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

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

After the introduction of Toradol, evidence accumulated suggesting that the analgesic and anti-inflammatory activities of certain NSAIDs (Brune et al., 1991), specifically (R,S)-ketorolac (McCormack and 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 k 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-

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.

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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 (Pening et al., 1997) were synthesized in the Institute of Organic Chemistry, Roche Biosciences (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 Biochemistry 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 Biosciences Institutional Animal Care and Use Committee.

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

Carrageenan-Induced Edema Formation. Rats (130–140 g) were randomly 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 the plantar surface of the left hindpaw, essentially, as described earlier (Vinage et al., 1976). Three hours later, the rats were euthanized and the difference in the weight (g) of the treated and untreated hindpaws was recorded as an expression of edema formation (g).

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

Pharmacokinetics Analysis of (R,S)-Ketorolac

Rats were anesthetized with 5% halothane and administered [3H](R,S)-ketorolac (100 nmol; 0.495 mCi/mmol) by i.c.v. injection. At 0, 5, 20, 35, 60, and 180 min after injection, the rats were anesthetized with CO2/O2 (60:40%); blood was withdrawn into a heparinized syringe and a plasma fraction was obtained by centrifugation of the blood at 2600g for 5 min in a clinical centrifuge. To determine the total [3H](R,S)-ketorolac in each sample, aliquots of plasma were subjected to scintillation spectroscopy and HPLC. Protein was removed from the samples by precipitation with 1 volume of acetonitrile and centrifugation (500g, 15 min). The resulting supernatant was applied to a BDS-Hypersil-C18 (4.6 × 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/min 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 were then applied to a 1 mL C18 Sep-pak column that had previously been equilibrated with 0.15 M KCl, using a SIRIUS PCA 101 (SIRIUS Analytical Instrumentally in a 1-octanol/water system at 25°C and an ionic strength of 1000 mOsm/l.

Putative inhibitors of COX were tested for their ability to interact with COX using a radiometric assay (Barnett et al., 1994). The assay involved the measurement of cyclooxygenase activity using the following equation:

\[ D = (P[H^+] + pK_a^s)/([H^+] + K_a) \]

**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})^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).

**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/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; 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 isofoms of COX in recombiant 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-

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

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

**Fig. 2.** Effect of (S)-ketorolac (0.01–1 mg/kg s.c.) and INDO (0.3–6 mg/kg s.c.) on carrageenan-induced paw hyperalgesia (A) and carrageenan-induced paw edema formation (B). Each point represents the mean ± S.E. percentage of inhibition; n = 10 to 18 rats/group.
phrine sulfate (6 mg/kg p.o.) completely blocked the carba-
prostacyclin-induced writhing response.

Central Actions of Ketorolac. To investigate the possi-
bility 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 prepara-
tion 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 constitut-
ive or inducible isoforms of NO at 10 μM synthase (2 and
−19% inhibition, respectively), phospholipase A₂ at 300 μM
(4% inhibition) or protein kinase C at 100 μM (−6% inhibi-
tion).

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 as-
sumed. (S)-ketorolac (1–100 nmol i.c.v.) significantly inhib-
ited the acid-induced writhing with an ID₅₀ 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 cen-
tral 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 periph-
eral blood was measured using both radiometric and HPLC
methods of quantification. As seen in Fig. 5, [³H](R,S)-ketor-
olac entered the peripheral circulation 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. admin-
istration via a chronically implanted cannula was virtually
indistinguishable from that described in Fig. 5 (data not
shown).

To further explore the possibility that peripherally admin-
istered (R,S)-ketorolac could exert its analgesic actions via
a central mechanism, the effect of peripherally administered
(S)-ketorolac on mononeuropathy-induced cold allodynia (Go-
gas 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 cen-
trally acting antiepileptic drug gabapentin (30, 100, and 300
mg/kg p.o.) effectively reversed the allodynia.

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

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
hyperalgiesia (B; see Table 1 for the ID₅₀ values). A Pearson correlation
coefficient was calculated for each comparison.
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

<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>H₁DPCPX</td>
<td>Brain</td>
<td>−11</td>
</tr>
<tr>
<td>Adenosine A₂B</td>
<td>H₁CGS-21680</td>
<td>Striatum</td>
<td>4</td>
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<tr>
<td>Adrenergic α₁ (nonselective)</td>
<td>H₁Prazosin</td>
<td>Brain</td>
<td>−17</td>
</tr>
<tr>
<td>Adrenergic α₂ (nonselective)</td>
<td>H₁Rauwolscine</td>
<td>Cortex</td>
<td>−7</td>
</tr>
<tr>
<td>Adrenergic β (nonselective)</td>
<td>H₁DHIA</td>
<td>Brain</td>
<td>2</td>
</tr>
<tr>
<td>CGRP</td>
<td>[125I]CGRP</td>
<td>Brain</td>
<td>−4</td>
</tr>
<tr>
<td>Type L calcium channel (benzothiazepine site)</td>
<td>H₁Diltiazem</td>
<td>Cortex</td>
<td>16</td>
</tr>
<tr>
<td>Type L calcium channel (dihydropyridine site)</td>
<td>H₁Nitrendipine</td>
<td>Cortex</td>
<td>27</td>
</tr>
<tr>
<td>Type N calcium channel</td>
<td>γ-aminobutyric acid (benzodiazepine site)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galanin</td>
<td>H₁Flunitrazepam</td>
<td>Brain</td>
<td>6</td>
</tr>
<tr>
<td>Glutamate-AMPA</td>
<td>H₁AMPA</td>
<td>Cortex</td>
<td>2</td>
</tr>
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<td>Glutamate-kainate</td>
<td>H₁Kainate</td>
<td>Brain</td>
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<td>H₁Pyrilamine</td>
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<td>−2</td>
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<tr>
<td>Histamine H₂</td>
<td>H₁NAMH</td>
<td>Brain</td>
<td>21</td>
</tr>
<tr>
<td>Mucarinc (nonselective)</td>
<td>QNB</td>
<td>Cortex</td>
<td>2</td>
</tr>
<tr>
<td>Muscarinic (nonselective)</td>
<td>H₁Substance P</td>
<td>Submaxillary glands</td>
<td>−16</td>
</tr>
<tr>
<td>Neurokinin NK₁</td>
<td>H₁FPUT</td>
<td>SK-N-MC cells</td>
<td>8</td>
</tr>
<tr>
<td>Neuropeptide Y₁</td>
<td>[125I]PYY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiate (nonselective)</td>
<td>Naloxone</td>
<td>Brain</td>
<td>6</td>
</tr>
<tr>
<td>5-HT₁</td>
<td>H₁5-HT</td>
<td>Cortex</td>
<td>−12</td>
</tr>
<tr>
<td>5-HT₁A</td>
<td>H₁8-OH-DPAT</td>
<td>Cortex</td>
<td>−7</td>
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<tr>
<td>5-HT₁B</td>
<td>H₁Retanserin</td>
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<td>16</td>
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<tr>
<td>5-HT₂</td>
<td>H₁GR-65630</td>
<td>Real muscularis</td>
<td>5</td>
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<tr>
<td>Sigma (nonselective)</td>
<td>H₁DTG</td>
<td>Brain</td>
<td>11</td>
</tr>
<tr>
<td>Sodium channel (site 2)</td>
<td>H₁BTX</td>
<td>Brain</td>
<td>3</td>
</tr>
</tbody>
</table>

*AMP A (±)-3-hydroxy-5-methylisoxazole-4-propionic acid
†Averaged from two determinations.
‡Except cerebellum
§From brain
¶Guinea pig
‖Human
¶¶Rabbit

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

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 (R,S)-ketorolac depends on whether the drug is administered prophylactically or therapeutically.

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-
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

| Compound        | pK_a | logP | logP | D^2 (pH 7.4) | Ratio
|-----------------|------|------|------|-------------|-------
| (R,S)-ketorolac | 3.5  | 2.74 ± 0.02 | -0.52 ± 0.07 | 0.40 | 1
| DS              | 4.0  | 4.33 ± 0.01 | 0.56 ± 0.02 | 14.1 | 35
| IND             | 4.2  | 4.08 ± 0.01 | 0.47 ± 0.02 | 12.3 | 30

*pK_a* values for (R,S)-ketorolac (Muchowski et al., 1985), DS, and INDO (Sallmann, 1979) were obtained from previously published reports.

$\log P$, partition coefficient of the neutral species.

$\log P^i$, partition coefficient of the ionized species.

$D^2$, Distribution coefficient determined at pH 7.4

$\text{Ratio} = \log_{10}(\text{D} / \text{D}_{\text{R,S-ketorolac}})$ where x is INDO or DS, respectively.
suggest that peripherally administered (R,S)-ketorolac acts peripherally to produce its analgesic actions.

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