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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; ID_{50} 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 Uquhart, 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 (Uphouse 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-

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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, although the mechanism by which it exerts these actions is unclear.

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 (Penning 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. [^{3}H](*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). Carbaprostacyclin 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 located in the lateral cerebral ventricle. A 3 μl volume of vehicle or drug was administered and the incision was closed with a wound clip. The rats were allowed to recover for 10 min before testing.

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

Writhing. The writhing tests were carried as described previously (Arrigoni-Martelli, 1979; 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 (Vinegar 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 (Stoelting 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 (Vinegar 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 andadministered [^{3}H](*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 CO_2/O_2 (60:40%); blood was withdrawn into a heparinized syringe and a plasma fraction was obtained by centrifugation of the blood at 2600*g* for 5 min in a clinical centrifuge. To determine the total [^{3}H](*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 (500*g*, 15 min). The resulting supernatant was applied to a BDS-Hypersil-C18 (4.6 × 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 [^{3}H](*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 (2–15 μl) were diluted in DMSO and preincubated with the appropriate recombinant COX (3–15 ng) at a final concentration of 0.01 to 1000 μM in a reaction mixture (150 μl) containing 50 mM Tris-HCl buffer (pH 7.9), 2 mM EDTA, 10% glycerol, 2 mM phenol, and 1 μM hematin for 10 min. The reaction was initiated by addition of [^{14}C]arachidonic acid (Amersham, 50–60 mCi/mmol in a final concentration of 20 μM) and was terminated 45 s later by the addition of 100 μl of 0.2 N HCl and 750 μl of distilled water. The total reaction volume was then applied to a 1 ml C_{18} 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 references: adenosine A_1 (Lohse et al., 1987), A_2 (Jarvis et al., 1989); α_1-adrenergic (Greengrass and Bremner, 1979); α_2-adrenergic (Boyajian and Leslie, 1987); β-adrenergic (U'Prichard et al., 1978); calcitonin gene-related peptide (Yoshizaki et al., 1987); γ-aminobutyric acid$_A$, benzodiazepine site (Damm et al., 1978); galanin (Servin et al., 1987); glutamate-($\pm$)α-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., 1988); glutamate-NMDA, phencyclidine site (Goldman et al., 1985); glycine (Young and Snyder, 1974); histamine H_1 (Hill, 1978); H_3 (Korte et al., 1990); muscarinic (Luthin and Wolfe, 1984); neurokinin NK_1 (Lee et al., 1983); neuropeptide Y_2 (Shelkh et al., 1989); opiate (Pasternak et al., 1975); hydroxytryptamine (serotonin; 5-HT)$_1$ (Middlemiss, 1984); 5-HT$_{1A}$ (Hall et al., 1985); 5-HT$_2$ (Leysen et al., 1982); 5-HT$_3$ (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, dihydropyridine site (Gould et al., 1982); N type calcium channel (Moresco 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 isoform of NO synthase from rat cerebellum (Nathan, 1992) and with the inducible isoform of NO synthase from mouse macrophages (Nathan, 1992). (*S*)-ketorolac (300 μM) was also evaluated for its effects on porcine pancreatic phospholipase A_2 (Katsumata et al., 1986), and rat brain protein kinase C (Hannun 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 0.15 M KCl, using a SIRIUS PCA 101 (SIRIUS Analytical Instruments Ltd., East Sussex, UK), and calculated as follows:

$$V_w/V_o = P\ 10^{(pKa - pKa')} - Pi$$

V_w: volume of water
V_o: volume of 1-octanol
P: partition coefficient of a compound as neutral species
P_i: partition coefficient of a compound as ionized species
pK_a: acidity constant in water
$pK_a^{\{prime\}}$: acidity constant in the presence of 1-octanol

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

$$D = (P\,[H^+] + P_iK_a)/([H^+] + K_a).$$

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/ID_{50})^N), where ID_{50} 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), or (*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 ID_{50} values of 0.04 $\pm$ 0.009 and 1.65 $\pm$ 0.72 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 ID_{50} value of 7.9 $\pm$ 1.2 mg/kg (Table 1).

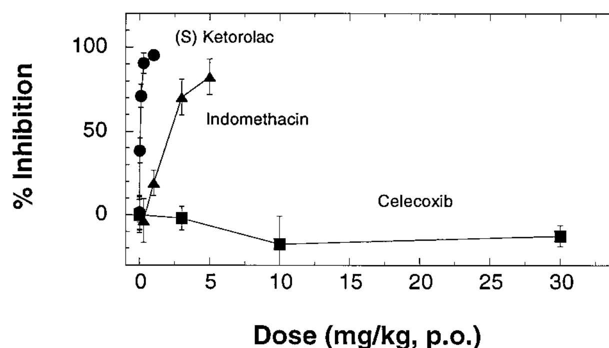

Fig. 1. Effects of (*S*)-ketorolac (0.01–1 mg/kg p.o.; ●), INDO (0.3–5 mg/kg p.o.; ▲), and celecoxib (1–30 mg/kg p.o.; ■) in the acid-induced abdominal constriction in rats. The results are expressed as a percentage of inhibition from control values, where the control writhing responses were 9.13 $\pm$ 0.83, 12.57 $\pm$ 1.34, and 14 $\pm$ 0.62 writhes/15 min for (*S*)-ketorolac, INDO, and celecoxib, respectively. Each point represents the mean $\pm$ S.E.; n = 5 to 8 per group.

TABLE 1
Antinociceptive, antihyperalgesic, and anti-inflammatory actions of (*R,S*)-, (*S*)-, and (*R*)-ketorolac

Compound	Route	Test of Nociception ID_{50}	Test of Hyperalgesia ID_{50}	Test of Edema Formation[a] ID_{50}
		mg/kg	*mg/kg*	*mg/kg*
(*R,S*)-ketorolac	s.c.	0.24 (0.20, 0.27)	0.29 (0.19, 0.39)	0.08 (0.05, 0.11)[b]
(*S*)-ketorolac	s.c.	0.06 (0.04, 0.07)[c]	0.07 (0.04, 0.09)[c]	0.02 (0.01, 0.03)[b]
(*R*)-ketorolac	s.c.	20.8 (14.1, 26.5)[c]	26.5 (16.9, 36.1)[c]	4.0 (1.4, 6.6)[b,c]
DS	s.c.	5.89 (4.46, 7.33)[c]	4.40 (1.62, 7.17)[c]	0.27 (0.17, 0.37)[b,c]
INDO	s.c.	1.10 (0.60, 1.60)[c]	2.6 (2.1, 3.1)[c]	0.46 (0.16, 0.76)[b,c]
Celecoxib	p.o.	>30	7.9 (5.5, 10.3)[c]	8.4 (4.9, 11.9)[c]
	i.v.		2.8 (1.7, 3.8)[c]	7.5 (4.0, 11.0)[c]

[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 ($p < .05$)

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.

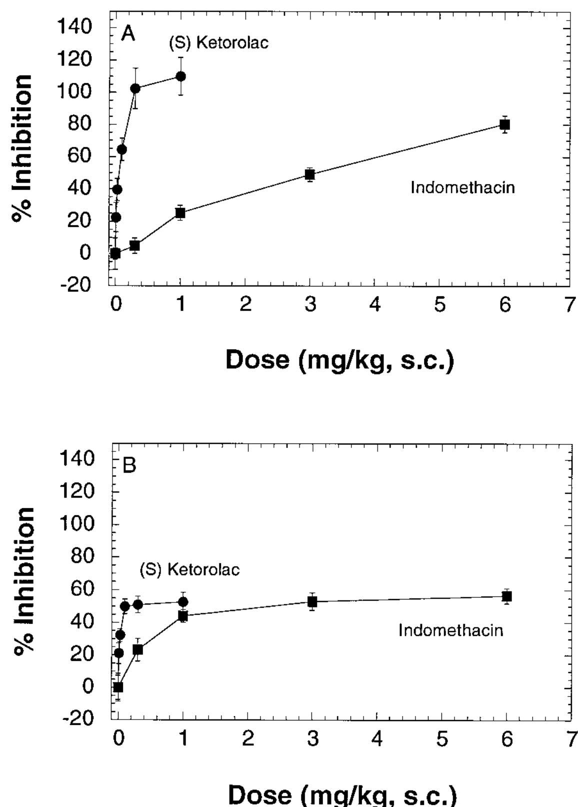

Fig. 2. Effect of (*S*)-ketorolac (0.01–1 mg/kg s.c.) and INDO (0.3–6 mg/kg s.c.) on carrageenan-induced paw hyperalgesia (A) and carrageenan-induced paw edema formation (B). Each point represents the mean ± S.E. percentage of inhibition; $n = 10$ to 16 rats/group.

Analgesic and Anti-Inflammatory Actions of Ketorolac

Under the conditions used, (*R,S*)-ketorolac was marginally, but significantly ($p < .05$), more potent as an anti-inflammatory than as an antinociceptive or an antihyperalgesic agent; ID_{50} values = 0.08 (0.05, 0.11), 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-

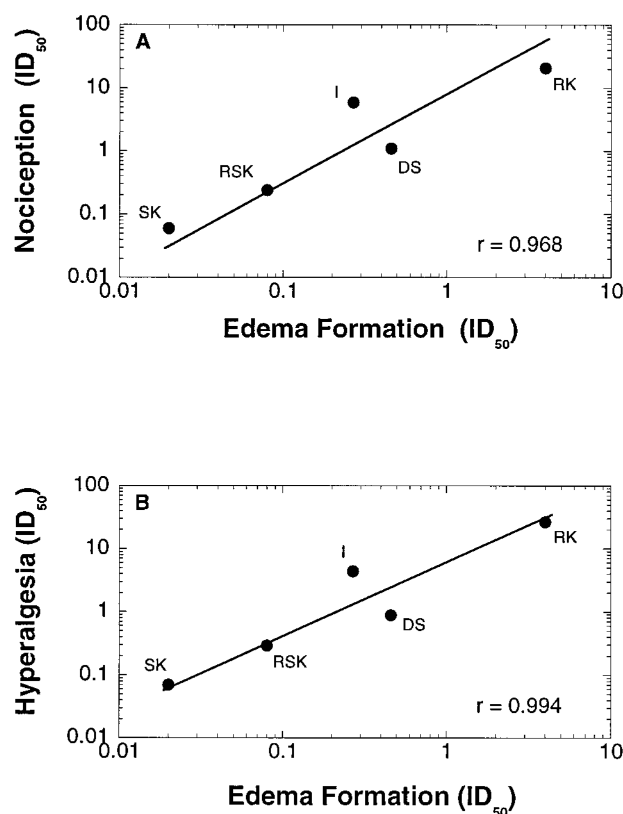

Fig. 3. Relationship between the potency of (*R,S*)-ketorolac (RSK), (*S*)-ketorolac (SK), (*R*)-ketorolac (RK), DS, and INDO (I) in the tests of nociception and edema formation (A) and in the tests of nociception and hyperalgesia (B; see Table 1 for the ID_{50} values). A Pearson correlation coefficient was calculated for each comparison.

imately twice as potent as the racemate, whereas the (*R*)-enantiomer with an IC_{50} value of > 100 μM was virtually without activity.

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, 0.03 and 0.04, 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 carbaprostacyclin-induced writhing (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 irritants (Doherty et al., 1987), acts at IP receptors [i.e., the receptors at which prostacyclin (PGI_2) binds selectively] on visceral afferent fibers stimulating a nociceptive response. The dose of carbaprostacyclin (30 μg) was selected to mimic the response elicited by the irritant, acetic acid (i.e., ~ 12 writhes/15 min). Under these conditions, (*S*)-ketorolac did not affect the writhing response elicited by carbaprostacyclin at a dose (i.e., 3 mg/kg p.o.) 100-fold greater than its ID_{50} value (i.e., 0.04 ± 0.009 mg/kg p.o.). INDO (6 mg/kg p.o.) and DS (100 mg/kg p.o.) were also ineffective. In contrast, morphine sulfate (6 mg/kg p.o.) completely blocked the carbaprostacyclin-induced writhing response.

Central Actions of Ketorolac. To investigate the possibility that the analgesic actions of ketorolac are mediated by central COX or as yet unidentified mechanisms within the central nervous system (CNS), the binding profile of (*S*)-ketorolac to ion channels and receptors known to be involved in central mechanisms of analgesia was determined (Table 3). In each case, the channel or receptor membrane preparation was incubated in the presence of 10 μM (*S*)-ketorolac. Under these conditions, (*S*)-ketorolac did not significantly inhibit selective ligand binding to the channels and receptors evaluated. Neither did it inhibit the activity of the constitutive or inducible isoforms of NO at 10 μM synthase (2 and −19% inhibition, respectively), phospholipase A_2 at 300 μM (4% inhibition) or protein kinase C at 100 μM (−6% inhibition).

Because central mechanisms, perhaps not included in the binding or enzyme activity assays, might contribute to the analgesic actions of ketorolac, the effect of (*S*)-ketorolac (i.c.v.) on the acetic acid-induced writhing response was assessed. (*S*)-ketorolac (1–100 nmol i.c.v.) significantly inhibited the acid-induced writhing with an ID_{50} of 21.1 ± 6.0 nmol. Under the same conditions, neither INDO nor DS, at 300 nmol, significantly affected the writhing response. These results suggest that either (*S*)-ketorolac had produced central analgesic actions or that centrally administered (*S*)-ketorolac had entered the systemic circulatory system and blocked the writhing response by inhibiting the peripheral COX-1.

To test this latter possibility, $[^3H]$(*R,S*)-ketorolac (100 nmol) was administered i.c.v. and its appearance in peripheral blood was measured using both radiometric and HPLC methods of quantification. As seen in Fig. 5, $[^3H]$(*R,S*)-ketorolac entered the peripheral circulatory system and reached peak levels of 0.3 to 0.4 μg/ml within 5 min of dosing and steady-state levels of 0.2 to 0.3 μg/ml within 30 min of dosing. These levels of (*R,S*)-ketorolac in peripheral blood must be considered significant, because the C_{max} achieved with a near maximally effective dose of (*R,S*)-ketorolac (1 mg/kg i.v.) is approximately 3 μg/ml (Mroszczak et al., 1987). The egress of radiolabeled (*R,S*)-ketorolac from the CNS after i.t. administration via a chronically implanted cannula was virtually indistinguishable from that described in Fig. 5 (data not shown).

To further explore the possibility that peripherally administered (*R,S*)-ketorolac could exert its analgesic actions via a central mechanism, the effect of peripherally administered (*S*)-ketorolac on mononeuropathy-induced cold allodynia (Gogas et al., 1997) was assessed. Normally, rats are able to 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

TABLE 2
Inhibition of rat and human COX-1 and COX-2 by (*R,S*)-, (*S*)-, and (*R*)-ketorolac

Compound	Rat COX ID_{50}[a]			Human COX ID_{50}[a]		
	rCOX-1	rCOX-2	rCOX-1/rCOX-2	hCOX-1	hCOX-2	hCOX-1/hCOX-2
		μM			μM	
(*R,S*)-ketorolac	0.27 ± 0.06	2.06 ± 0.95	0.13	1.23 ± 0.16	3.50 ± 0.63	0.35
(*S*)-ketorolac	0.10 ± 0.08	0.79 ± 0.60	0.13	0.46 ± 0.08	1.46 ± 0.43	0.32
(*R*)-ketorolac	>100[b]	>100[b]		>100[b]	>100[b]	
DS	0.20 ± 0.11	0.60 ± 0.18	0.33	0.90 ± 1.22	1.50 ± 0.80	0.60
INDO	0.22 ± 0.14	6.90 ± 2.00	0.03	0.66 ± 0.56	14.56 ± 9.30	0.04
Celecoxib	57.2 ± 24.9	0.04 ± 0.01	1431			

[a] Values are mean ± S.E. from at least three separate determinations.
[b] Due to the weakness of binding, an ID_{50} value could not be determined.
[c] Values previously reported (Ramesha, 1996).

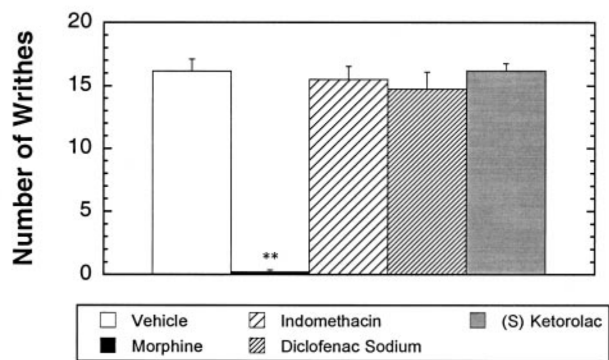

Fig. 4. Effects of vehicle at 2 ml/kg (white), (*S*)-ketorolac at 3 mg/kg (gray), morphine sulfate at 6 mg/kg (black), INDO at 6 mg/kg (wide lines), and DS at 100 mg/kg (narrow lines) on carbaprostacyclin-induced writhing. The drugs were administered (p.o.) 1 h before treatment with carbaprostacyclin (30 μg). Each point represents the mean ± S.E. incidence of writhes/15 min interval; n = 6 to 16 rats per group; $p < .01$ (**).

predicts 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 (Rooks 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 constriction, (*R,S*)-ketorolac, INDO, and DS most likely act by inhibiting COX-1, as this test was shown to depend on COX-1 and not COX-2 activity. In the carrageenan-induced hyperalgesia and edema tests involving both COX-1 and COX-2 activities (Seibert et al., 1994), it is not as clear whether (*R,S*)-ketorolac acts by inhibiting COX-1 alone or by inhibiting both COX-1 and COX-2. However, the rank-order potency for (*R,S*)-, (*S*)-, and (*R*)-ketorolac as well as INDO, DS, and celecoxib in vivo (i.e., (*S*)-ketorolac > (*R,S*)-ketorolac ≫ INDO = DS = celecoxib ≫ (*R*)-ketorolac) corresponds more closely with the rank-order potency for these same compounds as inhibitors of COX-1 in vitro (i.e., (*S*)-ketorolac > (*R,S*)-ketorolac = INDO = DS ≫ celecoxib = (*R*)-ketorolac) than as inhibitors of COX-2 in vitro (i.e., celecoxib ≫ DS = (*S*)-ketorolac > (*R,S*)-ketorolac = INDO ≫ (*R*)-ketorolac). Additional studies will be needed to delineate the differential effects of (*R,S*)-ketorolac in systems that involve both COX-1 and COX-2.

At present, inhibition of COX is clearly the most likely mechanism underlying the actions of (*R,S*)-ketorolac. This is based not only on its ability to inhibit COX in vitro and block prostaglandin production in vivo (Zhang et al., 1997), but also on its inability to produce COX-independent activities. Most notably, (*S*)-ketorolac did not interact with a variety of key receptors, channels, or enzymes known to be involved in pain transmission mechanisms. Furthermore, when (*S*)-ketorolac was examined for its ability to block a COX-independent writhing response (i.e., carbaprostacyclin-induced writhing), it was without effect. COX-independent mechanisms that may underlie the analgesic and anti-inflammatory actions of (*R,S*)-ketorolac were not identified, although this does not preclude their existence.

Potency of (*R,S*)-Ketorolac Relative to Other NSAIDs

Based on the relative potencies of (*R,S*)-ketorolac, INDO, and DS as inhibitors of rCOX-1 (i.e., ID_{50} values of 0.27, 0.20, and 0.22 μM, respectively), one would predict that (*R,S*)-ketorolac would be no more potent than INDO or DS as an inhibitor of nociception, hyperalgesia, and inflammation in vivo. Yet, (*R,S*)-ketorolac was ~ 3- to 24-fold more potent than these reference compounds in vivo, depending on the test. This greater potency for (*R,S*)-ketorolac in vivo may be due to important differences in the pharmacokinetics for the three compounds. Differences in plasma protein binding can not explain the differences in potency, as all three compounds are highly bound to plasma proteins in humans (Physicians' Desk Reference, 1995a,b,c). (*R,S*)-ketorolac is, however, 30- to 35-fold less lipophilic than INDO or DS. As such, it may partition less extensively into body

TABLE 3
Binding profile of (*S*)-ketorolac

Receptor	Selective Ligand	Tissue[a]	% Inhibition[b]
Adenosine A_1	[^{3}H]DPCPX	Brain	−11
Adenosine A_{2A}	[^{3}H]CGS-21680	Striatum	4
Adrenergic α_1 (nonselective)	[^{3}H]Prazosin	Brain[c]	−17
Adrenergic α_2 (nonselective)	[^{3}H]Rauwolscine	Cortex[d]	−7
Adrenergic β (nonselective)	[^{3}H]DHA	Brain	2
CGRP	[^{125}I]CGRP	Brain	−4
Type L calcium channel (benzothiazepine site)	[^{3}H]Diltiazem	Cortex[d]	16
Type L calcium channel (dihydropyridine site)	[^{3}H]Nitrendipine	Cortex[d]	27
Type N calcium channel	[^{125}I]ω-Conotoxin	Frontal lobe[d]	6
γ-aminobuytric acid$_A$ (benzodiazepine site)	[^{3}H]Flunitrazepam	Brain[c]	6
Galanin	[^{125}I]Galanin	Brain	14
Glutamate-AMPA	[^{3}H]AMPA	Cortex[d]	2
Glutamate-kainate	[^{3}H]Kainate	Brain[c]	13
Glutamate-NMDA (agonist site)	[^{3}H]CGS-19755	Cortex[d]	11
Glutamate-NMDA (glycine site)	[^{3}H]Glycine	Cortex[d]	−2
Glutamate-NMDA (phencyclidine site)	[^{3}H]TCP	Cortex[d]	22
Glycine	[^{3}H]Strychnine	Spinal cord	19
Histamine H_1	[^{3}H]Pyrilamine	Brain[e]	−2
Histamine H_3	[^{3}H]NAMH	Brain	21
Muscarinic (nonselective)	QNB	Cortex[d]	2
Neurokinin NK_1	[^{3}H]Substance P	Submaxillary glands[e]	−16
Neuropeptide Y_1	[^{125}I]PYY	SK-N-MC cells[f]	8
Opiate (nonselective)	Naloxone	Brain[c]	6
5-HT_1	[^{3}H]5-HT	Cortex[d]	−12
5-HT_{1A}	[^{3}H]8-OH-DPAT	Cortex[d]	−7
5-HT_2	[^{3}H]Ketanserin	Brain[c]	16
5-HT_3	[^{3}H]GR-65630	Ileal muscularis[g]	−5
Sigma (nonselective)	[^{3}H]DTG	Brain[e]	11
Sodium channel (site 2)	[^{3}H]BTX	Brain[c]	3

AMPA, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
[a] All tissues were from rat except where noted.
[b] Average of two determinations.
[c] Except cerebellum
[d] From brain
[e] Guinea pig
[f] Human
[g] Rabbit

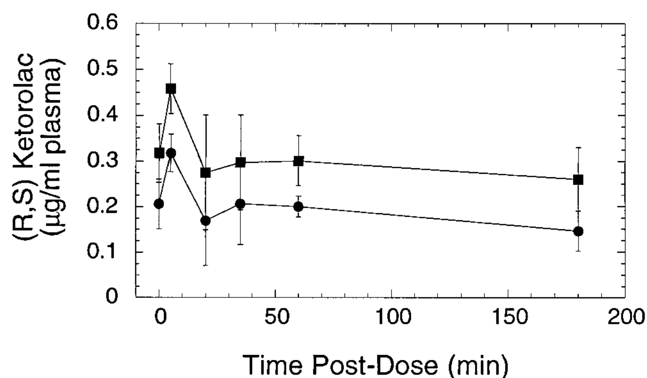

Fig. 5. Appearance of (*R,S*)-ketorolac (100 nmol) administered i.c.v. in the plasma as measured by radiometric (■) or HPLC (●) methods. Each point represents the mean ± S.E.; $n = 4$ per time point.

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 for (*R,S*)-ketorolac, (*R*)-ketorolac, (*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 produced 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-

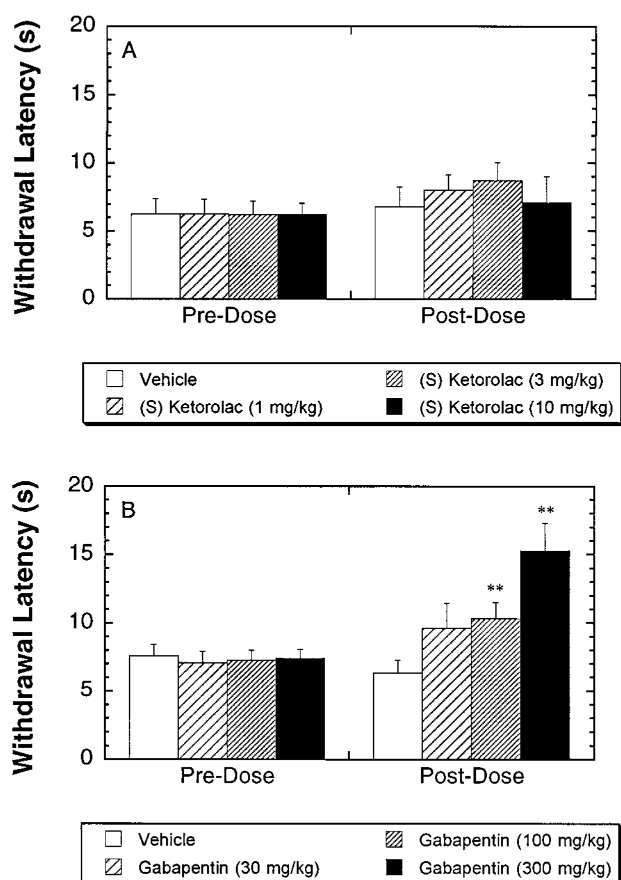

Fig. 6. Effects of vehicle at 2 ml/kg (white) or (*S*)-ketorolac at 1 mg/kg (wide lines), 3 mg/kg (narrow lines), and 10 mg/kg (black) (A) or vehicle at 10 ml/kg (white) or gabapentin at 30 mg/kg (wide lines), 100 mg/kg (narrow lines), and 300 mg/kg (black) (B) on cold allodynia. The drugs were administered (p.o.) 1 h before testing. Each point represents the mean ± S.E. withdrawal latency; $n = 9$ to 12 rats per group; $p < .01$ (**).

mack and Urquhart, 1995). The fact that (*R,S*)-ketorolac is less lipophilic than other NSAIDs, specifically INDO and DS, may help explain this apparent dissociation of activities. In chronic inflammatory conditions, NSAIDs, including (*R,S*)-ketorolac, are administered after the inflamed tissue is "walled off" by edema formation and swelling. (*R,S*)-ketorolac, being less lipophilic, does not distribute well beyond the vascular bed (Mroszczak et al., 1996) and may not partition into inflamed joints and tissues (Avdeef, 1996;) as well as INDO or DS. This would effectively reduce (*R,S*)-ketorolac's potency as an anti-inflammatory drug relative to INDO and DS, as has been shown previously (Young and Yee, 1994). If (*R,S*)-ketorolac exerted its analgesic actions only at the site of inflammation, then its analgesic and anti-inflammatory potencies should be similar. The fact that (*R,S*)-ketorolac 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

Distribution coefficients (*D*)- for (*R,S*)-ketorolac, INDO, and DS measured in a 1-octanol/water system at 25°C and an ionic strength of 0.15 M KCl (pH 7.4)

Compound	pK_a[a]	logP[b]	$logP_i$[c]	D[d] (pH 7.4)	Ratio[e]
(*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

[a] pK_a values for (*R,S*)-ketorolac (Muchowski et al., 1985), DS, and INDO (Sallmann, 1979) were obtained from previously published reports.
[b] logP, partition coefficient of the neutral species.
[c] $logP_1$, partition coefficient of the ionized species.
[d] D, Distribution coefficient determined at pH 7.4.
[e] Ratio: $(_{(x)}/D_{(ketorolac)})$, where x is INDO or DS, respectively.

TABLE 5
Distribution volumes of (*R*,*S*)-ketorolac, DS, and INDO

Compound	$V_{d\beta}$ (Route)
	l/kg ± S.E.
(*R*,*S*)-ketorolac	0.149 ± 0.05 (i.v.)[a]
	0.157 ± 0.023 (p.o.)[a]
	0.145 ± 0.032 (i.m.)[a]
DS	0.55 (p.o.)[b]
INDO	1.53 ± 1.27 (i.v.)[c]
	0.92 ± 0.53 (p.o.)[d]

[a] Mroszczak et al., 1996.
[b] Physicians' Desk Reference, 1995a.
[c] Olkkola et al., and Maunuksela, 1991.
[d] Alvan et al., 1975.

suggest that peripherally administered (*R,S*)-ketorolac acts peripherally to produce its analgesic actions.

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References

Akarsu ES, Palaoglu O and Ayhan IH (1989) Iloprost-induced writhing in mice and its suppression by morphine. *Methods Find Exp Clin Pharmacol* **11:**273–275.

Alvan G, Orme M, Bertilsson L, Ekstrand R and Palmer L (1975) Pharmacokinetics of Indomethacin. *Clin Pharmacol & Ther* **18:**364–373.

Arrigoni-Martelli E (1979) Screening and assessment of anti-inflammatory drugs. *Methods Find Exp Clin Pharmacol* **1:**157–177.

Avdeef A (1996) Assessment of Distribution-pH Profiles, in *Lipophilicity in Drug Action and Toxicology* (Pliska V, Testa B and van de Waterbeemd H eds), pp 109–138, VCH Publishers Inc., New York.

Barnett J, Chow J, Ives D, Chiou M, Mackenzie R, Osen E, Nguyen B, Tsing S, Bach C, Freire J, Chan H, Sigal E and Ramesha C (1994) Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. *Biochim Biophys Acta* **1209:**130–139.

Bennett GJ and Xie Y-K (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33:**87–107.

Boyajian CL and Leslie FM (1987) Pharmacological evidence for α_2-adrenoceptor heterogeneity: Differential binding properties of tritiated rauwolscine and tritiated idazoxan in rat brain. *J Pharmacol Exp Ther* **241:**1092–1098.

Brune K, Beck WS, Geisslinger G, Menzel-Soglowek S, Peskar BM and Peskar BA (1991) Aspirin-like drugs may block pain independently of prostaglandin synthesis inhibition. *Experientia (Basel)***47:**257–261.

Catterall WA, Morrow CS, Daly JW and Brown GB (1981) Binding of batrachotoxinin A 20-α-benzoate to a receptor site associated with sodium channels in synaptic nerve ending particles. *J Biol Chem* **256:**9822–9827.

Chavez R, Bravo C, Zazueta C, Pichardo J, Uribe A, Corona N, Reyes-Vivas H, Gonzalez C and Chavez E (1993) Ionophoretic-like properties of ketorolac for calcium. *J Pharmacol Exp Ther* **267:**1134–1139.

Damm HW, Muller WE, Schlafer U and Wollert U (1978) [^{3}H]flunitrazepam: Its advantages as a ligand for the identification of benzodiazepine receptors in rat brain membranes. *Res Commun Chem Pathol Pharmacol* **22:**449–457.

Doherty NS, Beaver TH, Chan KY, Coutant JE and Westrich GL (1987) The role of prostaglandins in the nociceptive response induced by intraperitoneal injection of zymosan in mice. *Br J Pharmacol* **91:**39–47.

Gogas KR, Jacobson LO, Waligora D, Martin B and Hunter JC (1997) The cold bath assay: A simple and reliable method to assess cold allodynia in neuropathic rats. *Analgesia* **3:**1–8.

Goldman ME, Jacobson AE, Rice KC and Paul SM (1985) Differentiation of [^{3}H] phencyclidine and (+)-[^{3}H]SKF-10047 binding sites in rat cerebral cortex. *FEBS Lett* **190:**333–336.

Gould RJ, Murphy KMM and Snyder SH (1982) [^{3}H]nitrendipine-labeled calcium channels discriminate inorganic calcium agonists and antagonists. *Proc Natl Acad Sci USA* **79:**3656–3660.

Granados-Soto V, Flores-Murrieta FJ, Castaneda-Hernandez G and Lopez-Munoz FJ (1995) Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac. *Eur J Pharmacol* **277:**281–284.

Greengrass P and Bremner R (1979) Binding characteristics of [^{3}H] prazosin to rat brain α-adrenergic receptors. *Eur J Pharmacol* **55:**323–326.

Guzman A, Yuste F, Toscano RA, Young JM, Van Horn AR and Muchowski JM (1986) Absolute configuration of (−)-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2-a]pyrrole-1-carboxylic acid, the active enantiomer of ketorolac. *J Med Chem* **29:** 589–591.

Hall MD, El Mestikawy S, Emerit MB, Pichat L, Hamon M and Gozlan H (1985) [^{3}H] 8-Hydroxy-2(di-*n*-propylamino)-tetralin binding to pre and post synaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J Neurochem* **44:**1685–1696.

Hannun YA, Loomis CR and Bell RM (1985) Activation of protein kinase C by Triton X-100 mixed micelles containing diacylglycerol and phosphatidylserine. *J Biol Chem* **260:**10039–10043.

Higgs GA, Harvey EA, Ferreira SH and Vane JR (1976) The effects of anti-inflammatory drugs on the production of prostaglandins *in vivo*. *Adv Prostaglandin Thromboxane Res* **1:**105–110.

Hill SJ (1978) The binding of tritiated mephyramine to histamine H_1 receptors in guinea pig brain. *J Neurochem* **31:**997–1004.

Jarvis MF, Schulz R, Hutchison AJ, Do UH, Sills MA and Williams M (1989) Tritiated CGS-21680 a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J Pharmacol Exp Ther* **251:**888–893.

Jones SM, Snell LD and Johnson KM (1989) Characterization of the binding of radioligands to the N-methyl-D-aspartate, phencyclidine, and glycine receptors in buffy coat membranes. *J Pharmacol Methods* **21:**161–168.

Katsumata M, Gupta C and Goldman AS (1986) A rapid assay for activity of phospholipase A_2 using radioactive substrate. *Anal Biochem* **154:**676–681.

Korte A, Myers J, Shih NY, Egan RW and Clark MA (1990) Characterization and tissue distribution of H_3 histamine receptors in guinea pigs by *N*-α-Methylhistamine. *Biochem Biophys Res Commun* **168:**979–986.

Lee CM, Javitch JA and Snyder SH (1982) [^{3}H]substance P binding to salivary gland membranes. *Mol Pharmacol* **23:**563–569.

Leysen JE, Niemegeers JE, Van Nueten JM and Laduron PM (1982) [^{3}H] ketanserin (R 41 468), a selective [^{3}H] ligand for serotonin 2 receptor binding sites. *Mol Pharmacol* **21:**301–314.

Lohse MJ, Klotz K-N, Lindenborn-Fotinos J, Reddington M, Schwabe U and Olsson RA (1987) 8-cyclopentyl-1, 3-dipropylxanthine DPCPX: A selective high affinity antagonist radioligand for A_1 adenosine receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **336:**204–210.

London ED and Coyle JT (1979) Specific binding of [^{3}H] kainic acid to receptor sites in rat brain. *Mol Pharmacol* **15:**492–505.

LoPachin RM, Rudy TA and Yaksh TL (1981) An improved method for chronic catheterization of the rat spinal subarachnoid space. *Physiol Behav* **27:**559–561.

Luthin GR and Wolfe BB (1984) Comparison of [^{3}H] quinuclidinyl benzilate binding to muscarine cholinergic receptors in rat brain. *J Pharmacol Exp Ther* **228:**648–655.

Malmberg AB and Yaksh TL (1992) Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. *J Pharmacol Exp Ther* **263:**136–146.

Malmberg AB and Yaksh TL (1993) Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. *Anesthesiology* **79:**270–281.

McCormack K and Urquhart E (1995) Correlation between nonsteroidal anti-inflammatory drug efficacy in a clinical pain model and the dissociation of their anti-inflammatory and analgesic properties in animal models. *Clin Drug Invest* **9:**88–97.

Middlemiss DN (1984) Stereoselective blockade at [^{3}H] 5-HT binding sites and at the 5-HT autoreceptor by propranolol. *Eur J Pharmacol* **101:**289–293.

Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ and Vane JR (1994) Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclo-oxygenase. *Proc Natl Acad Sci USA* **90:**11693–11697.

Moresco RM, Govoni S, Battaini F, Trivulzio S and Trabucchi M (1990) Omegaconotoxin binding decreases in aged rat brain. *Neurobiol Aging* **11:**433–436.

Mroszczak EJ, Lee FW, Combs D, Sarnquist FH, Huang BL, Wu AT, Tokes LG, Maddox ML and Cho DK (1987) Ketorolac tromethamine absorption, distribution, metabolism, excretion, and pharmacokinetics in animals and humans. *Drug Metab Dispos* **15:**618–626.

Mroszczak EJ, Combs D, Chaplin M, Tsina I, Tarnowski T, Rocha C, Yuen T, Boyd A, Young J and Depass L (1996) Chiral kinetics and dynamics of ketorolac. *J Clin Pharmacol* **36:**521–539.

Muchowski JM, Unger SH, Ackrell J, Cheung P, Cooper GF, Cook J, Gallegra P, Halpern O, Koehler R, Kluge AF, Van Horn AR, Carpio YAH, Franco F, Galeazzi E, Garcia I, Greenhouse R, Guzman A, Iriarte J, Leon A, Pena A, Perez V, Valdez D, Ackerman N, Ballaron SA, Krishna-Murthy DV, Rovito JR, Tomolonis AJ, Young JM and Rooks WH (1985) Synthesis and anti-inflammatory and analgesic activity of 5-aroyl-1,2-dihydro-[^{3}H]-pyrrolo[1,2-a]pyrrole-1-carboxylic acids and related compounds. *J Med Chem* **28:**1037–1049.

Nathan C (1992) Nitric oxide as a secretory product of mammalian cells. *FASEB J* **6:**3051–3064.

O'Hara DA, Fragen RJ, Kinzer M and Pemberton D (1987) Ketorolac tromethamine as compared with morphine sulfate for treatment of postoperative pain. *Clin Pharmacol Ther* **41:**556–561.

Olkkola KT and Maunuksela EL (1991) The pharmacokinetics of postoperative intravenous ketorolac tromethamine in children. *Br J Clin Pharmacol* **31:**182–184.

Olsen RW, Szamraj O and Houser CR (1987) Tritiated AMPA binding to glutamate receptor subpopulations in rat brain. *Brain Res* **402:**243–254.

Pallapies D, Salinger A, Meyer Zum Gottesberge A, Atkins DJ, Rohleder G, Nagyivanyi P and Peskar BA (1995) Effects of lysine clonixinate and ketorolac tromethamine on prostanoid release from various rat organs incubated ex vivo. *Life Sci* **57:**83–89.

Pardridge, WM (1991) Overview of blood-brain barrier transport biology and experimental methodologies, in *Peptide Drug Delivery to the Brain* (Pardridge WM ed) pp 52–98, Raven Press, New York.

Parnham MJ (1993) Inflammation '93. *Drug News and Perspect* **6:**737–742.

Parris WC, Janicki PK, Johnson B Jr and Horn JL (1996) Intrathecal ketorolac tromethamine produces analgesia after chronic constriction injury of sciatic nerve in rat. *Can J Anaesth* **43:**867–870.

Pasternak GW, Wilson HA and Snyder SH (1975) Differential effects of protein-modifying reagents on receptor binding of opiate agonists and antagonists. *Mol Pharmacol* **11:**340–351.

Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, Anderson GD, Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen AW, Zhang YY and Isakson PC (1997) Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: Identification

of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide (SC-58635, celecoxib). *J Med Chem* **40:**1347–1365.
Physicians' Desk Reference (1995a) 49th ed., pp 1061–1064, Medical Economics Data Production Co., Montvale, NJ.
Physicians' Desk Reference (1995b) 49th ed., pp 2492–2496, Medical Economics Data Production Co., Montvale, NJ.
Physicians' Desk Reference (1995c) 49th ed., pp 1566–1570, Medical Economics Data Production Co., Montvale, NJ.
Pinkus LM, Sarbin NS, Barefoot DS and Gordon JC (1989) Association of [^{3}H] zacopride with 5-HT$_3$ binding sites. *Eur J Pharmacol* **168:**355–362.
Ramesha CS (1996) Human and rat cyclooxygenases are pharmacologically distinct, in *Eicosaniods and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury* (Honn K, Marnett LJ, Nigam S, Jones RL and Wong PY-K eds) pp 67–71, Plenum Press, New York.
Randall LW and Selitto JJ (1957) A method for measurement of analgesic activity on inflamed tissue. *Arch Int Pharmacodyn* **111:**409–419.
Rooks WH, Tomolonis AJ, Maloney PJ, Wallach MB and Schuler ME (1982) The analgesic and anti-inflammatory profile of (±)-5-benzoyl-1, 2-dihydro-[^{3}H]-pyrrolo[1,2a]pyrrole-1-carboxylic acid (RS-37619). *Agents Actions* **12:**684–690.
Rooks WH, Maloney PJ, Shott LD, Schuler ME, Sevelius H, Strosberg AM, Tanenbaum L, Tomolonis AJ, Wallach MB, Waterbury D and Yee JP (1985) The analgesic and anti-inflammatory profile of ketorolac and its tromethamine salt. *Drugs Exp Clin Res* **11:**479–492.
Sallmann A (1979) The chemistry of diclofenac sodium (Voltarol). *Rheumatol Rehabil* **(Suppl) 2:**4–10.
SAS Institute Inc. (1989) *SAS/STAT User's Guide*, Version 6, 4th ed., Volume 2, SAS Institute Inc., Cary, NC.
Schoemaker H and Langer SZ (1985) [^{3}H] diltiazem binding to calcium channel antagonists recognition sites in rat cerebral cortex. *Eur J Pharmacol* **111:**273–277.
Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L and Isakson P (1994) Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci USA* **91:**12013–12017.
Servin AL, Amiranoff B, Rouyer-Fessard C, Tatemoto K and Laburthe M (1987) Identification and molecular characterization of galanin receptor sites in rat brain. *Biochem Biophys Res Commun* **144:**298–306.
Shelkh SP, O'Hare MM, Tortroa O and Schwartz TW (1989) Binding of monoiodinated neuropeptide Y to hippocampal membranes and human neuroblastoma cell line. *J Biol Chem* **246:**6648–6654.
Snell LD, Morter RS and Johnson KM (1988) Structural requirements for activation of the glycine receptor that modulates the *N*-methyl-D-aspartate operated ion channel. *Eur J Pharmacol* **156:**105–110.
Stanski DR, Cherry C, Bradley R, Sarnquist FH and Yee JP (1990) Efficacy and safety of single doses of intramuscular ketorolac tromethamine compared with meperidine for postoperative pain. *Pharmacotherapy* **10:**S40–S44.
Tripathi A and Welch SP (1995) Blockade of the antinociceptive activity of centrally administered ketorolac by nor-binaltorphimine. *Eur J Pharmacol* **278:**27–32.
U'Prichard DC, Bylund DB and Snyder SH (1978) (+)-[^{3}H] epinephrine and (−)-dihydroalprenolol binding to b$_1$ and b$_2$-noradrenergic receptors in brain, heart, and lung membranes. *J Biol Chem* **253:**5090–5012.
Uphouse LA, Welch SP, Ward CR, Ellis EF and Embrey JP (1993) Antinociceptive activity of intrathecal ketorolac is blocked by the kappa-opioid receptor antagonist, nor-binaltorphimine. *Eur J Pharmacol* **242:**53–58.
Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* **231:**232–235.
Vinegar R, Truax JF and Selph JL (1976) Quantitative comparison of the analgesic and anti-inflammatory activities of aspirin, phenacetin and acetaminophen in rodents. *Eur J Pharmacol* **37:**23–30.
Weber E, Sonders M, Quarum M, McLean S, Pou S and Keana JFW (1986) 1,3-Di-(2-[5-^{3}H] tolyl)-guanidine: A selective ligand that labels sigma-type receptors for psychotomimetic opiates and antipsychotic drugs. *Proc Natl Acad Sci USA* **83:** 8784–8788.
Willingdale HL, Gardiner NJ, McLymont N, Giblett S and Grubb BD (1997) Prostanoids synthesized by cyclo-oxygenase isoforms in rat spinal cord and their contribution to the development of neuronal hyperexcitability. *Br J Pharmacol* **122:** 1593–1604.
Yee JP, Koshiver JE, Allbon C and Brown CR (1986) Comparison of intramuscular ketorolac tromethamine and morphine sulfate for analgesia of pain after major surgery. *Pharmacotherapy* **6:**253–261.
Yoshizaki H, Takamiya M and Okada T (1987) Characterization of picomolar affinity binding sites for [^{125}] human calcitonin gene-related peptide in rat brain and heart. *Biochem Biophys Res Commun* **146:**443–451.
Young A and Snyder S (1974) Strychnine binding in rat spinal cord membranes associated with the synaptic glycine receptor cooperativity of glycine interactions. *Mol Pharmacol* **10:**790–809.
Young JM and Yee JP (1994) Ketorolac, in *Nonsteroidal Anti-Inflammatory Drugs* (Lewis AJ and Furst DE eds) pp. 247–266, Marcel Dekker, Inc., New York.
Zhang Y, Shaffer A, Portanova J, Seibert K and Isakson PC (1997) Inhibition of cyclooxygenase-2 rapidly reverses inflammatory hyperalgesia and prostaglandin E$_2$ production. *J Pharmacol Exp Ther* **283:**1069–1075.

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