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

MARY-FRANCES JETT, CHAKK S. RAMESHA, CARL D. BROWN, SOPHIE CHIU, CAROLINE EMMETT, TATYANA VORONIN, THOMAS SUN, COUNDE O’YANG, JOHN C. HUNTER, RICHARD M. EGLEN, and RANDOLPH M. JOHNSON

Center for Biological Research (M.-F.J., C.E., J.C.H., R.M.E., R.M.J.), Preclinical Research and Development (C.D.B., T.S.), Medicinal Chemistry (C.O.), Radiochemistry Group (T.V.), and Central R & D (S.C.) in the Neurobiology Unit or Inflammatory Disease Unit (C.S.R.) of Roche Bioscience, Palo Alto, California

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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.

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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 (Mróz-Szczak 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 the possible mechanism(s) underlying its analgesic efficacy.

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 efficiency.

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. [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, MD). (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%) and administered 50 μl of vehicle or carrageenan (0.5% in saline) s.c. on the plantar surface of the left hindpaw (Vinog et al., 1976). Three hours later, the rats were euthanized and the difference in the weight 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 × 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 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/acetate 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). Non-specific binding was estimated using an excess of unlabeled, receptor-selective ligands. Details of the specific binding assays are described in the appendices: adenosine A1 (Lohse et al., 1987), A2 (Jarvis et al., 1989); α1-adrenergic (Greengrass and Bremner, 1979); α2-adrenergic (Boyaajian and Leslie, 1987); β-adrenergic (UPrichard et al., 1978); calcitonin gene-related peptide (Yoshizaki et al., 1987); γ-aminobutyric acid, benzodiazepine site (Damm et al., 1978); galanin (Servin et al., 1987); glutamate-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (Olsen et al., 1987); glutamate-NMDA, glycine site (Snell et al., 1987); glutamate-NMDA, phencyclidine site (Goldman et al., 1982); 5-HT3 (Pinkus et al., 1989); sigma (Weber et al., 1986) and the cyclooxygenase activity was measured following sigmoidal model was used: % Inhibition = MAX/1 + (dose/ ID_{50})^{P}, 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 ± 0.009 and 1.65 ± 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 ± 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:

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

**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 ± 0.83, 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 anti-inflammatory than as an antinociceptive or an antihyperalgesic agent; ID₅₀ 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 isomers 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 IC₅₀ 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 IC₅₀ value of 0.10 ± 0.08 μM was approx-

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**TABLE 1**

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Test of Nociception ID₅₀</th>
<th>Test of Hyperalgesia ID₅₀</th>
<th>Test of Edema Formation ID₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>mg/kg</td>
</tr>
<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)</td>
</tr>
<tr>
<td>(S)-ketorolac</td>
<td>s.c.</td>
<td>0.06 (0.04, 0.07)</td>
<td>0.07 (0.04, 0.09)</td>
<td>0.02 (0.01, 0.03)</td>
</tr>
<tr>
<td>(R)-ketorolac</td>
<td>s.c.</td>
<td>0.01 (0.01, 0.01)</td>
<td>0.02 (0.01, 0.02)</td>
<td>0.02 (0.01, 0.03)</td>
</tr>
<tr>
<td>DS</td>
<td>s.c.</td>
<td>4.40 (1.62, 7.17)</td>
<td>4.40 (1.62, 7.17)</td>
<td>0.27 (0.17, 0.37)</td>
</tr>
<tr>
<td>INDO</td>
<td>s.c.</td>
<td>1.10 (0.60, 1.60)</td>
<td>2.6 (2.1, 3.1)</td>
<td>0.46 (0.16, 0.76)</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>i.v.</td>
<td>2.8 (1.7, 3.8)</td>
<td>7.5 (4.0, 11.0)</td>
<td>8.4 (4.9, 11.9)</td>
</tr>
</tbody>
</table>

* Estimation of ID₅₀ values assumes a maximum response of 50 to 60% inhibition.
* Significantly different from values in the test of nociception (p < .05).
* Significantly different from (R,S)-ketorolac (p < .05).
A coefficient was calculated for each comparison. DS (100 mg/kg p.o.) were also ineffective. In contrast, mor-

approximately twice as potent as the racemate, whereas the (S)-ketorolac had nearly maximally effective dose of (R,S)-ketorolac (1 mg/kg i.v.) was 100-fold greater than its ID_{50} value (i.e., 1292 Jett et al.). The dis-

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.

dictively 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 carbacholstacyclin-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 (PGL_2) binds selectively] on visceral afferent fibers stimulating a nociceptive response. The dose of carbacholstacyclin (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 carbacholstacyclin 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, mor-

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, [^{3}H](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, [^{3}H](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...
Under these conditions, the respective distribution coefficients of the three compounds were evaluated in a 1-octanol/water system (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 con-
TABLE 3

Binding profile of (S)-ketorolac

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Selective Ligand</th>
<th>Tissue</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A₁</td>
<td>[H]DPCPX</td>
<td>Brain</td>
<td>-11</td>
</tr>
<tr>
<td>Adenosine A₂A</td>
<td>[H]CGS-21680</td>
<td>Striatum</td>
<td>4</td>
</tr>
<tr>
<td>Adrenergic α₁ (nonselective)</td>
<td>[H]Prazosin</td>
<td>Brain¹</td>
<td>-17</td>
</tr>
<tr>
<td>Adrenergic α₂ (nonselective)</td>
<td>[H]RAuwolscine</td>
<td>Cortex¹</td>
<td>-7</td>
</tr>
<tr>
<td>Adrenergic β (nonselective)</td>
<td>[H]DHH</td>
<td>Brain</td>
<td>2</td>
</tr>
<tr>
<td>CGRP</td>
<td>[¹¹¹]I CGRP</td>
<td>Brain</td>
<td>-4</td>
</tr>
<tr>
<td>Type L calcium channel (benzothiazepine site)</td>
<td>[H]Diltiazem</td>
<td>Cortex¹</td>
<td>16</td>
</tr>
<tr>
<td>Type L calcium channel (dihydropyridine site)</td>
<td>[H]Nitrendipine</td>
<td>Cortex¹</td>
<td>27</td>
</tr>
<tr>
<td>Type N calcium channel</td>
<td>[¹¹¹]Iα-Conotoxin</td>
<td>Frontal lobe²</td>
<td>6</td>
</tr>
<tr>
<td>γ-aminobutyric acid (benzodiazepine site)</td>
<td>[H]Flunitrazepam</td>
<td>Brain¹</td>
<td>6</td>
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<tr>
<td>Galanin</td>
<td>[¹¹¹]IGalanin</td>
<td>Brain</td>
<td>14</td>
</tr>
<tr>
<td>Glutamate-AMPA</td>
<td>[H]AMPA</td>
<td>Cortex¹</td>
<td>2</td>
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<td>Glutamate-kainate</td>
<td>[H]Kainate</td>
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<td>Glutamate-NMDA (agonist site)</td>
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<td>Cortex¹</td>
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<tr>
<td>Glutamate-NMDA (glycine site)</td>
<td>[H]Glycine</td>
<td>Cortex¹</td>
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<td>Glutamate-NMDA (phencyclidine site)</td>
<td>[H]TCP</td>
<td>Cortex¹</td>
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<tr>
<td>Glycine</td>
<td>[H]Strychnine</td>
<td>Spinal cord</td>
<td>19</td>
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<tr>
<td>Histamine H₁</td>
<td>[H]Pyrilamine</td>
<td>Brain¹</td>
<td>-2</td>
</tr>
<tr>
<td>Histamine H₃</td>
<td>[H]NAMH</td>
<td>Brain</td>
<td>21</td>
</tr>
<tr>
<td>Muscarinic (nonselective)</td>
<td>QNB</td>
<td>Cortex¹</td>
<td>2</td>
</tr>
<tr>
<td>Neurokinin NK₁</td>
<td>[H]Substance P</td>
<td>Submaxillary glands³</td>
<td>-16</td>
</tr>
<tr>
<td>Neuropeptide Y₁</td>
<td>[¹¹¹]IYF</td>
<td>SK-N-MC cells¹</td>
<td>8</td>
</tr>
<tr>
<td>Opiate (nonselective)</td>
<td>Naloxone</td>
<td>Brain¹</td>
<td>6</td>
</tr>
<tr>
<td>5-HT₁</td>
<td>[H]5-HT</td>
<td>Cortex¹</td>
<td>-12</td>
</tr>
<tr>
<td>5-HT₁A</td>
<td>[H]8-OH-DPAT</td>
<td>Cortex¹</td>
<td>-7</td>
</tr>
<tr>
<td>5-HT₂</td>
<td>[H]Reticulin</td>
<td>Brain¹</td>
<td>16</td>
</tr>
<tr>
<td>Sigma (nonselective)</td>
<td>[H]5-6530</td>
<td>Real muscularis⁶</td>
<td>5</td>
</tr>
<tr>
<td>Sodium channel (site 2)</td>
<td>[H]BDTG</td>
<td>Brain¹</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>[H]IBTX</td>
<td>Brain¹</td>
<td>3</td>
</tr>
</tbody>
</table>

AMPA (2)-α-aminobutyric acid 4-propionic acid

¹ Average of two determinations.
² Except cerebellum
³ From brain
⁴ Guinea pig
⁵ Human
⁶ 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.

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-
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 properties 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).

<table>
<thead>
<tr>
<th>Compound</th>
<th>pK(_a) (6)</th>
<th>(\log P^d)</th>
<th>(\log P^b)</th>
<th>(D^b/(\text{pH} 7.4))</th>
<th>Ratio(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>((R,S))-ketorolac</td>
<td>3.5</td>
<td>2.74 ± 0.02</td>
<td>-0.52 ± 0.07</td>
<td>0.40</td>
<td>1</td>
</tr>
<tr>
<td>DS</td>
<td>4.0</td>
<td>4.33 ± 0.01</td>
<td>0.56 ± 0.02</td>
<td>14.1</td>
<td>35</td>
</tr>
<tr>
<td>IND</td>
<td>4.2</td>
<td>4.08 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>12.3</td>
<td>30</td>
</tr>
</tbody>
</table>

\(^a\) pK\(_a\) values for \((R,S)\)-ketorolac (Muchowski et al., 1985), DS, and INDO (Sallmann, 1979) were obtained from previously published reports.

\(^b\) \(\log P^d\), partition coefficient of the neutral species.

\(^c\) \(\log P^b\), partition coefficient of the ionized species.

\(^d\) \(D^b\), Distribution coefficient determined at pH 7.4.

\(^c\) Ratio: \(\frac{1/D(K\text{etorolac})}{x}\) 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


Randall LW and Selitto JJ (1957) A method for measurement of analgesic activity on


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Send reprint requests to: Mary Frances Jett, Ph.D., Department of Analgesia, Center for Biological Research, Neurobiology Unit, Roche Bioscience (R2-101), 3401 Hillview Ave., Palo Alto, CA 94304. E-mail: mary-frances.jett@roche.com

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