Antagonistic Modulation Between the *Delta* Opioid Agonist BW373U86 and the *Mu* Opioid Agonist Fentanyl in Mice

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ABSTRACT

This study was performed to assess the interactions that occur between *delta*- and *mu* opioid receptors by studying effects of the systemically active nonpeptide *delta* agonist BW373U86 and the *mu* agonist fentanyl in mice. Concentrations of the compounds were varied, and analgesic responses were determined by 55°C hot-plate assays. BW373U86 produced hot-plate antinociceptive activity along with convulsive side effects. These effects could be antagonized by the selective *delta* antagonist naltrindole. Fentanyl produced hot-plate antinociceptive activity with Straub tail and hyperactivity as side effects. When BW373U86 and fentanyl were coadministered, BW373U86 convulsive activity was attenuated by fentanyl in a dose-dependent manner and the fentanyl-induced Straub tail effect was antagonized by BW373U86, also in a dose-dependent manner. Hot-plate analgesic activity was additive between the two compounds. The *delta* antagonist naltrindole partially antagonized the ability of BW373U86 to block the fentanyl-induced Straub tail effect. The *mu* antagonist β-funaltrexamine antagonized the fentanyl-induced blockade of the convulsive effects of BW373U86. These data suggest that complex inhibitory interactions take place between *mu* and *delta* receptors in mice. Future studies are clearly needed to study the neuromodulatory effects of *mu* and *delta* receptors. The widespread use of *mu* agonists in the clinic indicates that a large number of patients exist who could greatly benefit from the conjunctive use of *delta* pharmaceuticals.

Accumulating evidence suggests that interactions occur between *mu* - and *delta* opioid receptors (Lee and Smith, 1980; Rothman and Westfall, 1982; Vaught et al., 1982). There are also considerable data indicating that the antinociceptive effects of certain *mu* agonists can be increased or decreased by *delta* agonists. The first investigators to report such action (Vaught and Takemori, 1979) showed that an intracerebroventricular injection of 

\[ \text{Leu}^5 \text{enkephalin} \]

potentiates the antinociceptive action of morphine. Later, studies showed that the antinociceptive action of morphine can be antagonized by 

\[ \text{Met}^5 \text{enkephalin} \]

(Lee et al., 1980; Vaught et al., 1982). The agonistic and antagonistic effects of 

\[ \text{Leu}^5 \] and \[ \text{Met}^5 \] enkephalins apparently occur through the actions of *delta* receptors, because the effects can be blocked by selective *delta* antagonists (Cotton et al., 1984; Heyman et al., 1989a, 1989b; Ji et al. et al., 1990b; Portoghese et al., 1988b). All of these results indicate that antinociception can be modulated by an interaction between *delta* and *mu* receptors. However, only a few studies have investigated the interactive effects of *delta* receptor activation on other *mu* opioid effects; these studies include modulation of endotoxic shock in rats (D’Amato and Holaday, 1984; Holaday et al., 1986; Holaday and D’Amato, 1983) and modulation of *mu*-mediated changes in urinary bladder motility (Sheldon et al., 1989).

BW373U86 [(\pm)-4-(α-R)-α-(2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl]-N,N-diethylbenzamide] is a novel nonpeptide *delta* agonist that can cross the blood-brain barrier. *In vitro* evidence shows that BW373U86 has a higher affinity for *delta* opioid receptors (\(K_i = 1.8 \text{nM}\)) than for *mu* opioid receptors (\(K_i = 15 \text{nM}\)) in rat brain (Chang et al., 1993). BW373U86 is \(-700\) times more potent at *delta* receptors of mouse vas deferens than at *mu* receptors of guinea pig ileum (Chang et al., 1993). *In vivo* pharmacological studies indicate that BW373U86 primarily acts at *delta* receptors to produce a variety of effects in different species (Comer et al., 1993a, 1993b; Dykstra et al., 1993; Lee et al., 1993; Negus et al., 1993). The mild analgesic effects of BW373U86 are primarily mediated by *delta* opioid receptors at the spinal level (Wild et al., 1993). In rats, increased locomotor activity and inhibition of acoustic startle reflex are seen after *s.c.* or intraperitoneal administration of BW373U86, but no antinociceptive activity occurs in the hot-plate, tail-withdrawal, tail-flick or tail-pinch assays (Chang et al., 1993). *In vivo* characteristics of BW373U86 in mice include antinociceptive activity for the acetic acid-induced writhing and hot-plate assays, along with increased locomotor activity and convulsions (Comer et al., 1993a; Wild et al., 1993). We now report...
that data obtained with BW373U86 indicate that mu and delta receptors interact, resulting in a modulation of opioid side effects as well as antinociception in mice.

**Methods**

**Animals.** Male CD-1 mice (Charles River Laboratories, Raleigh, NC) weighing 24 to 34 g on injection were housed in groups of 15 in a temperature-controlled room (22±1°C) on a 12-hr dark/light cycle with food and water available ad libitum.

**Analgesic testing.** At the time of testing, 10 animals were placed in a clean cage and transported to the laboratory. The mice were given 1 hr to adapt to the new environment. Analgesic responses were determined using a hot plate with timer (Ugo Basile, Varese, Italy) and a temperature of 55±0.5°C as the noxious stimulus. Each mouse was removed from the hot plate when either a jumping escape response occurred, hind paws were licked or a maximal cutoff time of 50 sec was reached. Two initial preinjection analgesia readings were collected as control measures. The first was used as a habituation procedure and was disregarded. The second, taken 5 to 10 min later, was used as the pre-injection control baseline response time. Drugs were administered by an s.c. route of injection at the back of neck. Hot-plate measures were performed at 10-, 30- and 60-min time intervals after drug treatment. Thereafter, measurements were taken in 30-min intervals until drug action had terminated.

**Convulsion scoring.** Groups of 10 mice were injected s.c. with test drugs, and the percentage of animals that showed a seizure response was scored. To achieve a positive seizure score, subjects must have exhibited convulsive activity followed by a period of catalepsy. Convulsive activity was characterized by clonic movements that encompassed the entire body. The postictal period of catalepsy was characterized by a loss of locomotor activity for a 5- to 15-min period and a loss of the righting reflex. Normal behavior patterns were typically resumed after the cataleptic period.

**Straub tail measures.** Straub tail effect values were recorded by visual observation with the use of a protractor. Straub tail scores were recorded using the horizontal as 0 degrees and measuring the increase of tail curvature in 15-degree increments up to 90 degrees.

**Drugs.** Drugs used in the study were BW373U86 (Burroughs Wellcome, Research Triangle Park, NC), fentanyl citrate (Sigma Chemical, St. Louis, MO), midazolam (Hoffmann-LaRoche, Nutley, NJ), β-FNA hydrochloride (Research Biochemicals, Natick, MA) and NTI (synthesized according to Portoghese et al., 1988a). BW373U86 and the delta antagonist NTI (Portoghese et al., 1988b) were dissolved in a 0.9% NaCl solution to concentrations used in the text. β-FNA and fentanyl were dissolved using water to the required concentrations. Some dimethylsulfoxide (15% of the total volume) was used to enhance the solubility of midazolam. Fentanyl and BW373U86 were often coadministered. They were each dissolved in a stock solution, and then the required volume of each was pipetted to a separate vial to reach final concentrations. To ensure that receptors were saturated, the opioid antagonists and midazolam were administered at a set time before the individual agonist or mixed agonists were injected. Midazolam was injected 10 min and NTI was injected 20 min before mu and delta agonist administration. β-FNA was injected once every 24 hr over a 3-day period (total of three injections). Mu and delta agonists were administered 24 hr after the last β-FNA injection.

**Data analysis.** Statistical analyses were performed on all data using an analysis of variance, the Student’s t test or a χ² test, depending on the nature of the data. Differences were considered to be significant at a P < .05 level. ED₅₀ values were calculated with regression equations plotted on a probit scale. The following equation was used to determine the MPE score:

\[
MPE = \frac{\text{Postdrug latency} - \text{Predrug latency}}{50 - \text{Predrug latency}} \times 100\% 
\]  

(1)

**Results**

**Hot-plate antinociceptive response.** Although the delta opioid agonist BW373U86 was inactive in the hot-plate assay with rats (Chang et al., 1993), in mice the compound induced a time- and dose-dependent antinociceptive response in the hot-plate assay (figs. 1 and 2). As in the rat, BW373U86 appeared (by visual observation) to substantially increase locomotor activity. In mice, hyperactivity persisted for ~30 min, or during the period before the occurrence of a convulsion. The antinociceptive response of BW373U86 (F = 23.65, df = 4/2, P < .0001) peaked at 10 min after the injection, and the duration of action was short, lasting ~45 to 60 min at the highest dose tested (fig. 1). The antinociceptive effects of BW373U86 were produced with a 0.5 mg/kg dose and reached a nearly maximal effect at an s.c. dose of 10 mg/kg (fig. 2). The ED₅₀ values for the production of analgesic and seizure effects by BW373U86 were 2 and 5 mg/kg, respectively.

The mu agonist fentanyl was also observed to produce analgesia (ED₅₀ = 50 μg/kg), and the coadministration of BW373U86 and fentanyl produced additive antinociceptive effects (fig. 3). Results do not demonstrate a clear potentiality of analgesic activity (note the similarity of slopes between doses in fig. 3). Most importantly, analgesic effects of fentanyl were not decreased after BW373U86 administration, and thus the delta and mu agonists do not have antagonistic effects in a hot-plate paradigm. The BW373U86-induced hot-plate antinociceptive response was antagonized by the delta antagonist NTI (F = 27.8, df = 3/7, P < .0003), which did not affect fentanyl analgesia (F = 0.55, df = 2/6, P = NS; fig. 4A). Analgesia induced by 10 mg/kg BW373U86 s.c. was effectively blocked by a 0.5 mg/kg dose of NTI, suggesting that the hot-plate antinociceptive activity of BW373U86 was mediated by delta receptors. Antagonism of delta receptors does not, however, play a significant role in modulating the analgesic effects produced by fentanyl (fig. 4A). The anticonvulsant midazolam did not have analgesic
Convulsions produced by BW373U86 in mice were brief, nonreoccurring incidents that are typically followed by a period of catalepsy (Comer et al., 1993a). In the present study, the minimal dose needed to produce convulsions in at least 1 mouse in a group of 10 was 1 mg/kg s.c. Convulsive activity occurred in 80% of the animals with a dose of 10 mg/kg s.c. (fig. 5). The convulsive activity of BW373U86 is about 2.5-fold less potent than the antinociceptive activity (fig. 2). The administration of the delta antagonist NTI completely eliminated convulsions in a dose-dependent manner (fig. 3; \( \chi^2 = 30.9, df = 3, P < .0001 \)). A dose of 1 mg/kg of NTI completely eliminated convulsions induced by 10 mg/kg BW373U86. As observed by Comer et al. (1993a), BW373U86-induced convulsive activity was completely blocked by the anticonvulsant midazolam at a dose of 3.2 mg/kg s.c. (fig. 5; \( \chi^2 = 36.7, df = 1, P < .0001 \)).

The data from figure 2 do not reveal whether 373U86-induced increases in hot-plate latencies represent true antinociception or are, in part, a reflection of animals being behaviorally compromised by some degree of convulsive activity. Most importantly, the data from figures 4B and 5 indicate that the antinociceptive effect of BW373U86 is not a consequence of convulsive effects. When BW373U86 seizure activity is blocked by midazolam (fig. 5), the hot-plate antinociceptive response of BW373U86 still occurs (see fig. 4B).

Fentanyl did not have convulsive activity at doses tested up to 500 

\( \mu \)g/kg s.c. Administration of the mu agonist fentanyl did, in a dose-dependent manner, reduce the percentage of mice that had BW373U86-induced seizures (McNemar’s \( \chi^2 = 193.3, df = 6, P < .0001 \)) but did not completely eliminate all convulsions (fig. 6). These results lead to the conclusion that mu receptors can modulate BW373U86-induced convulsive activity. To confirm this, a study was performed using \( \beta-FNA, \) a specific and irreversible mu opioid antagonist (Jiang et al., 1990a, 1990b; Takemori and Portughese, 1985; Ward et al., 1982) to test the hypothesis that mu receptors can modulate BW373U86-induced convulsive activity.

Mice were injected (s.c.) a total of three times with 10 

\( \mu \)g/kg \( \beta-FNA, \) once a day over a 3-day period. The antinociceptive effect of fentanyl was significantly reduced from maximum levels to \( \sim \)50% of the MPE after \( \beta-FNA \) pretreatment (\( t = 4.36, df = 2, P < .05; \) fig 7A). The antinociceptive effect of BW373U86 was not affected by \( \beta-FNA \) pretreatment (\( t = 0.67, df = 5, NS \)). These data confirm that \( \beta-FNA \) treatment under the present protocol produced selective antagonistic effects at mu-, but not delta-, opioid receptors.

BW373U86-induced seizure activity was again observed to be significantly attenuated by fentanyl administration (fig. 7; \( \chi^2 = 21.5, df = 1, P < .0001 \)). Interestingly, the convulsive effect of BW373U86 appeared to be slightly decreased from 80% to 67% after \( \beta-FNA \) treatment, but the effect was not observed to be significant (\( \chi^2 = 0.63, df = 1, P = NS; \) fig. 7B).

It is unknown whether the current \( \beta-FNA \) treatment, by
itself, would have altered nociceptive or seizure thresholds; however, published reports of studies that used similar methods do not reveal any such effects of \( \beta \)-FNA (Heyman et al., 1989a; Jiang et al., 1991). \( \beta \)-FNA treatment did, however, antagonize the ability of BW373U86 (10 mg/kg s.c.) to produce seizures. The anticonvulsant midazolam (3.2 mg/kg s.c.) also blocked BW373U86-induced seizure activity at a dose that does not affect analgesia (see fig. 4). * Significant difference from BW373U86-treated control animals that did not receive NTI. Convulsive scores of BW373U86 in mice pretreated with NTI or midazolam are presented as mean ± S.E. with 30 mice in each dose group.

**Fig. 5.** Effect of the \( \delta \) antagonist NTI and midazolam on BW373U86-induced convulsions. NTI (0.2–1 mg/kg s.c.) dose-dependently antagonized the ability of BW373U86 (10 mg/kg s.c.) to produce seizures. The anticonvulsant midazolam (3.2 mg/kg s.c.) also blocked BW373U86-induced seizure activity at a dose that does not affect analgesia (see fig. 4). * Significant difference from BW373U86-treated control animals that did not receive NTI. Convulsive scores of BW373U86 in mice pretreated with NTI or midazolam are presented as mean ± S.E. with 30 mice in each dose group.

**Fig. 6.** Dose-response curves of NTI (0.1–1 mg/kg s.c.) on BW373U86 (10 mg/kg s.c.) and fentanyl (150 \( \mu \)g/kg s.c.) analgesic effects in a hot-plate assay. Data are presented as mean ± S.E. with 20 to 30 mice in each experiment. The administration of NTI dose-dependently antagonized BW373U86- but not fentanyl-induced analgesic effects. B, Hot-plate analgesic effects of BW373U86 (10 mg/kg s.c.) in mice pretreated with midazolam (3.2 mg/kg s.c.) and for midazolam alone. Analgesic effects of BW373U86 were still observed after midazolam administration at a dose that does block seizure activity (see fig. 5). Data are presented as mean ± S.E. with 30 mice in each dose group.

**Fig. 4.** A, Dose-response curves of NTI (0.1–1 mg/kg s.c.) on BW373U86 (10 mg/kg s.c.) and fentanyl (150 \( \mu \)g/kg s.c.) analgesic effects in a hot-plate assay. Data are presented as mean ± S.E. with 20 to 30 mice in each experiment. The administration of NTI dose-dependently antagonized BW373U86- but not fentanyl-induced analgesic effects. B, Hot-plate analgesic effects of BW373U86 (10 mg/kg s.c.) in mice pretreated with midazolam (3.2 mg/kg s.c.) and for midazolam alone. Analgesic effects of BW373U86 were still observed after midazolam administration at a dose that does block seizure activity (see fig. 5). Data are presented as mean ± S.E. with 30 mice in each dose group.

results indicate that the ability of fentanyl to block BW373U86-induced seizures is due to \( \mu \) receptor activity.

**BW373U86 inhibits fentanyl-induced Straub tail effect.** Muscle rigidity is one of the side effects of fentanyl that, in mice, results in a Straub tail effect. The degree of tail curvature increases proportionally as the dose of fentanyl is increased (fig. 8). The administration of BW373U86 decreased fentanyl-induced Straub tail in a dose-dependent manner \( (F = 69.4, df = 5/458, P < .0001; \text{fig. } 8) \). NTI was administered to mice in combination with BW373U86 and fentanyl to test the hypothesis that the BW373U86 inhibitory effect on fentanyl-induced muscle rigidity is the result of \( \delta \) receptor activation. Neither BW373U86 nor NTI alone induced the Straub tail effect in mice (fig. 9). Results depicted in figure 9 illustrate that fentanyl produced ~30 degrees of Straub tail curvature at a 150 \( \mu \)g/kg dose s.c. BW373U86, at a 10 mg/kg dose, almost completely inhibited the Straub tail effect induced by 150 \( \mu \)g/kg fentanyl \( (t = 8.3, df = 58, P < .0001) \). NTI (0.5 mg/kg s.c.) also reduced Straub tail curva-
ture from 30 degrees to ~18 degrees \( (t = 3.23, df = 58, P < .002) \) but did not eliminate the occurrence of Straub tail (fig. 9). A possible explanation for this effect is that NTI does have some affinity for \( \mu \) receptors and thus can exhibit \( \mu \) antagonist effects (Portoghese et al., 1988b). When fentanyl, BW373U86 and NTI were coadministered, the degree of curvature was ~15 degrees, which was significantly different from the 5-degree curvature observed in subjects treated with fentanyl alone (fig. 9). Results indicate that NTI administration can, at least partially, antagonize the ability of BW373U86 to block fentanyl-induced Straub tail. The degree of tail curvature was returned to the level produced by NTI alone (fig. 9). Results indicate that the \( \delta \) agonist BW373U86 can inhibit fentanyl-induced Straub tail through direct effects on \( \delta \) receptors.

**Discussion**

Previous reports (Comer et al., 1993a; Wild et al., 1993) described antinociceptive effects of BW373U86 in acetic acid-induced writhing and tail-flick tests in mice. The present studies confirm the antinociceptive activity of BW373U86 in a 55°C hot-plate test in mice. Analgesic effects of BW373U86 do not appear to be a consequence of seizure activity, because antinociceptive effects are still observed when seizure activity is blocked with the anticonvulsant midazolam (figs. 4 and 5). The hot-plate antinociceptive activity of BW373U86 was antagonized by NTI at doses of <1 mg/kg. In contrast, the irreversible \( \mu \) antagonist \( \beta \)-FNA, under conditions that significantly reduce the antinociceptive response of the \( \mu \) agonist fentanyl, did not affect the antinociceptive effect of BW373U86. Convulsive activity induced by BW373U86 was also antagonized by NTI in a dose-dependent manner (fig. 5). The data provide evidence that BW373U86 produces antinociceptive and convulsive effects in mice through actions at NTI-selective \( \delta \) receptors.
A potentiation and modulation of the antinociceptive response of morphine by delta agonist peptides has been previously documented (Heyman et al., 1989a, 1989b; Vaught and Takemori, 1979). Dykstra et al. (1993) also demonstrated that BW373U86 potentiates morphine and l-methadone antinociception in an electric shock titration assay in monkeys. In the present study, we were unable to demonstrate a clear potentiation by BW373U86 of the antinociceptive response of fentanyl in the mouse hot-plate test. The antinociceptive effect of fentanyl and BW373U86 in our hot plate test was purely additive. This finding is not surprising because the potentiating effect of delta agonists has not been reported for highly efficacious mu agonists (i.e., [d-Ala²,N-MePhe⁴,Gly⁵]enkephalin, PL 017 and sufentanil; Heyman et al., 1989b). Fentanyl is known to be a highly effective mu agonist (Jiang et al., 1990b; Porreca et al., 1992; Stevens and Yaksh, 1989), and thus BW373U86 would not be expected to potentiate analgesia induced by fentanyl.

The interactive modulation of mu and delta receptor effects can be extended to effects of the nonpeptide delta agonist BW373U86 and the selective mu agonist fentanyl. In mice, BW373U86 induces seizure activity via delta receptor activation. The mu opioid agonist fentanyl can antagonize the ability of BW373U86 to produce seizures. Additionally, the selective mu agonist β-FNA significantly blocked the ability of fentanyl to inhibit seizures produced by BW373U86 (fig. 7). Thus, the ability of BW373U86 to produce seizures can be attenuated by fentanyl through an agonist action at mu receptors.

Fentanyl also induces muscle rigidity, such as Straub tail, through mu receptor activation. Conversely, fentanyl-induced muscle rigidity can be inhibited by the delta agonist BW373U86. Apparently, delta receptor effects of BW373U86 inhibit mu agonist-induced muscle rigidity, because the delta antagonist NTI can partially antagonize the ability of BW373U86 to inhibit fentanyl-induced Straub tail (fig. 9). The present data indicate that a delta opioid agonist can attenuate some effects of a mu agonist (i.e., muscle rigidity) and a mu agonist can antagonize some effects of a delta agonist (i.e., seizure activity). Interestingly, the delta agonist BW373U86 and the mu agonist fentanyl can selectively antagonize each other's effects because a combination of the two agonists results in additive analgesic effects.

It is curious that Straub tail was not observed after fentanyl and BW373U86 administration, when Straub tail has been reported to be produced by the delta peptide DPDPDE (Murray and Cowan, 1990). We have never observed a Straub tail response in male CD1 mice after the administration of specific nonpeptide delta agonists. As suggested by Murray and Cowan (1990), the Straub tail response seen after DPDPDE administration is probably due to mu receptor activation. The question of why delta effects of DPDPDE failed to block the mu-induced Straub tail remains unanswered, but critical factors could be the strain of mouse used or pharmacodynamic differences produced by the route of administration (s.c. vs. intracerebroventricular).

A previous report described the existence of a mu and delta interaction in a rat model of opioid dependence. Lee et al. (1993) demonstrated that BW373U86 does not produce physical dependence alone but can attenuate the development of abstinence precipitated by naloxone in morphine-dependent rats. Our data clearly indicate that a complex in vivo interaction takes place between delta- and mu-mediated effects in mice. It will be interesting to find out whether similar interactions occur in other species and with other opioid effects.

In conclusion, many complex interactions occur between mu and delta receptors. These effects contribute to the modulation of antinociception and side effects. In our study, BW373U86 decreased fentanyl-induced muscle rigidity (e.g., Straub tail), whereas fentanyl decreased the convulsions caused by BW373U86. Coadministration of both compounds produced an additive analgesic effect in a mouse hot-plate assay. Further studies are clearly needed to provide a detailed understanding of the intricate interaction that takes place between delta- and mu opioid receptors.

References


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