Comparison of Receptor Mechanisms and Efficacy Requirements for δ-Agonist-Induced Convulsive Activity and Antinociception in Mice

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ABSTRACT

δ-Opioid receptor-selective agonists produce antinociception and convulsions in several species, including mice. This article examines two hypotheses in mice: 1) that antinociception and convulsive activity are mediated through the same type of δ-receptor and 2) that greater δ-agonist efficacy is required for antinociception than for convulsive activity. δ-Mediated antinociception was evaluated in the acetic acid-induced abdominal constriction assay, which involves a low-intensity noxious stimulus; convulsive activity was indicated as a mild tonic-clonic convulsive episode followed by a period of catalepsy. In δ-opioid receptor knockout mice [DOR-1(+/−)], the nonpeptidic δ-agonists (±)-4-[([R])-2S,5R]-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-[3-hydroxyphenyl]methyl]-N,N-diethylbenzamide hydrochloride (BW373U86) and (+)-4-[([S,5R])-2S,5R]-2,5-dimethyl-4-(2-propenyl)-1-piperazinyl]-[3-methoxyphenyl]methyl]-N,N-diethylbenzamide (SNC80) failed to produce convulsive behavior demonstrating the absolute involvement of DOR-1 in this effect. In NIH Swiss mice expressing δ-opioid receptors, BW373U86 produced both antinociception and convulsive activity. These effects were antagonized by the putative δ₁-receptor-selective antagonist 7-benzylidenenaltrexone and the putative δ₂-receptor-selective antagonist naltrexone. Tolerance developed to both the convulsive and antinociceptive effects of BW373U86. Tolerance to the convulsive, but not the antinociceptive, effects of BW373U86 was largely prevented when the antagonist naltrexone was given 20 min after each dose of the agonist in a 3-day treatment paradigm. The convulsive action of BW373U86 was also less sensitive than the antinociceptive action to treatment with the irreversible δ-agonist naltrexone isothiocyanate. Collectively, these data suggest that the convulsive and antinociceptive activities of δ-agonists are mediated through the same receptor but that the receptor reserve for δ-mediated convulsive activity is greater than for δ-mediated antinociceptive activity.

The nonpeptidic, selective, δ-opioid receptor agonist BW373U86 has antinociceptive properties without pharmacological effects associated with μ-opioid agonists. For example, BW373U86 stimulates respiration and has little abuse potential in models of self-administration (Wild et al., 1993; Negus et al., 1994). Thus, δ-opioid receptor agonists may have therapeutic potential as analgesic agents, especially in hyperalgesia and allodynia (e.g., Negus et al., 1994; Fraser et al., 2000). Unfortunately, BW373U86 displays dose-dependent convulsive activity upon systemic administration to mice (Comer et al., 1993a), rats (Broom et al., 2002), and monkeys (Dykstra et al., 1993; Negus et al., 1994; Pakarinen et al., 1996).

In mice, the convulsions are mild, clonic-tonic, and nonlethal, followed by a short period of catalepsy, after which the mice recover and are indistinguishable from vehicle-treated controls (Comer et al., 1993a). The convulsions are naltrindole-sensitive, confirming a role for the δ-opioid receptor. Indeed, SNC80, the (+)-isomer of the methyl ether of BW373U86 (Calderon et al., 1994) is more selective for the δ-receptor than BW373U86 and shows both antinociceptive (Bilsky et al., 1995) and convulsive activity (Hong et al., 1998).

The δ-partial agonist BU48 (Broom et al., 2000) produces...
dose-dependent convulsive activity in mice via a naltrindole-sensitive mechanism. The convulsions are identical to those seen with BW373U86 and SNC80. BU48 also produces antinociception, but this is not reversed by naltrindole. The reason why BU48 has only one of the two predominant effects of δ-agonists is unknown; it can be hypothesized, however, that subtypes of the δ-receptor mediate the two effects or that a single type of receptor is involved, but with antinociception having a higher agonist efficacy requirement. The existence of δ-opioid receptor subtypes has been suggested by a number of studies using the putative subtype-selective antagonists 7-benzylidenenaltrexone (BNTX; δ₁) and naltriben (NTB; δ₂) (Sofuoglu et al., 1991; Portoghese et al., 1992a, Hammond et al., 1997). This pharmacological evidence is not supported by the existence of more than one δ-opioid receptor clone, but this discrepancy may be explained by the cellular environment and/or the presence of receptor complexes such as dimers and heterodimers (Jordan and Devi, 1999; George et al., 2000; Gomes et al., 2000).

In the present studies, a variety of pharmacological approaches were used in an attempt to understand the relationship between the undesirable convulsive and beneficial antinociceptive properties of nonpeptide δ-agonists and to test the two hypotheses regarding these properties. The importance of the DOR-1 receptor was tested using δ-opioid receptor knockout mice [DOR-1(−/−); Zhu et al., 1999]. To determine whether the convulsive and antinociceptive effects were caused by actions at two different δ-receptor subtypes, the potency of the putative δ₁ and δ₂ agonists BNTX and NTB were compared in NIH Swiss mice treated with BW373U86. Using NIH Swiss mice, two procedures were used to see if greater δ-agonist efficacy is needed for antinociception than for convulsions. First, the development of tolerance to the antinociceptive versus the convulsive effects of acute or chronic BW373U86 was measured; more tolerance would be expected to the effect with the higher agonist efficacy requirement. Second, the effects of the irreversible antagonist naltrindole isothiocyanate (5-NTII; Portoghese et al., 1990) were measured to establish whether there was a difference in the receptor reserve for antinociception compared with convulsive effects of BW373U86. BW373U86 was chosen for the majority of the studies because it is a water-soluble drug and it has a pharmacology that is very similar to SNC80; indeed, BW373U86 is the major metabolite of SNC80 (Schetz et al., 1996).

The criteria used to score the two behaviors were relatively mild. For a convulsive episode, mice had to show a single tonic-clonic convolution followed by a period of catalepsy. This is a lenient measure because convulsive episodes following administration of nonpeptide δ-agonists are much less severe than, for example, with pentyleneetrazone (Hong et al., 1998). Antinociception was determined as the ability to inhibit the abdominal stretch response to an i.p. injection of 0.6% acetic acid. Compounds with low efficacy produce antinociception in this assay. Thus, the low-efficacy agonist naldorphine, which is ineffective against heat nociceptive stimuli, is active in this test (Ward and Takemori, 1983), as are nonsteroidal anti-inflammatory drugs (al-Swayeh et al., 2000). Indeed, BW373U86 given peripherally is not generally active against heat or mechanical stimuli in the mouse but is active in the acetic acid-induced stretch assay (Wild et al., 1993). Results from the various studies imply that the same δ-receptor (DOR-1) is responsible for the convulsive and antinoceceptive actions of δ-agonists, but the two responses may have different agonist efficacy requirements.

Materials and Methods

Chemicals. [3H]DPDPE ([d-Pen²,d-Pen⁵]enkephalin; 45 Ci/mmol) was purchased from PerkinElmer Life Science Products (Boston, MA). BNTX (Portoghese et al., 1992) and SNC80 (Calderon et al., 1994) were synthesized as previously described. nor-Binaltorphimine was provided by Dr. H. Mosberg (School of Pharmacy, University of Michigan, Ann Arbor, MI). Naltrindole, BW373U86, NTB, 5-NTII, pentylentetrazole, and all other chemicals were from Sigma-Aldrich (St. Louis, MO) and were of analytical grade. NTB and BNTX were dissolved in 10% dimethyl sulfoxide. SNC80 base was dissolved in sterile water with minimum 1.13 N hydrochloric acid. 5-NTII was dissolved in 45% 2-hydroxypropyl-β-cyclodextrin. All other drugs were dissolved in sterile water.

Animals. Male wild-type [DOR-1(+/+) and knockout mice homozygous for a deletion of exon 2 of DOR-1 generated from mating outbred heterozygous mice were used (Zhu et al., 1999). Briefly, a 2.1-kilobase SpeI-BamHI fragment corresponding to the 5′-translated region just before exon 2 and a 4.8-kilobase KpnI-HindIII fragment corresponding to the 3′-region outside of exon 2 of DOR-1 were cloned into the targeting vector containing a neomycin resistant sequence and the HSV-TK gene. The targeting vector was introduced into embryonic stem cells from the 129SvEv mouse line. Correctly targeted embryonic stem cells were injected into C57BL/6J blastocysts to give germine-transmitting male chimeras. Male chimera mice were mated with C57BL/6J females to produce wild-type and heterozygous mutant mice. The heterozygous mice were in turn mated to provide littermate DOR-1(+/+) and DOR-1(−/−) mice. Male 129SvEv and C57BL/6J mice (20–30 g) were obtained from Taconic Farms (Germantown, NY). For all other in vivo assays, male NIH Swiss mice (20–35 g; Harlan Sprague-Dawley Indianapolis, IN) were used. All animals were fed on a standard laboratory diet and kept on a 12-h light/dark cycle at a temperature of 20°C. Studies were performed in accordance with the Declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The experimental protocols were approved by the University of Michigan University Committee on the Use and Care of Animals.

In Vivo Assays. Measurement of convulsant activity was performed as previously described (Hong et al., 1998). Mice were injected s.c. with BW373U86 or SNC80 at doses up to 32 mg/kg (limited by drug availability) and placed in individual Plexiglas boxes (18 × 28 × 13 cm) for the duration of the observation period. Mice were observed for convulsant activity for 20 min after drug injection. For a positive convulsion score, a mouse had to exhibit a convulsive episode and a subsequent period of catalepsy. Postconvulsion catalepsy was assessed by placing the forepaws of a mouse on a horizontal rod; a positive catalepsy score was assigned if the mouse had not removed its paws within 15 s. The putative δ₁ antagonist BNTX and the putative δ₂ antagonist NTB were administered s.c. 20 min before δ-agonist administration. The irreversible antagonist 5-NTII was administered s.c. 24 h before the start of the assay. Data are expressed as a percentage of number of animals convulsing.

For the DOR(+/+) and DOR(−/−) mice, a cumulative dosing procedure was used in which doses of SNC80 were administered every 20 min and the animals were observed for convulsive activity between injections.

Antinociception was evaluated immediately following observation for convulsive activity using the acetic acid-induced abdominal stretch method (Hong et al., 1998). Following recovery from convulsion and catalepsy (i.e., 20 min after injection of the δ-agonist), 0.4 ml of 0.6% acetic acid was injected by the i.p. route. Five minutes after administration, the animals were observed for abdominal stretches.
for 5 min. Abdominal stretches were characterized by a wave of contraction of the abdominal musculature followed by extension of the hind legs. Data were expressed as percent antinociception in drug-treated animals compared with vehicle-treated animals as follows: % antinociception = (vehicle treated stretches – drug treated stretches/vehicle treated stretches) × 100.

**Tolerance Studies.** Two paradigms were used. First, various doses of BW373U86 (1–32 mg/kg, s.c.) were given as single injections 24 h before a challenge dose of 10 mg/kg s.c. BW373U86. Second, BW373U86 (32 mg/kg, s.c.) was given as daily injections for 3 days before a challenge dose of BW373U86 (10 mg/kg, s.c.) on day 4. In the daily dosing paradigm, mice were given an injection of either 10 mg/kg naltrindole or sterile water (s.c.) 20 min after each dose of BW373U86, except for the final challenge dose. Animals were observed for convulsive activity on each day, and 20 min after the final challenge dose, 0.6% acetic acid solution was administered as described above and the number of abdominal stretches measured.

**Ex Vivo Binding Assays.** Mice were treated s.c. with 5′-NTTII (32 mg/kg) or vehicle (45% 2-hydroxypropyl-β-cyclodextrin). Twenty-four hours later, the mice were sacrificed, and their brains (minus cerebellum) were rapidly removed, quickly frozen in liquid nitrogen, and stored at −80°C. Brain homogenates were prepared in Tris-HCl (pH 7.4, 50 mM) and centrifuged at 18,000 g for 20 min. The resultant pellet was resuspended in Tris-HCl, warmed to 37°C for 20 min, then recentrifuged. The pellet was again suspended in Tris-HCl buffer at a protein concentration of 1 mg/ml and stored at −80°C. Saturation-binding assays were performed in Tris-HCl buffer with [3H]DPDPE (0.1 to 20 nM) for 1 h at 25°C using 100 μg of protein. Nonspecific binding was defined with 10 μM naloxone. Bound and free ligands were rapidly separated by vacuum filtration through glass fiber filters (32; Scheiber and Schuell, Keene, NH), radioactivity retained on the filters, and quantified by liquid scintillation counting. The data were analyzed as a one-site binding isotherm using GraphPad Prism (GraphPad Software, Inc., San Diego, CA) to provide K_D and B_max values with 95% confidence intervals (CI).

**Results**

**δ-Receptor Knockout Mice.** SNC80 administration to DOR-1(+/+) mice caused a dose-dependent increase in the number of mice convulsing, up to a maximum of 33% (Fig. 1). Of the embryonic stem cell donor strain 129SvEv mice, three of six male and two of six female convulsed when given a dose of 32 mg/kg SNC80, but with the C57BL6/J blastocyst donor strain, all mice (six of six) convulsed at this dose (data not shown). In contrast to its effects in DOR-1(+/+) mice, SNC80 produced no convulsions in DOR-1(−/−) mice even at a dose of 100 mg/kg (Fig. 1). A single, supramaximal dose of 32 mg/kg BW373U86 that produced convulsions in 100% of male NIH Swiss mice, produced convulsions in only three of nine of the DOR-1(+/+) animals and no convulsions in six DOR-1(−/−) mice tested (data not shown), confirming the results seen with SNC80. All DOR-1(+/+) and DOR-1(−/−) mice displayed severe convulsions on injection of 100 mg/kg of the nonopioid convulsant, pentylenetetrazole (s.c.; data not shown).

**δ-Receptor Antagonists.** BW373U86 caused dose-dependent convulsions (Fig. 2, a and c) and dose-dependent antinociception in NIH Swiss mice (Fig. 2, b and d). The ED_50 for BW373U86 in the acetic acid-induced writhing assay (2.2 mg/kg; 95% confidence limits 2.1–2.5 mg/kg) was shifted approximately 4-fold to the right in a parallel manner by either 0.3 mg/kg BNTX or 0.01 mg/kg NTB (Fig. 2, b and d). Higher doses of antagonist did cause a further shift, but this was smaller than expected. Based on the shift (3.7-fold) in the presence of 0.3 mg/kg BNTX, a shift of 10-fold was predicted for 1 mg/kg BNTX, yet only a 5-fold shift was obtained. Similarly, 0.1 mg/kg NTB gave a 5.9-fold shift rather than the 15.5-fold shift predicted on the basis of the shift (2.4-fold) caused by the 0.01 mg/kg dose. The ED_50 for the convulsive action of BW373U86 was estimated to be 2.1 mg/kg since no mice convulsed at 1 mg/kg and all mice convulsed at 3.2 mg/kg. This was shifted approximately 3-fold to the right and downward by either 0.3 mg/kg BNTX or 0.01 mg/kg NTB. Higher antagonist doses completely prevented convulsions when tested up to 32 mg/kg BW373U86 (Fig. 2, a and c).

**Tolerance.** Rapid tolerance to the convulsive and antinociceptive effects of BW373U86 was observed. When a single dose of BW373U86 (1, 3.2, 10, or 32 mg/kg) was followed 24 h

![Fig. 1. The convulsive properties of SNC80 in δ-opioid receptor wild-type (DOR-1(+/+)) mice and δ-opioid receptor knockout (DOR-1(−/−)) mice. Cumulative doses of SNC80 were administered by s.c. injection 20 min apart. Animals were observed for convulsive activity between injections. n = 3–9 animals for each point.](image1)

![Fig. 2. The effect of putative antagonists for δ-receptor subtypes on the convulsive and antinociceptive properties of BW373U86. Antagonists (NTB or BNTX) were administered at the indicated doses (milligrams per kilogram s.c., see below) 20 min before BW373U86. Mice were observed for convulsions for 20 min (a and c) and then subjected to the acetic acid-induced abdominal stretch assay (b and d), as described under Materials and Methods. n = 6 for each group. ○, vehicle; △, +0.3 BNTX; □, +1 BNTX; ●, vehicle; ▽, +0.01 NTB; ◆, +0.1 NTB.](image2)
later by a challenge dose of 10 mg/kg BW373U86, both convulsions and antinociception were similarly reduced. The degree of tolerance that developed was dependent on the pretreatment dose (Fig. 3) such that complete tolerance was observed following a pretreatment dose of 32 mg/kg BW373U86. Tolerance to δ-agonist-mediated convulsions is reduced by administration of the antagonist naltrindole up to 1 h after the agonist (Comer et al., 1993a). A 3-day dosing paradigm was used to determine whether tolerance to more chronic treatment was also prevented and whether antinociceptive tolerance was similarly controlled by this procedure. A complete loss of the antinociceptive (Fig. 4a) and convulsive (Fig. 4b) effects of a challenge dose of 10 mg/kg BW373U86 was observed following 3 consecutive days of treatment with 32 mg/kg BW373U86. Naltrindole (10 mg/kg) given 20 min after the first three doses of BW373U86 did not prevent the development of tolerance to the antinociceptive effects of 10 mg/kg BW373U86 (Fig. 4a) but did prevent

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**Fig. 3.** Behavioral responses in mice to 10 mg/kg BW373U86 (s.c.) administered 24 h after treatment with a single dose of 1, 3.2, 10, or 32 mg/kg BW373U86 (s.c.). Convulsive activity (a) and antinociception (b) + S.E.M, as described under Materials and Methods. n = 6–9 animals/group.

**Fig. 4.** Tolerance to the antinociceptive and convulsive effects of BW373U86 (BW) and the effect of postagonist naltrindole (NTI) treatment. Animals (five to six per group) were injected with vehicle (sterile water) or 32 mg/kg BW373U86 s.c. every 24 h for 3 days and observed for convulsions. Twenty minutes following BW373U86 administration (when animals had recovered from convulsions) either 10 mg/kg naltrindole or vehicle was administered s.c. On day 4, animals were observed for convulsive activity following a challenge dose of 10 mg/kg BW373U86 and then tested in the acetic acid-induced abdominal stretch assay, as described in Materials and Methods. Antinociceptive effect (a) of 10 mg/kg BW373U86 following 3 days of vehicle or 32 mg/kg BW373U86 treatment with or without naltrindole. Bars are means ± S.E.M. Convulsive action (b) of 10 mg/kg BW373U86 following 3 days of vehicle or 32 mg/kg BW373U86 treatment with or without naltrindole. Tolerance development (c) to the convulsive effects of 32 mg/kg BW373U86 with or without naltrindole.
tolerance to the convulsive effects of this dose (Fig. 4b). In fact, each daily dose of 32 mg/kg BW373U86 produced convulsive activity in all animals on days 1 through 3 (Fig. 4c), and on day 4, four of five animals convulsed to the 10 mg/kg challenge dose of BW373U86 (Fig. 4c).

Irreversible Antagonist Studies. The δ-selective irreversible antagonist 5'-NTII (32 mg/kg) administered 24 h before BW373U86 caused a 40% reduction in the percentage of mice convulsing when given a dose of 10 mg/kg BW373U86. On the other hand, this pretreatment dose of 5'-NTII completely prevented BW373U86-mediated antinociception (Fig. 5). Lower doses of 5'-NTII (3.2 and 10 mg/kg) that had no effect on the convulsive properties of BW373U86 caused significant decreases in BW373U86-mediated antinociception (Fig. 5).

Saturation binding of the δ-specific ligand [3H]DPDPE to homogenates of mouse brain afforded a Bmax value of 156 fmol/mg of protein (CI 146–166) with a KD value of 4.23 nM (CI 2.65–5.9) (Fig. 6). The specific binding varied from 68% at concentrations of 1 nM or less to 36% at the highest concentration used (20 nM). In contrast, brain homogenates from 5'-NTII-treated (32 mg/kg) mice revealed a Bmax value for [3H]DPDPE of 61.8 fmol/mg of protein (CI 8.3–115) and a KD value of 8.89 nM (CI –7.3–30.2). The large variability in KD values was caused by the low level of specific binding of [3H]DPDPE in brain homogenates from the 5'-NTII-treated animals (13.4 ± 1.7%). If the KD value was assumed not to change, then a Bmax value of 40.4 fmol/mg of protein (CI 31.9–48.8) was determined (Fig. 6), representing a 74% reduction in δ-receptor number.

Discussion

Previous studies have identified the δ-opioid receptor as the source of convulsive activity following administration of BW373U86 and SNC80 to mice (Comer et al., 1993a; Hong et al., 1998). The present study confirmed these observations because both BW373U86 and SNC80 failed to produce convulsions in DOR-1(−/−) mice. In addition, SNC80 is reported to be ineffective as an antinociceptive agent in DOR-1(−/−) mice (Nitsche et al., 2000). Thus, the convulsive and antinociception activity of SNC80 are mediated through the δ-opioid receptor. Surprisingly, robust BW373U86 (i.c.v and s.c.) and DPDPE (i.c.v.) antinociception is reported in DOR-1(−/−) mice. That this is not seen with the more δ-selective agonist SNC80, however, suggests that BW373U86 and DPEP may be producing these effects through a different system (Zhu et al., 1999).

SNC80 and BW373U86 caused convulsions in only 33% of the DOR(+/+) mice. Thus, the presence of the δ receptor does not guarantee convulsive activity following administration of a nonpeptide δ agonist. The low responsiveness in the DOR(+/+) mice was surprising but was consistent with the susceptibility to convulsions of the 129SvEv stem cell donor strain rather than the C57BL/6J blastocyst donor mice. In line with this low sensitivity to BW373U86-mediated convulsions in the DOR(+/+) mice, previous studies have shown a low antinociceptive response to BW373U86 in these mice (Zhu et al., 1999) compared with ICR mice, for example (Wild et al., 1993). This genetic variation is unlikely to be at the level of the δ receptor since the DOR-1(+/+) mice have 56 to 144 fmol of receptor/mg of protein, depending on the ligand used for the measurement (Zhu et al., 1999), which is similar to the receptor number in the NIH Swiss mice (156 fmol/mg of protein), a strain that is highly susceptible to convulsions in response to SNC80 or BW373U86 (Comer et al., 1993a; Hong et al., 1998). All animals responded robustly to pentylenetetrazole, suggesting that resistance to δ-mediated convulsions was not due to an inability to convulse. Genetic differences, however, might be manifested downstream of the δ system such that the correlation between antinociception and convulsive susceptibility in the DOR-1(+/+) mice is a coincidence. For example, SNC80 administered i.p. to male ICR mice causes a full antinociceptive response (with an ED50 value of 57 mg/kg), yet only 4 to 10 mice show convulsive behavior at 100 mg/kg SNC80 (Bilsky et al., 1995). It will be of obvious interest to compare mice of different genetic backgrounds to determine whether there is a consistent re-

![Fig. 5. The effect of naltrindole isothiocyanate (5'-NTII) on BW373U86-mediated convulsions and antinociception ± S.E.M. Single doses of 5'-NTII were administered 24 h before 10 mg/kg BW373U86 (s.c.). After BW373U86 administration, animals were observed for agonist-induced convulsive activity for 20 min and then subjected to the acetic acid-induced abdominal stretch assay, as described under Materials and Methods. n = 6 animals/group. * P < 0.01; ** P < 0.001 compared with BW373U86 alone.](image)

![Fig. 6. Saturation binding of [3H]DPDPE to membranes prepared from whole brain (cerebellum) of mice treated with vehicle (45% 2-hydroxypropyl-β-cyclodextrin) or 32 mg/kg 5'-NTII 24 h before sacrifice, as described in Materials and Methods. Points represent data from three drug-treated and three vehicle-treated animals, each assayed in duplicate. The curve for the 5'-NTII-treated group was constructed assuming no change in ligand affinity (see text).](image)
relationship between δ-mediated convulsions and δ-mediated antinociception.

To provide clinically useful δ-opioid analgesics, it will be necessary to separate convulsive activity from antinociceptive activity. This would be feasible if different receptor subtypes were involved. In the current assays, however, the putative δ-subtype-selective antagonists NTB and BNTX were qualitatively similar in their action. The higher potency of NTB compared with BNTX in preventing the convulsive and antinociceptive effects of BW373U86 can be explained by a higher δ-receptor affinity (17- to 40-fold; Toll et al., 1998; Neilan et al., 1999) and a 4-fold better penetration into central nervous tissue (Lever et al., 1996) of NTB. Both NTB and BNTX afforded a complete antagonism of the convulsive activity of BW373U86 (up to 32 mg/kg; the maximum dose examined) but produced only a rightward shift in the dose-effect curve for antinociception. As the antagonists are reversible, this suggests a greater degree of shift for convulsive activity (at least 30-fold with 1 mg/kg BNTX and 100-fold with 0.1 mg/kg NTB) compared with antinociception (a 10-fold shift). The greater susceptibility of δ-mediated convulsions to the antagonists is unexpected if both actions are mediated by the same receptor. It is possible that BW373U86 is acting at non-δ receptors to produce antinociception. Certainly, the dose-effect curve for antinociception is shifted to a relatively greater extent by lower doses of antagonist than by higher doses, suggesting that the agonist is acting through an alternative mechanism in the presence of δ-receptor blockade by BNTX or NTB. This conclusion is supported by the known actions of BW373U86 at μ-opioid receptors (Chang et al., 1993; Comer et al., 1993b) and the fact that BW373U86 is an effective antinociceptive agent in DOR-1 knockout mice (Zhu et al., 1999).

The convulsive and antinociceptive effects of δ-agonists may have different receptor reserves and therefore different efficacy requirements. Both effects have similar EC50 values, so the difference in efficacy is not especially marked. If there were differences, however, then the system requiring higher efficacy would be more sensitive to the development of tolerance and to a reduction in δ-receptor number. There was a similar reduction in the susceptibility of mice to the acute convulsive and antinociceptive effects of a challenge dose of BW373U86 after single or chronic (3-day) dosing with BW373U86. Profound tolerance to BW373U86 developed rapidly, which is in agreement with previous observations (Comer et al., 1993a; Hong et al., 1998). Therefore, small differences in tolerance within the two systems were not readily identified. Administration of the δ-antagonist naltrindole 20 min after each daily dose of BW373U86 limited tolerance development to convulsions, presumably by displacing agonist from receptor such that a reduced level of adaptation was afforded (Comer et al., 1993a). Similar effects could presumably be obtained with lower doses of BW373U86. The procedure used, however, allowed convulsions to be scored to confirm that there was no refractoriness to convulsions independent of δ-opioid tolerance. In contrast, tolerance to the antinociceptive effect of BW373U86 was not reduced by postagonist administration of naltrindole. The continued convulsive response in the face of antinociceptive tolerance suggests a higher receptor reserve for convulsive activity that allowed this response to be maintained in the less efficient signaling environment induced by previous drug exposure. These findings are not likely to be due to the involvement of the putative δ1 and δ2 subtypes because naltrindole is not selective across δ-receptor subtypes and, as stated above, BNTX and NTB have similar effects on δ-mediated convulsions and antinociception.

In support of the hypothesis that convulsions and antinociception have different efficacy requirements, the irreversible antagonist 5'-NTII at a dose of 32 mg/kg completely suppressed the antinociceptive activity of BW373U86 but did not completely prevent convulsions. Lower doses of 5'-NTII (3 and 10 mg/kg) were able to partially antagonize δ-mediated antinociception without affecting convulsive activity. δ-Opioid receptor levels in brains from mice treated with 32 mg/kg 5'-NTII were approximately 25% of those in brains from vehicle-treated animals. Although a full dose-effect curve for BW373U86 was not constructed, these data are consistent with the notion that a greater receptor number, and therefore activation, is required for δ-mediated antinociception than for the δ-mediated convulsions.

Previous studies have suggested that 5'-NTII given intracerebroventricularly is a δ2 antagonist. This is based on the greater sensitivity of antinociception mediated by the putative δ2 agonists [d-Ala2]deltorphin II and [d-Ser4,Leu5,Thr6]enkephalin than the putative δ1 agonist DPDPE to pretreatment with 5'-NTII (Jiang et al., 1991; Mattia et al., 1992). Nevertheless, the present results show that peripherally administered 5'-NTII prevents the binding of [3H]DPDPE and the actions of peripherally administered BW373U86. This agrees with the finding that in vitro treatment of guinea pig brain membranes with 5'-NTII reduces the Bmax value for [3H]DPDPE binding (Portoghese et al., 1992b) but is in contrast to a report that treatment of mice with 10 mg/kg 5'-NTII does not alter [3H]DPDPE binding to striatal slices (Chakrabarti et al., 1993). The reasons for these discrepancies are unknown but may be due to the use of membranes rather than slice preparations or region-specific effects of 5'-NTII. Nevertheless, the current findings with 5'-NTII confirm the usefulness of the compound for reducing δ-receptor numbers following peripheral administration.

The present studies have demonstrated that the convulsions seen following administration of nonpeptidic δ-receptor agonists are mediated through the δ-receptor and that convulsive effects may be less susceptible to tolerance and a reduction in δ-opioid receptor numbers than antinociceptive effects. These data have been collected using a single measure of convulsive activity and a single measure of antinociception. Although blockade of the nociceptive stimulus due to i.p. injection of acetic acid requires low agonist efficacy, it is possible that differences could be minimized if other stimuli were used, for example hyperalgesia or allodynia. Nevertheless, the findings are consistent with the hypothesis that a greater ligand efficacy is required for antinociception than for convulsive activity of δ-opioid receptor agonists.

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References


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