (R,S)-ketorolac acts at a central site to produce its analgesic effects.

Although the physicochemical and pharmacokinetic properties of (R,S)-ketorolac greatly limit its ability to enter the CNS, this does not preclude the possibility that the drug can act centrally. Several studies have shown that central administration of (R,S)-ketorolac reduces pain-related behaviors in both rats (Malmberg and Yaksh, 1993; Parris et al., 1996) and mice (Uphouse et al., 1993; Tripathi and Welch, 1995). Our efforts to further characterize the centrally mediated antinociceptive actions of (R,S)-ketorolac were confounded by the rapid egress of drug from the CNS to the periphery. This work demonstrates some of the difficulties associated with studying the central actions of (R,S)-ketorolac, particularly when the pain-related behavior being measured depends on peripheral COX activity.

The fact that (R,S)-ketorolac acts centrally to block pain-related behaviors in rat models involving central sensitization (Malmberg and Yaksh, 1993; Parris et al., 1996) provides a basis for determining whether peripherally administered (R,S)-ketorolac can act centrally to exert its analgesic actions. We demonstrated that peripherally administered (S)-ketorolac at a supramaximal dose (10 mg/kg) does not ameliorate the neuropathy-induced cold allodynia. This contrasts with a previous report showing that centrally administered (R,S)-ketorolac decreases thermal hyperalgesia in the same neuropathic rat model (Parris et al., 1996). These results

**Table 4**

| Compound        | pKᵢ,ᵣ | logPᵢ | logPᵢ⁺ | Dᵢ (pH 7.4) | Ratio
|-----------------|--------|-------|--------|-------------|-------|
| (R,S)-ketorolac | 3.5    | 2.74 ± 0.02 | -0.52 ± 0.07 | 0.40 | 1
| DS              | 4.0    | 4.33 ± 0.01 | 0.56 ± 0.02 | 14.1 | 35
| IND             | 4.2    | 4.08 ± 0.01 | 0.47 ± 0.02 | 12.3 | 30

* *pKᵢ,ᵣ* values for (R,S)-ketorolac (Muchowski et al., 1985), DS, and INDO (Sallmann, 1979) were obtained from previously published reports.
* logPᵢ, partition coefficient of the neutral species.
* logPᵢ⁺, partition coefficient of the ionized species.
* Dᵢ, Distribution coefficient determined at pH 7.4.
* Ratio: Dᵢ/[(x)Ketorolac], where x is INDO or DS, respectively.
suggest that peripherally administered (R,S)-ketorolac acts peripherally to produce its analgesic actions.

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References


Alvan et al., 1975.

All of the reference citations have been omitted. The text seems to be discussing the analgesic properties of morphine and its suppression by ketorolac.

TABLE 5

Distribution volumes of (R,S)-ketorolac, DS, and INDO

<table>
<thead>
<tr>
<th>Compound</th>
<th>$V_{ds}$/Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R,S)-ketorolac</td>
<td>$0.149 \pm 0.05$ (i.v.) $0.157 \pm 0.023$ (p.o.) $0.114 \pm 0.027$ (i.m.)</td>
</tr>
<tr>
<td>DS</td>
<td>$0.55 \pm 0.08$</td>
</tr>
<tr>
<td>INDO</td>
<td>$1.53 \pm 1.27$ (i.v.) $0.92 \pm 0.53$ (p.o.)</td>
</tr>
</tbody>
</table>

* Mroszczak et al., 1996.
* Alkkola et al., and Mauzuikela, 1991.
* Alvan et al., 1975.

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Moresco RM, Geveni S, Battaini F, Trivalzio S and Travuschi M (1990) Omepramoge-


McCormack K and Urquhart E (1995) Correlation between nonsteroidal anti-


Moresco RM, Geveni S, Battaini F, Trivalzio S and Travuschi M (1990) Omepramoge-


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McCormack K and Urquhart E (1995) Correlation between nonsteroidal anti-


Moresco RM, Geveni S, Battaini F, Trivalzio S and Travuschi M (1990) Omepramoge-

