The Novel, Orally Active, Delta Opioid RWJ-394674 Is Biotransformed to the Potent Mu Opioid RWJ-413216


Research and Early Development, Johnson & Johnson Pharmaceutical Research and Development, Spring House, Pennsylvania

ABSTRACT

Although the mu opioid receptor is the primary target of marketed opioid analgesics, several studies suggest the advantageous effect of combinations of mu and delta opioids. The novel compound RWJ-394674 [N,N-diethyl-4-[(8-phenethyl-8-azabicyclo[3.2.1]oct-3-ylidene)-phenylmethyl]-benzamide] bound with high affinity to the delta opioid receptor (0.2 nM) and with weaker affinity to the mu opioid receptor (72 nM). 5′-O-[(3-[35S]thio)triphosphate binding assay demonstrated its delta agonist function. Surprisingly given this pharmacologic profile, RWJ-394674 exhibited potent oral antinociception (ED50 = 10.5 μmol/kg or 5 mg/kg) in the mouse hot-plate (48°C) test and produced a moderate Straub tail. Antagonist studies in the more stringent 55°C hot-plate test demonstrated the antinociception produced by RWJ-394674 to be sensitive to the nonselective opioid antagonist naloxone as well as to the delta- and mu-selective antagonists, naltrindole and β-funaltrexamine, respectively. In vitro studies demonstrated that RWJ-394674 was metabolized by hepatic microsomes to its N-desethyl analog, RWJ-413216 [N-ethyl-4-[(8-phenethyl-8-azabicyclo[3.2.1]oct-3-ylidene)-phenylmethyl]-benzamide], which, in contrast to RWJ-394674, had a high affinity for the mu rather than the delta opioid receptor and was an agonist at both. Pharmacokinetic studies in the rat revealed that oral administration of RWJ-394674 rapidly gave rise to detectable plasma levels of RWJ-413216, which reached levels equivalent to those of RWJ-394674 by 1 h. RWJ-413216 itself demonstrated a potent oral antinociceptive effect. Thus, RWJ-394674 is a delta opioid receptor agonist that appears to augment its antinociceptive effect through biotransformation to a novel mu opioid receptor-selective agonist.

Morphine’s widespread use as an analgesic long preceded the discovery of peptidic mu-selective ligands, whereas small-molecule delta opioids are of more recent discovery than their peptidic counterparts. BW373U86, the first potent, nonpeptide delta agonist, pioneered the design of new small-molecule delta opioids (Bernard et al., 2003), such as SNC-80 (Bilsky et al., 1995), SB219825 (Dondio et al., 2003), all of which make use of this structural feature.

The present work describes the pharmacology of a tropanyl-dine-containing, tricyclic, delta opioid receptor-selective ligand, RWJ-394674. Biotransformation of the compound to the monodesethyl analog, RWJ-413216, revealed a novel antinociceptive agent with a mu opioid receptor-selective pharmacology.

Materials and Methods

Chemicals. The radiochemicals [D-Ala2,Nme-Phe4,Gly-ol5]-enkephalin ([3H]DAMGO), [D-Pen2,D-Pen5]-enkephalin ([3H]DPDPE), [35S]GTPγS, 5′-O-[(3-[35S]thiotriphosphate; pFNA, β-funaltrexamine; DOR, delta opioid receptor; MPE, maximum possible effect; MS, mass spectrometry; MS/MS, tandem mass spectrometry; TAN-67, (4aS,12aR)-4a-(3-hydroxyphenyl)-2-methyl-1,2,3,4,4a,5,12,12a-octahydroprido[3,4-b]cinnoline; i.v., intravenous; s.c., subcutaneous; i.p., intraperitoneal; SB219825, (−)-(4aS,8aR)-trans-2-[diethylamino)carbonyl]-6-ethyl-8a-(3-hydroxyphenyl)-3-methyl-4,4a, 5,6,7,8,9-octahydro-1H-pyrrolo[2,3-g]isoquinoline; AR-M390, N,N-diethyl-4-[(8-phenethyl-8-azabicyclo[3.2.1]oct-3-ylidene)-phenylmethyl]-benzamide; RWJ-394674, N,N-diethyl-4-[(8-phenethyl-8-azabicyclo[3.2.1]oct-3-ylidene)-phenylmethyl]-benzamide; RWJ-413216, N-ethyl-4-[(8-phenethyl-8-azabicyclo[3.2.1]oct-3-ylidene)-phenylmethyl]-benzamide.
and 5'-O-[3-[35S]thio)triphosphate ([35S]GTPyS) were purchased from Perkin Elmer (Boston, MA), and the neurochemicals [DMG], DPDP, SNC-80, β-funaltrexamine (βFNA), naloxone, naltrindole, morphine, and GDP were obtained from Sigma-Aldrich (St. Louis, MO) or Toecis (Ellisville, MO).

**Animals.** Mice and rats used in these studies were obtained from Charles River (Kingston, NY) and housed according to the recommendations in the National Research Council’s Guide for the Care and Use of Laboratory Animals. The protocols used, explained in the several sections below, were reviewed and approved by the Institutional Animal Care and Use Committee of J&J PRD.

In Vitro Opioid Receptor Binding and Functional Assays

**Delta and Mu Opioid Receptor Binding Assays.** Male Wistar rats (150–250 g, virus- and antibody-free; Charles River) were euthanized by cervical dislocation, and their brains were removed and placed immediately in 50 mM ice-cold Tris HCl buffer, pH 7.4. The forebrains were separated from the remainder of the brain by a coronal transection, beginning dorsally at the colliculi and passing ventrally through the midbrain-pontine junction. After dissection, the forebrains were homogenized in Tris buffer in a Teflon-glass homogenizer. The homogenate was diluted to a concentration of 1 g of forebrain tissue per 80 ml of Tris and centrifuged at 39,000 g for 10 min. The pellet was resuspended in the same volume of Tris buffer containing 5 mM MgCl2 with several brief pulses from a Polytron homogenizer. This particulate preparation was used for the delta and mu opioid binding assays, at a concentration of 5 mg/ml. Following incubation with either the delta-selective ligand [3H]DPDP [Kd = 5.4 nM; ~4 nM used in assay] or the mu-selective ligand [3H]DMG [Kd = 1.75 nM; ~0.8 nM used in assay] at 25°C for 2.5 h in a 96-well plate in a total volume of 1 ml, the plate contents were filtered through Wallac filter mat B sheets (GE Healthcare, Little Chalfont, Buckinghamshire, UK) on a Tomtec 96-well harvester (Tomtec, Orange, CT). The filters were rinsed three times with 2 ml of filtered TRIS-HCl, pH 7.2, 2 mM EDTA, and 10% sucrose. Membranes were purchased from Receptor Biology, Inc. (Baltimore, MD), and 10 mg/ml membrane protein was suspended in 10 mM TRIS-HCl, pH 7.2, 2 mM EDTA, and 10% sucrose. Membranes were maintained at 4 to 8°C. One milliliter of membranes was added into 15 ml of cold assay buffer (50 mM HEPES, pH 7.6, 5 mM MgCl2, 100 mM NaCl, 1 mM dithiothreitol, and 1 mM EDTA). The membrane suspension was homogenized with a Polytron and centrifuged at 3000 rpm for 10 min. The supernatant was then centrifuged at 18,000 rpm for 20 min. The pellet was resuspended in 10 ml of assay buffer and mixed with a Polytron.

The membranes (20 μg/ml) were preincubated with scintillation proximity assay beads (10 mg/ml) at 25°C for 45 min in the assay buffer, and the membrane-coupled scintillation proximity assay beads (5 mg/ml) were then incubated with 0.5 nM [35S]GTPyS in HEPES buffer containing 50 μM GDP in a total volume of 200 μl. A range of concentrations of receptor agonists was used to stimulate [35S]GTPyS binding. Basal binding was tested in the absence of agonists, and nonspecific binding was measured in the presence of 10 μM unlabeled GTPyS. Radioactivity was evaluated on a Packard Top Count, and Ki values were calculated using GraphPad PRISM (GraphPad Software Inc., San Diego, CA). The relative efficacy of delta agonists was based on the stimulation of GTPyS incorporation by SNC-80, whereas the relative efficacy of mu opioid agonists was based on the stimulation of GTPyS incorporation by DMG.

**Antinociceptive Tests.**

**Animals and Drug Preparation.** Male Crl:CD-1 (Charles River) mice, weighing 18 to 24 g, and male rats (Sprague-Dawley), weighing 80 to 120 g, were housed 5 to 10 per container in a climate-controlled, virus-free environment for at least 5 days prior to testing. Food and water were available during this time. The animals were individually weighed and allowed to acclimate to conditions before testing. Test drug was dissolved in sterile water (vehicle) and administered in a volume of 10 ml/kg (mouse) or 2 ml/kg (rats).

**Mouse Abdominal Irritant Test.** The procedure used was that described by (Collier et al., 1968), with minor modifications. Thirty minutes after the administration of test drug, the animals received an i.p. injection of 5.5 mg/kg acetylcholine bromide. The mice were then placed into large glass animal jars and were continuously observed for the first occurrence of a characteristic behavioral response (twisting and elongation of the body that extends throughout the hind limbs) within the specified observation period of 10 min. The percentage of inhibition of this response was calculated as follows:

\[
\text{Percent inhibition} = 100 \times \frac{\text{nonresponders}/(\text{number of animals in group})}{\text{test latency} - \text{cutoff time} - \text{predrug latency}}
\]

The estimated ED50 value (the dose of agonist calculated to produce 50% inhibition) was determined using a probit analysis (Litchfield and Wilcoxon, 1949).

**Mouse and Rat Hot-Plate Tests.** The hot-plate test was that described previously (Eddy and Leimbach, 1953; O’Callaghan and Holtzman, 1975), with minor modifications. Animals were placed on a heated surface (mice, 48 or 55°C; rats, 51°C), and the time interval (seconds) between placement and a shaking, licking, or tucking of the hind paw was recorded as the predrug latency response. This same procedure was repeated at specified times after the oral administration of drug. Cutoff times, designed to prevent injury to the animal, were 60 s for the 55°C test (with control latencies of approximately 10–20 s) and 90 s for the 48 and 51°C tests (with control latencies of approximately 25–40 s). The percent maximum possible antinociceptive effect (MPE) was determined using the formula:

\[
\text{Percent MPE} = 100 \times \frac{\text{test latency}}{\text{predrug latency} - \text{cutoff time} - \text{predrug latency}}
\]

using the predrug latency of each animal and cutoff times noted above. The ED50 values were determined using a computer-assisted linear regression analysis of the dose-response curve, including an analysis of variance test for linearity.

**Antagonist Study in the Mouse 55°C Hot-Plate Test.** The opioid antagonist naloxone, δ-selective antagonist naltrindole, and irreversible μ-selective antagonist βFNA were used to probe the receptor pharmacology mediating the antinociceptive effect of RWJ-394674 in the mouse 55°C hot-plate test. Morphine-induced antinociception was studied in parallel. The antinociceptive agents were used at doses near their 50% effect level, 300 μmol/kg for morphine and 60 μmol/kg for RWJ-394674, both administered orally. The antagonists were administered at doses and times determined in preliminary studies to be appropriate: naloxone, 1 and 10 mg/kg s.c., 20 min prior to drug; naltrindole, 1 and 10 mg/kg s.c., 30 min prior to drug; and βFNA, 30 and 60 mg/kg s.c., 24 h prior to drug administration. Antinociception was assessed 30 min after the administration of morphine or RWJ-394674.

**Metabolic Profile.**

**In Vitro Metabolism.** RWJ-394674 hydrochloride was incubated at a concentration of 10 μg/ml with male CD-1 mouse, male Sprague-Dawley rat, and human (male and female) pooled hepatic S9 fractions. Concurrent incubations took place for 90 min in a Dubnoff Metabolic Shaker Incubator at 37°C with an NADPH-generating system.
system using freshly made ingredients (1.15% KCl in 0.05 M Tris buffer, pH 7.4, 5 mM MgCl₂, 0.5 mM NADP, and 5 mM glucose-6-phosphate). Control samples containing each drug but no hepatic subcellular fraction were also incubated. Aliquots (1 ml) of the incubations were removed at 0, 60, or 90 min. All aliquots and remaining incubation mixtures were transferred to vials containing cold ethyl acetate to terminate the reaction, placed in a dry ice-acetone bath to quickly freeze the samples, and stored at −20°C. Following ethyl acetate (3 ml) extraction of each NH₄OH-basified (~pH 9) incubate, unchanged drug and metabolites were characterized, tentatively identified, and quantified from these samples using Sciex API-3000 (ion spray)-MS (MDS Sciex, Concord, ON, Canada) and MS/MS techniques.

**Rat Pharmacokinetic Studies.** Male Sprague-Dawley rats received a single i.v. or oral dose of RWJ-394674 or RWJ-413216 hydrochloride following an overnight fasting period. The i.v. dose was prepared as a solution (10 mg of RWJ-394674 or RWJ-413216/ml) in 20% hydroxypropyl-β-cyclodextrin, whereas the oral dose was prepared as a solution (15 mg of RWJ-394674 or RWJ-413216/ml) in 0.5% Methocel (hydroxypropyl methyl cellulose). Following drug administration, blood samples were collected from each animal by jugular venipuncture under slight isoflurane anesthesia. Blood samples, collected using lithium heparin as the anticoagulant, were placed on ice pending centrifugation. Following centrifugation, plasma was harvested and stored at −20°C until use. Plasma samples were analyzed for parent drug (RWJ-394674 or RWJ-413216), and, in the RWJ-394674 study, the major metabolite (RWJ-413216), using a liquid chromatography-MS/MS assay with a lower limit of quantification of 1 ng/ml for each analyte. Pharmacokinetic analysis of the concentrations of analytes in plasma was performed using the WinNonlin Software Program (version 3.1; Pharsight, Palo Alto, CA). Behavioral observations were made throughout the studies.

**Results**

**Structures, in Vitro Receptor Binding, and Functional Assays**

The structures of RWJ-394674 (a tertiary amide) and RWJ-413216 (a secondary amide) are shown in Fig. 1; their synthesis has been described previously (Carson et al., 2004a,b). RWJ-394674 exhibited a high affinity for the delta opioid receptor (Table 1) and a 300-fold weaker affinity for the mu opioid receptor. In contrast, the N-desethyl analog RWJ-413216 bound with high affinity to the mu opioid receptor and a 180-fold weaker affinity to the delta receptor. In vitro GTPγS functional assay of RWJ-394674 revealed that it was a delta opioid partial agonist, whereas RWJ-413216 was a near full agonist at the mu and a partial agonist at the delta opioid receptor (Table 1). Table 1 also shows the mu and delta opioid affinities of selected peptidic and nonpeptidic delta and mu opioid reference compounds. In mu and delta opioid GTPγS functional assays, each of these reference compounds demonstrated agonist action at the receptor at which it bound with higher affinity, whereas functionality could not be determined (over the range of concentrations tested) at the receptor to which it bound with relatively lower affinity.

**Antinociceptive Tests**

**Mouse Abdominal Irritant Test.** RWJ-394674 dose-dependently inhibited the mouse abdominal irritant response over the range of 1 to 30 μmol/kg p.o. (Fig. 2), fully inhibiting the response at the highest dose tested. The calculated ED₅₀ value was 5.5 μmol/kg (2.8 mg/kg).

**Mouse 48°C Hot-Plate Test.** In an initial time course experiment, RWJ-394674 and RWJ-413216 at oral doses of 10 μmol/kg were each antinociceptive in the mouse 48°C hot-plate test, the maximum responses at that dose being ~45 and ~80%, respectively (Fig. 3). The time of maximal antinociceptive effect following the dosing of each compound was 1 h, and this effect persisted near or above the 50% level for at least 3 h. Both RWJ-394674 and RWJ-413216 elicited dose-dependent antinociception in the mouse 48°C hot-plate test, with ED₅₀ values of 10.5 and 4.7 μmol/kg, respectively (Fig. 4).

**Mouse 55°C Hot-Plate Test.** In the more stringent 55°C hot-plate test, RWJ-394674 and RWJ-413216 each elicited dose-dependent antinociception, reaching full effect at the highest dose tested, 100 μmol/kg (Fig. 5). The ED₅₀ values for the two compounds were 25.2 and 26.7 μmol/kg, respectively.

**Antagonist Study in the Mouse 55°C Hot-Plate Test.** The broad opioid antagonist naloxone, δ-selective antagonist naltindrole, and mu selective antagonist βFNA were used to probe the opioid subtype underlying the antinociceptive effect of RWJ-394674 in the mouse 55°C hot-plate test. The antinociceptive effect of morphine was studied in parallel as a mu opioid-selective reference comparator. The antinociceptive agents were used at a dose corresponding to their approximate 50% level of effect, 300 μmol/kg for morphine and 60 μmol/kg for RWJ-394674. As shown in Fig. 6, naloxone, naltindrole, and βFNA each dose dependently blocked the antinociceptive effect of both morphine and RWJ-394674.

**Rat 51°C Hot-Plate Test.** In the rat 51°C hot-plate test, both RWJ-394674 and RWJ-413216 were antinociceptive, although with different time courses (Fig. 7). At oral doses of 60 μmol/kg, the antinociception of the mu-preferring opioid RWJ-413216 reached its peak effect by 60 min, whereas that of the delta-selective RWJ-394674 did not reach its peak effect until 2 h after dosing (Fig. 7). The antinociception of RWJ-413216 diminished more rapidly than that of RWJ-394674. Both compounds exhibited an MPE of approximately 60% at this dose; lower doses were less effective (data not shown), and evaluation at higher doses was precluded by sedation/catatonia.

**In Vitro Metabolism**

To probe the metabolic relationship between RWJ-394674 and RWJ-413216, an in vitro study was conducted. Following the incubation of RWJ-394674 with hepatic S9 fractions from three species, unchanged RWJ-394674 (12, 45, and 7% of the sample in mouse, rat, and human, respectively) and several minor oxidized metabolites including N-desethyl-RWJ-

---

**Fig. 1.** Structures of RWJ-394674 and RWJ-413216.
were identified. The major metabolite, M1, was identical to a synthetic sample of RWJ-413216 on the basis of MS and MS/MS analysis. The relative percent of sample for RWJ-394674 and its major metabolite RWJ-413216 at two incubation times is listed in Table 2.

**Rat PK Studies**

Table 3 summarizes the parameters determined in a rat PK study. RWJ-394674 was absorbed slowly following an oral dose of 30 mg/kg RWJ-394674 to male rats with a mean \( T_{\text{max}} \) (time of maximal plasma concentration) value of 3.3 h. RWJ-394674 also appeared to be eliminated slowly following oral administration with a mean half-life value of 17 h. The mean oral bioavailability of RWJ-394674 was moderate at 48%. The mean volume of distribution was calculated to be 27 l/kg following i.v. administration. This value greatly exceeds the volume for total body water in the rat (approximately 670 ml/kg), suggesting that RWJ-394674 was extensively distributed outside of plasma. Table 3 summarizes the PK parameters determined. After oral administration of RWJ-394674, its peak plasma levels and those of its des-ethyl metabolite RWJ-413216 were similar (Tables 3 and 4), both being near the \( C_{\text{max}} \) value through the 24-h time point (Fig. 8). Oral rat PK on the metabolite, RWJ-413216, itself yielded similar plasma levels of the compound to those seen following oral administration.

**TABLE 1**

*In vitro receptor binding and GTP\(_{\text{S}}\) functional profile of RWJ-394674, RWJ-413216, and reference mu and delta agonists*

The relative efficacy of delta agonists was based on the stimulation of GTP\(_{\text{S}}\) incorporation by SNC-80, whereas the relative efficacy of mu opioid agonists was based on the stimulation of GTP\(_{\text{S}}\) binding by DAMGO. \( K_{i} \) values were determined from data obtained over the concentration range 1E\(^{-9}\) to 1E\(^{-3}\) M. The inhibition of binding at each concentration was determined in duplicate, and the results shown are the mean of two separate experiments. The concentration range studied for the GTP\(_{\text{S}}\) assays was 1E\(^{-9}\) to 1E\(^{-3}\) M. The stimulation of GTP\(_{\text{S}}\) incorporation at each concentration was determined in duplicate, and the values given are the mean of the results of two separate experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Binding Affinity ((K_{i}))</th>
<th>GTP(_{\text{S}}) Functional Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delta ( nM )</td>
<td>Mu ( nM )</td>
</tr>
<tr>
<td>RWJ-394674</td>
<td>0.24</td>
<td>72</td>
</tr>
<tr>
<td>RWJ-413216</td>
<td>46.7</td>
<td>0.26</td>
</tr>
<tr>
<td>Morphine</td>
<td>654</td>
<td>1.39</td>
</tr>
<tr>
<td>DAMGO</td>
<td>859</td>
<td>1.33</td>
</tr>
<tr>
<td>SNC-80</td>
<td>0.42</td>
<td>7620</td>
</tr>
<tr>
<td>DPDPE</td>
<td>3.57</td>
<td>241</td>
</tr>
</tbody>
</table>

\(^a\) Selectivity, \(K_{i}\) at higher affinity receptor/\(K_{i}\) at lower affinity receptor.

\(^b\) N.D., not determined. Rel. Eff., relative efficacy.

---

Fig. 2. Dose-response profile of RWJ-394674 in the mouse abdominal irritant test (\(n = 10\) per dose).

Fig. 3. Time course of the antinociceptive effect of orally administered doses of 10 \(\mu\)mol/kg each of RWJ-394674 (5.15 mg/kg) and RWJ-413216 (4.87 mg/kg) in the mouse 48°C hot-plate test. Shown are the means ± S.E.M.; \(n = 10\) per compound.

394674 were identified. The major metabolite, M1, was identical to a synthetic sample of RWJ-413216 on the basis of MS and MS/MS analysis. The relative percent of sample for RWJ-394674 and its major metabolite RWJ-413216 at two incubation times is listed in Table 2.

**Rat PK Studies**

Table 3 summarizes the parameters determined in a rat PK study. RWJ-394674 was absorbed slowly following an oral dose of 30 mg/kg RWJ-394674 to male rats with a mean \( T_{\text{max}} \) (time of maximal plasma concentration) value of 3.3 h. RWJ-394674 also appeared to be eliminated slowly following oral administration with a mean half-life value of 17 h. The mean oral bioavailability of RWJ-394674 was moderate at 48%. The mean volume of distribution was calculated to be 27 l/kg following i.v. administration. This value greatly exceeds the volume for total body water in the rat (approximately 670 ml/kg), suggesting that RWJ-394674 was extensively distributed outside of plasma. Table 3 summarizes the PK parameters determined. After oral administration of RWJ-394674, its peak plasma levels and those of its des-ethyl metabolite RWJ-413216 were similar (Tables 3 and 4), both being near the \( C_{\text{max}} \) value through the 24-h time point (Fig. 8). Oral rat PK on the metabolite, RWJ-413216, itself yielded similar plasma levels of the compound to those seen following oral administration.
administration of the parent compound (see Fig. 8 and Table 3).

The behavioral observations made during the in life phase of the rat PK studies significantly distinguished the two compounds. Although all animals dosed i.v. with 1 mg/kg RWJ-413216 died of respiratory depression during the dosing procedure, the animals dosed with RWJ-394674 i.v. exhibited no adverse responses (Table 5). Striking differences between the compounds' behavioral effects were also evident following oral dosing. Oral administration of the potent mu opioid RWJ-413216 induced respiratory depression and reduced motor activity in all animals by the time of the first blood draw, 15 min. By 4 h postdosing, all animals exhibited varying degrees of whole body rigidity. Loss of pain perception was evident by the absence of a toe pinch reflex, but a startle reflex (to finger snap) was retained. In marked contrast, dosing with RWJ-394674 resulted in minimal to no mu opioid-associated adverse effects, sedation in only one orally dosed animal.

**Discussion**

Administration of the delta opioid agonist RWJ-394674 to mice provided a dose-dependent, protective effect in the abdominal irritant test. Antinociception has also been observed in abdominal irritant tests with other delta opioid-selective agonists including BW373U86 (Wild et al., 1993), TAN-67 (Kamei et al., 1995; Nagase et al., 1998), SNC-80 (Hong et al., 1998), and (±)-SB219825 (Dondio et al., 1997), suggesting that this pharmacology is characteristic of delta opioid agonists. Although these delta-selective compounds demonstrated efficacy following administration by the i.p. (BW373U86, TAN-67, SNC-80) or intracerebroventricular [BW373U86, (±)-SB219825] routes, RWJ-394674 exhibited its antinociceptive effect in the abdominal irritant test subsequent to oral administration, most likely evidencing its enhanced bioavailability or central nervous system penetration, compared with that of SB-213698 (TAN-67) and SB219825 (Chaturvedi et al., 2000). The potency of RWJ-394674 in the abdominal irritant test was similar to that of the centrally acting analgesic, tramadol, which exhibited an ED\textsubscript{50} value in this test of 5.67 μmol/kg p.o. (Codd et al., 1995).

Somewhat surprisingly, not only the mu opioid agonist RWJ-413216 but also the delta opioid agonist RWJ-394674 was antinociceptive in the mouse 48 and 55°C hot-plate tests, stringent nociceptive models in which delta-selective compounds such as SB-236863 have not proven effective (Petrillo et al., 2003). Although RWJ-413216 was twice as potent as RWJ-394674 in the 48°C hot-plate test, the compounds had virtually identical potencies in the 55°C test. Notably, the antinociception induced by the delta opioid RWJ-394674 was accompanied by moderate Straub tail and increased locomotor activity, behaviors characteristic of mu opioid agonists in mice.

The longer duration of the antinociceptive effect of the parent compound RWJ-394674 than that of RWJ-413216 in the hot-plate tests may be due to the continued generation of the mu-preferring metabolite from the parent compound. However, the sustained plasma levels of both parent compound and metabolite observed in rat PK studies would advocate the prolonged pharmacologic effect of both compounds. Alternatively, the diminution of RWJ-413216 antinociception may be due to the development of tolerance to its mu opioid pharmacology, whereas the more persistent antinociceptive effect of RWJ-394674 may be due to attenuation of mu opioid tolerance development by the delta opioid parent compound, RWJ-394674.

Antagonist studies in the 55°C mouse hot-plate test confirmed the opioid nature of the antinociceptive effect of both morphine and RWJ-394674. Understandably, the effect of both compounds was blocked by naloxone and the mu-selective agent βFNA, the mu component for RWJ-394674 likely due to its mu-preferring metabolite, RWJ-413216. The minimal delta opioid role for RWJ-394674 in this test may be due
TABLE 2
In vitro metabolism of RWJ-394674
The relative percentage of sample for RWJ-394674 and its major desethyl metabolite, RWJ-413216, incubated with the hepatic S9 fraction from mouse, rat, and human. Data were derived from the integrated ion chromatograms via Q scan MS determinations.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mouse 60 min</th>
<th>Mouse 90 min</th>
<th>Rat 60 min</th>
<th>Rat 90 min</th>
<th>Human 60 min</th>
<th>Human 90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWJ-394674</td>
<td>12</td>
<td>12</td>
<td>48</td>
<td>45</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>M1 RWJ-413216</td>
<td>55</td>
<td>55</td>
<td>31</td>
<td>32</td>
<td>43</td>
<td>37</td>
</tr>
</tbody>
</table>

TABLE 3
Calculated parameters [mean (±S.D.)] from rat PK study of RWJ-394674

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Route</th>
<th>Dose</th>
<th>(C_{\text{max}})</th>
<th>(T_{\text{max}})</th>
<th>AUC(0–24 h)</th>
<th>AUC(0–4 h)</th>
<th>AUC(0–\infty)</th>
<th>(t_{1/2})</th>
<th>CL/F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWJ-394674</td>
<td>Oral</td>
<td>30</td>
<td>176 (67)</td>
<td>3.3 (3.6)</td>
<td>2489 (1801)</td>
<td>495 (192)</td>
<td>5302c</td>
<td>16.7c</td>
<td>9108c</td>
<td>48.1c</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>10</td>
<td>1020 (212)</td>
<td>NA</td>
<td>3677 (877)</td>
<td>1739 (306)</td>
<td>4464 (1192)</td>
<td>10.7 (1.7)</td>
<td>2368 (637)</td>
<td></td>
</tr>
<tr>
<td>RWJ-413216</td>
<td>Oral</td>
<td>30f</td>
<td>162 (60)</td>
<td>5.5 (3.0)</td>
<td>2389 (1705)</td>
<td>422 (160)</td>
<td>289 (54)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>10f</td>
<td>88 (20)</td>
<td>2.5 (1.7)</td>
<td>1337 (338)</td>
<td>289 (54)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a\) \(C_0\) for i.v. dose
\(b\) Calculated using AUC(0–\infty).
\(c\) \(n = 2\) for these parameters.
\(d\) Refers to dose of RWJ-394674.

MC, not calculated.
\(n = 3\)

Fig. 8. Plasma levels of RWJ-394674 and RWJ-413216 following oral administration of a dose of 58 \(\mu\)mol/kg (30 mg/kg) RWJ-394674 or RWJ-413216 to male rats.

to the compound’s rapid biotransformation to its mu potent metabolite in mouse.

Unlike their peptidic counterparts, the first nonpeptide delta-selective agonists induced convulsions. Studied as a racemate, BW373U86 elicited brief, nonlethal convulsions; seizure-response curves were right-shifted by the nonselective opioid antagonist naltrexone and the delta opioid-selective antagonist naltrindole (Comer et al., 1993). Administration of SNC-80 by the s.c. or i.p. routes produced antinociception and induced seizures in overlapping dose ranges (Bilsky et al., 1995). Pharmacokinetic studies with a radiolabeled SNC-80 analog, SNC-121, revealed a BW373U86-like metabolite that may be responsible for SNC-80-induced convulsions (Schetz et al., 1996). Importantly, seizures were not observed following the administration of either RWJ-394674 or RWJ-413216 by the oral or i.v. route, at any dose, to mice or rats. Lack of proconvulsant activity also characterized SB-236863, another nonpeptidic delta opioid compound (Petrillo et al., 2003).

Although their oral dosing leads to their similar, plasma levels beyond 2 h postdose (Fig. 8), RWJ-413216 and RWJ-394674 show divergent antinociceptive durations: that of RWJ-394674 persists as does its plasma level, whereas the effect of RWJ-413216 wanes (Fig. 7) despite its rising plasma levels. It is not presently known whether the diminution in RWJ-413216 induced antinociception is due to decreased levels of the compound in a key tissue such as brain, or if the reduction in antinociceptive effect is due to the development of tolerance to RWJ-413216. If tolerance development was to underlie this diminution, however, then the relatively sustained effect of the parent compound RWJ-394674 may suggest the mitigation by its delta pharmacology of the tolerance that would otherwise develop to the mu effect of its metabolite RWJ-413216. Indeed, others have observed that the concurrent administration of a delta opioid agonist (e.g., DP-DPE) with the mu opioid agonist morphine resulted in less tolerance development in the mouse tail-flick test than resulted from administration of the morphine alone (Jiang et al., 1990). Other studies with DOR knockout mice point to an essential role for DOR in the development of tolerance; analgesic tolerance development was complete by day 3 following implantation of a morphine pellet in wild type mice, whereas little or no tolerance developed in similarly implanted DOR knockout mice (Nitsche et al., 2002). Thus, compounds embodying both delta and mu opioid agonist pharmacologies may serve to mitigate the significant clinical
issues attendant to dose escalation that are necessitated by the development of mu opioid analgesic tolerance.

The virtual absence of mu opioid liabilities noted when the delta- and mu-selective compounds were simultaneously present (following the oral administration of RWJ-394674), as compared with the prominent mu opioid adverse effects (e.g., sedation, muscular rigidity, respiratory depression, and death), observed following dosing with the mu opioid compound alone (RWJ-413216), suggests a key role for delta opioid agonists in the reduction of mu opioid side effects. Relying on separate administration of mu and delta opioid agonists, others have found evidence of delta agonist-mediated attenuation of mu opioid adverse effects such as respiratory depression and muscle rigidity. For example, the delta-selective agent BW373U86 reversed the hypoxic effect of alfentanil in rat but, importantly, did not reduce alfentanil-induced anticonvulsion (Su et al., 1998). In addition, in a naltrindole-sensitive manner, administration of the delta opioid agonist DPDE attenuated muscle rigidity induced in rat by the mu agonist DAMGO (Vankova et al., 1996). Thus, the structural, metabolic, and pharmacologic relationship between RWJ-394674 and RWJ-413216 more broadly substantiates the advantageous role of delta opioid agonists in the reduction of mu opioid side effects and in attenuating the development of tolerance to mu opioid analgesia. These findings suggest that the discovery and development of dual delta/mu opioid agonists may provide the potent pain relief of mu opioids with attenuated respiratory depression and minimal development of pharmacologic tolerance.

References


Address correspondence to: Ellen E. Codd, J&J PRD, P.O. Box 776, Welsh and McKeen Roads, Spring House, PA 19477. E-mail: ecodd@prdus.jnj.com