Butorphanol-Mediated Antinociception in Mice: Partial Agonist Effects and Mu Receptor Involvement1,2

H. R. GARNER,3 TIMOTHY F. BURKE,4 C. DAVID LAWHORN,5 JOANNE M. STONER and WILLIAM D. WESSINGER

Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas

Accepted for publication May 23, 1997

ABSTRACT

In the present experiments, we characterized the agonist and antagonist effects of butorphanol in mice. In the mouse radiant-heat tail-flick test, the mu agonists morphine and fentanyl and the kappa agonist U50,488H were fully effective as analgesics, whereas butorphanol was partially effective (producing 82% of maximal possible analgesic effect). Naltrexone was approximately equipotent in antagonizing the effects of morphine, fentanyl and butorphanol; in vivo apparent pKA values for these naltrexone/agonist interactions were 7.5 (unconstrained). Naltrexone was ~10 times less potent in antagonizing the effect of U50,488H (average apparent pKb = 6.7). The selective mu antagonist b-funaltrexamine (0.1–1.0 mg/kg) antagonized the effects of butorphanol in a dose-dependent insurmountable manner. Pretreatment with nor-binaltorphimine (32 mg/kg), a kappa-selective antagonist, did not reliably antagonize butorphanol, and naltrindole (20 and 32 mg/kg), a delta-selective antagonist, failed to antagonize the effects of butorphanol. Low doses of butorphanol (1.0, 1.8 or 3.2 mg/kg) caused parallel, rightward shifts in the dose-effect curve for morphine and parallel leftward shifts in the dose-effect curve for U50,488H. Taken together, the results of this study suggest that butorphanol is a partial agonist in the mouse radiant-heat tail-flick test and that activity at mu receptors accounts for the majority of its antinociceptive effects.

Clinically, opioids are most commonly used to provide relief of moderate-to-severe pain. The analgesics used for this purpose are predominantly mu agonists, although some effective analgesics possess significant activity at other opioid receptors. A vast number of analogs and congeners have been synthesized in an attempt to obtain compounds that retain the analgesic properties of morphine but have fewer adverse effects and lower abuse liability. Although these efforts have not resulted in the development of the “ideal opioid,” they have generated numerous unique compounds that vary widely in terms of opioid receptor-related properties such as selectivity, affinity and intrinsic efficacy. In addition to possessing substantial clinical utility, a number of these synthetic compounds have been instrumental in advancing basic research knowledge of opioid mechanisms. Some synthetic compounds, such as butorphanol and buprenorphine, appear to have “mixed” opioid actions; that is, they act as agonists or antagonists at multiple opioid receptors. Butorphanol is a widely used and potent analgesic with lower, although still significant, abuse potential than morphine and fentanyl (Brown, 1985; Evans et al., 1985; Smith and Davis, 1984). Over the past few years, butorphanol has gained increased clinical importance as an analgesic, a fact made clear by its recent release in a transnasal formulation (Shyu et al., 1993). Moreover, it was recently reported that butorphanol is effective in preventing the adverse side effects associated with morphine use in adults (Lawhorn et al., 1991) and in children (Lawhorn and Brown, 1994), suggesting that the antagonist actions of butorphanol can also have a clinical benefit.

Butorphanol exhibits both mu and kappa agonist actions depending on the animal species and experimental conditions used (e.g., Butelman et al., 1995; Dykstra, 1990; Preston and Bigelow, 1994). In radioligand binding experiments in monkey brain, butorphanol displaces mu, kappa and delta opioids with >10-fold binding selectivity for mu vs. kappa and >30-fold mu vs. delta selectivity (Butelman et al., 1995). A similar profile of binding selectivity was observed in rodent brain (Chen et al., 1992; Horan and Ho, 1989). Moreover, butorphanol is characterized as a low efficacy or partial agonist and exhibits antagonist action at mu and kappa recep-

ABBREVIATIONS: %MPE, % maximum possible effect; \( \beta \)-FNA, \( \beta \)-funaltrexamine; nor-BNI, nor-binaltorphimine; CL, confidence limit.
The particular effect is mediated. For example, in a study in opioid receptors renders this analysis a useful tool in characterizing the opioid receptor population through which a particular effect is mediated. Several investigators have compared the potency of competitive antagonists such as naltrexone, naltrexone and quazocine to block various effects of opioid agonists to make inferences about mechanisms of action. For example, in a study in which the response rate-decreasing effects of various opioid agonists were examined, naltrexone is more potent as an antagonist of the effects of morphine, a mu agonist, than of ethylketocyclazocine, a kappa agonist (Harris, 1980). These results were interpreted as evidence that the rate-decreasing effects of morphine and ethylketocyclazocine are mediated by separate opioid receptor populations for which naltrexone has different affinities. Analyses of results from this type of competitive antagonism study have broadened to include the in vivo apparent pA2 analysis (Tallarida et al., 1979). This analysis has been used to determine homogeneity of receptor populations and make inferences about agonists and antagonists with respect to the pharmacological receptor through which they produce their effects (Bertalario and Woods, 1987; Shannon et al., 1986; Walker et al., 1994; Wessinger and McMillan, 1986). The apparent pA2 analysis provides a measure of the potency of an antagonist in blocking the effects of an agonist and is defined as the negative logarithm of the dose of the antagonist that produces a 2-fold rightward shift in the dose-effect curve of the agonist alone. The differential affinity of the competitive opioid antagonists for the mu, kappa and delta opioid receptors renders this analysis a useful tool in characterizing the opioid receptor population through which a particular effect is mediated. For example, in a study in which the antinociceptive effects of various opioid compounds in mice were examined, apparent pA2 values for naltrexone in combination with mu agonists were higher than pA2 values for naloxone in combination with kappa agonists (Ward and Takemori, 1983). Determination of apparent pA2 values requires that the dose-effect curve of an agonist be redetermined several times in the presence of different doses of the antagonist. A less widely used analysis, the apparent pK2 analysis, requires the determination of an agonist dose-effect curve in the presence of only one dose of the antagonist (Nagus et al., 1993). Like the apparent pA2 analysis, the apparent pK2 analysis is used to compare the potency of antagonists in blocking the effects of agonists, but it is typically used in situations in which it is not possible to redetermine an agonist dose-effect curve in the presence of multiple doses of the antagonist (e.g., limited supply of the antagonist). In the present study, these analyses are used to compare the antinociceptive effects of morphine, fentanyl and U50,498H with those of butorphanol in the mouse radiant-heat tail-flick test and to make inferences about the receptor population or populations that mediate these effects. To this end, the effects of the competitive antagonist naltrexone on the antinociceptive effects of these agonists were examined in the mouse radiant-heat tail-flick test.

Another goal of the present study was to use highly specific antagonists for mu, kappa and delta receptors (e.g., beta-FNA, nor-BNI or naltirindole, respectively) to further define the receptor population that mediates the antinociceptive effects of butorphanol. The development of opioid antagonists highly specific for the mu, kappa and delta receptors has played a critical role in distinguishing the individual opioid receptor types that mediate the effects of various opioids (e.g., Broadbear et al., 1994; Sofuoglu et al., 1991; Spanagel et al., 1994; Ward et al., 1982). In the mouse abdominal stretch test for antinociception, beta-FNA pretreatment produced a marked rightward shift in the dose-effect curve for butorphanol, suggesting a major role for the mu receptor in its analgesic actions (Zimmerman et al., 1987). However, other investigators have classified the analgesic effects of butorphanol as being kappa mediated (e.g., Houde, 1979; Vogelsang and Hayes, 1991). Given the uncertainty surrounding the mechanisms of the analgesic action of butorphanol, we examined these effects after pretreatment with naltrexone, beta-FNA, nor-BNI or naltirindole in the mouse radiant-heat tail-flick test. Finally, because it has been classified as a low efficacy agonist with antagonist properties and because the antagonist effects of butorphanol may have clinical importance, the ability of butorphanol to antagonize the antinociceptive effects of morphine and U50,498H was also examined.

**Methods**

**Animals.** The animals were experimentally naive, male ND4 Swiss-Webster mice (Harlan Sprague Dawley, Indianapolis, IN). A total of five mice were used for each point on all dose-effect curves unless otherwise indicated. At the time of use, mice weighed ~25 to 30 g. Before use, mice had unlimited access to food (PMI Feed, St. Louis, MO) and water and were housed in groups of five in a vivarium maintained on a normal phase 12-hr light/dark cycle.

**Apparatus and antinociception tests.** An adaptation of the radiant-heat tail-flick procedure of D’Amour and Smith (1941) was used. Analgesia testing was conducted with a tail-flick apparatus (model TP-6; Emdie Instruments, Richmond, VA) that used a beam of light as the thermal nociceptive stimulus. An animal’s tail was positioned covering a photocell under the light beam. Illumination of the light started an automatic timer. The lamp was extinguished and the timer was stopped when the photocell was exposed after a mouse “flicked” its tail out of the beam. The lamp automatically extinguished after 10 sec to prevent thermal injury to the tail. The intensity of the lamp was adjustable and set so control tail-flick reaction times fell within 2 to 4 sec in control measurements. A small...
number of animals (<2%) with control reaction times outside this range were excluded from the study.

Mice were initially injected with saline; ~15 min later, a base-line (control) reaction time was determined. Agonist dose-effect curves were determined by the administration of fentanyl, morphine, butorphanol or U50,488H 20 min before redetermination of the tail-flick reaction times. In antagonism experiments, a pretreatment dose of the antagonist (naltrexone, β-FNA, nor-BNI or naltrindole) was initially administered. After the appropriate antagonist pretreatment time (i.e., naltrexone, 20 min; naltrindole, 25 min; β-FNA or nor-BNI, 24 hr), the tail-flick reaction time was measured and then a dose of the agonist was administered. At 20 min after the agonist injection, tail-flick reaction times were measured. Injections of β-FNA, nor-BNI or naltrindole were administered subcutaneously, whereas all other agonists and antagonists were administered.intraperitoneally.

In other experiments, butorphanol (1.0, 1.8 or 3.2 mg/kg) was administered before morphine or U50,488H. At 15 min later, the tail-flick reaction time was measured, and then a dose of the agonist was administered. At 20 min after administration of the agonist, tail-flick reaction times were redetermined.

Data analysis. The analgesic response was calculated as %MPE using the following equation:

\[
\% \text{MPE} = \frac{\text{Test reaction time} - \text{control reaction time}}{10 \text{ sec} - \text{Control reaction time}} \times 100
\]

After administration of an agonist or antagonist, if a mouse removed its tail faster than the control latency, a value of 0%MPE was assigned. Group mean ± S.E.M. values are presented and were determined by averaging individual data from all mice tested under a given condition. A test drug was considered to be fully effective if ≥90%MPE was obtained.

Least-squares linear regression analysis of the linear portion of the dose-effect curves was used to estimate the ED_{50} value, or the dose that would be expected to result in 50%MPE. The slopes of the dose-effect curves for the agonists in combination with antagonists were compared with those of the agonists alone using a parallel line assay (Tallarida and Murray, 1987). If slopes did not differ, apparent pA_{2} values were determined by constructing Schild plots (Arunlaksana and Schild, 1959) using drug doses instead of drug concentrations (Takemori, 1974). The apparent pA_{2} value represents the dose of the antagonist that would be expected to produce a 2-fold shift to the right of the dose-effect curve for the agonist alone. For Schild plot analysis, dose ratios were calculated by dividing the ED_{50} value of each agonist in combination with each dose of naltrexone by the ED_{50} for each agonist alone. The log of the dose ratios ~1 (log DR = 1) was plotted as a function of the negative log of the molar dose of the antagonist (mol/kg). A regression line was fitted to these points. Slopes of the Schild plots were considered different from unity if the 95% CL of the slope did not include ~1. If the slopes of Schild regression were not significantly different from unity, the regression line was redetermined with the slope constrained to ~1. The intercept of the Schild regression line on the abscissa is the apparent pA_{2} value. Apparent pA_{2} values from unconstrained and constrained Schild plots are reported here for comparison.

In one instance, the slope of the regression line was determined to be statistically different from ~1 (i.e., dose-effect curves were not all parallel to the initial control curves). In this case, an apparent pK_{B} value was calculated using a modification of the equation: DR = B/K_{B} (Tallarida et al., 1979), where B is the antagonist dose in mol/kg. Rearranging and taking the negative logarithm of both sides yield the following equation for determining pK_{B}: pK_{B} = -\log[B/(DR - 1)], where DR refers to the dose ratio. The apparent pK_{B} value is used to estimate the potency of an antagonist such as naltrexone to attenuate the effect of various agonists (Kenakin, 1987).

Drugs. Morphone sulfate was obtained from Mallinkrodt (St. Louis, MO). Butorphanol tartrate was a gift from Bristol-Myers Squibb Pharmaceutical Research Institute (Princeton, NJ). Naltrexone hydrochloride and fentanyl hydrochloride were provided by the National Institute on Drug Abuse (Rockville, MD). β-FNA, nor-BNI and naltrindole, all as hydrochloride salts, were obtained from Tocris-Cookson (Langford, Bristol, UK). U50,488H (trans-2-(3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulfonate hydrate) was generously supplied by The Upjohn Co. (Kalamazoo, MI). Drugs were dissolved in 0.9% physiological saline to an injection volume of 10 ml/kg. For nor-BNI to dissolve, a drop of lactic acid was added to the solution. Doses administered are expressed as mg/kg and refer to the salt, except in the apparent pA_{2} analyses, in which naltrexone doses were converted to mol/kg.

Results

Effects of agonists alone. Morphine, fentanyl, U50,488H and butorphanol produced dose-dependent increases in tail-flick latency (fig. 1). Maximal antinociception (i.e., >90%MPE) was observed after morphine, fentanyl and U50,488H. The dose-effect curve for butorphanol in figure 1 is the mean of two determinations [from the naltrexone antagonism studies and from the selective antagonist studies]. The ED_{50} (95% CL) for this mean curve was 27.6 mg/kg (19.7–38.8). Butorphanol, at the highest dose that could be administered (100 mg/kg), produced only partial analgesia (82%MPE). Doses of butorphanol of >100 mg/kg produced convulsions and were lethal within ~2 to 5 min. High doses of naltrexone (10 mg/kg) were unable to prevent this toxicity, suggesting it was a nonopioid effect. Likewise, doses of U50,488H of >180 mg/kg also produced convulsions and were lethal in all mice within ~5 to 10 min. Again, high doses of naltrexone (10 mg/kg) were unable to prevent this effect. The relative order of potency of these agonists in the mouse radiant-heat tail-flick test was fentanyl >> morphine >> U50,488H ≈ butorphanol.

Naltrexone antagonism of agonist effects. Naltrexone pretreatment (0.003–1.0 mg/kg) produced dose-dependent, parallel rightward shifts in the dose-effect curves for morphine, fentanyl and butorphanol alone (fig. 2, table 1). There was a significant difference (at P < .01; 26 df) between the slopes of the dose-effect curves for U50,488H alone and for U50,488H plus 1.0 mg/kg naltrexone; however, the slopes of the dose-effect curves for the other agonists (morphine, fentanyl or butorphanol) in combination with naltrexone were
not statistically different from parallel to the dose-effect curve for the agonists alone. Doses of morphine of 0.560 mg/kg (e.g., 1000 mg/kg), administered after pretreatment with 1.0 mg/kg naltrexone, were lethal to all animals.

Figure 3 shows the Schild plots for naltrexone as an antagonist of the antinociceptive effects of morphine, fentanyl and butorphanol. Apparent pA₂ values (determined from Schild plots with the slopes unconstrained) for these interactions were 7.5. Table 2 shows the similar apparent pA₂ values obtained from unconstrained and constrained Schild plot analyses. Because naltrexone did not produce consistent parallel shifts in the U50,488H dose-effect curve, apparent pA₂ values were not determined. However, to obtain an estimate of the potency of naltrexone as an antagonist of the analgesic effects of U50,488H, pKB values for each dose of naltrexone (0.01, 0.1 and 1.0 mg/kg) in combination with U50,488H were calculated. Apparent pKB values for U50,488H in combination with 0.01, 0.1 or 1.0 mg/kg naltrexone were 7.4, 6.6 and 6.2, respectively, and the average apparent pKB value was 6.7.

**Antagonism of butorphanol effects with β-FNA, nor-BNI and naltrindole.** Doses of 0.1, 1.0 and 10 mg/kg β-FNA administered 24 hr before butorphanol produced a dose-related antagonism of the analgesic effects of butorphanol in the mouse radiant-heat tail-flick test (fig. 4, top). Pretreatment with 0.1 mg/kg β-FNA produced a nonparallel, rightward shift in the dose-effect curve for butorphanol alone (at P < .05, 31 df) and caused a decrease in the maximum analgesic effect produced by higher doses of butorphanol (i.e., butorphanol alone produced 82%MPE; butorphanol plus 0.1 mg/kg β-FNA produced 64%MPE). The ED₅₀ (95% CL) values for butorphanol alone and butorphanol plus 0.1 mg/kg β-FNA were 31.9 mg/kg (20.6–57.4) and 82.3 mg/kg (60.7–136.2), respectively. Pretreatment with 1.0 mg/kg β-FNA produced a further decrease in the maximum level of analgesic effect (i.e., 37%MPE). Nearly complete antagonism of butorphanol antinociception was achieved with 10 mg/kg β-FNA pretreatment (i.e., 17%MPE).

Pretreatment with 10 mg/kg nor-BNI produced a significant, ~2-fold, rightward shift in the dose-effect curve for butorphanol alone (fig. 4, center). The ED₅₀ (95% CL) values for butorphanol alone and butorphanol plus 10 mg/kg nor-BNI were 31.9 mg/kg (20.6–57.4) and 74.4 mg/kg (38.6–299.7), respectively. In contrast, a higher dose of nor-BNI, 32 mg/kg, failed to significantly shift the dose-effect curve for butorphanol; the ED₅₀ (95% CL) was 35.2 mg/kg

**TABLE 1**

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED₅₀ (95% CL) values for the analgesic effects of opioid agonists alone and in combination with naltrexone in the mouse tail-flick analgesic test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Naltrexone dose</td>
</tr>
<tr>
<td></td>
<td>Alone</td>
</tr>
<tr>
<td>Morphine</td>
<td>11.8  (8.7–16.7)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.11  (0.09–0.13)</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>24.3  (14.2–45.8)</td>
</tr>
<tr>
<td>U50,488H</td>
<td>17.4  (12.6–23.9)</td>
</tr>
</tbody>
</table>

Data are from dose-effect curves shown in figure 2.
Neither pretreatment dose of nor-BNI caused a decrease in the maximum level of analgesic effect produced by higher doses of butorphanol. The %MPE was 82% after 100 mg/kg butorphanol alone and 83% and 82% after 100 mg/kg butorphanol plus 10 or 32 mg/kg nor-BNI, respectively.

Pretreatment with naltrindole (20 or 32 mg/kg) had no effect on the dose-effect curve for butorphanol alone (fig. 4, bottom). The ED₅₀ (95% CL) values for butorphanol in combination with 20 and 32 mg/kg naltrindole were 29.8 mg/kg (17.7–44.8) and 41.5 mg/kg (28.2–61.2). Pretreatment with naltrindole also had little effect on the maximum level of antinociception produced by higher doses of butorphanol alone. The analgesic effect of 100 mg/kg butorphanol alone was 82% MPE, and in combination with 20 or 32 mg/kg naltrindole, the analgesic effects were 91.4% MPE and 78% MPE, respectively.

**Discussion**

Many of the actions of butorphanol in the mouse radiant-heat tail-flick test were consistent with those of a partial μ agonist. In vivo apparent pA₂ analyses showed that the interactions of naltrexone and butorphanol were similar to the interactions of naltrexone with other μ agonists. β-FNA, the selective μ antagonist, produced an insurmountable antagonism of butorphanol analgesic effects, whereas antagonists specific for κappa or delta opioids produced inconsis-

---

**Fig. 4.** Antinociceptive effects of butorphanol alone and after the administration of the antagonists: β-FNA (top), nor-BNI (middle) and naltrindole (bottom). The same butorphanol dose-effect curve is depicted in each panel. The antagonists were administered (subcutaneously) 24 hr (β-FNA or nor-BNI) or 25 min (naltrindole) before administration (intraperitoneally) of butorphanol. Antinociceptive effects were assessed 20 min later and are expressed as %MPE. All points represent mean ± S.E.M. values (n = 5).

**Fig. 5.** Effects of various doses of butorphanol administered 15 min before morphine (top) or U50,488H (bottom). Antinociceptive effects were assessed 20 min after morphine or U50,488H administration and are expressed as %MPE. All drugs were administered intraperitoneally. All points represent mean ± S.E.M. values (n = 5).
tent effects or were ineffective. The partial mu agonist effects of butorphanol were also manifested by its antagonist actions when low doses of butorphanol were combined with morphine. There was some evidence that actions of butorphanol in the present studies could also be mediated by kappa receptors. First, the selective kappa antagonist nor-BNI was effective in antagonizing the analgesic actions of butorphanol at 10 mg/kg (however, it was not effective at 32 mg/kg). Second, when low doses of butorphanol were combined with doses of U50,488H, the dose-effect curve for U50,488H shifted to the left, suggesting a coagonist effect.

Butorphanol was effective as an analgesic in the mouse radiant-heat tail-flick test, but less-than-maximal analgesia was obtained. It should be noted that the inability of butorphanol to produce maximal analgesic effects may have been confounded by a ceiling effect in that doses of >100 mg/kg were lethal. In other studies with assays in which efficacy requirements are high (e.g., 55–56°C water in the tail-withdrawal test or a schedule of shock titration), butorphanol is only partially or ineffective in producing analgesia (Butelman et al., 1995; Dykstra, 1990; Morgan and Picker, 1996; O’Callaghan and Holtzman, 1975). In contrast, butorphanol produces maximum analgesia in the mouse abdominal stretch test (Zimmerman et al., 1987) and in the warm water tail-withdrawal test when lower temperatures were used (Butelman et al., 1995; Morgan and Picker, 1996). In current studies, the selective mu agonists morphine and fentanyl produced dose-related increases in antinociception and were fully effective. This is consistent with other studies using different animal models and high efficacy requiring assays (Morgan and Picker, 1996; Walker et al., 1994; Ward and Takemore, 1983). Likewise, U50,488H also produced dose-related increases in antinociception and maximal analgesia (i.e., 100% MPE was obtained). This finding is in contrast to previous investigations suggesting that kappa agonists are not as effective as mu agonists in rodent analgesia assays (Dykstra, 1985; Porreca et al., 1984; Upton et al., 1982); however, it is consistent with reports that kappa agonists are highly effective analgesics in rhesus monkeys (Dykstra et al., 1987a, 1987b).

The antinociceptive effects of butorphanol were sensitive to the antagonist effects of naltrexone and permitted the use of in vivo apparent $p_A^2$ analysis for characterization of the receptor populations through which butorphanol produces its agonist effects (Dykstra et al., 1988; Shannon et al., 1986; Takemori, 1974). Classification of agonists in terms of the opioid receptor population through which they produce their effects (i.e., analgesic, discriminative-stimulus, respiratory depressive effects, etc.) is an important step in understanding tolerance and physical dependence (Feng et al., 1994; Horan and Ho, 1991), drug interactions (Dykstra, 1990; Young et al., 1992) and efficacy questions (Morgan and Picker, 1996; Picker et al., 1990). If similar $p_A^2$ values for a given antagonist against different agonists (either full or partial) are obtained, then it is considered presumptive evidence that the agonist/antagonist interactions are mediated through the same receptor population (e.g., Tallarida et al., 1979). It should be emphasized that the utility of apparent $p_A^2$ analyses in the present study depends on the differential affinity of naltrexone for mu, kappa and delta opioid receptors; this approach would not be useful to distinguish between receptors or receptor subtypes for which the antago-
ing the range over which the dose-effect curve could be
shifted by naltrexone. This limitation of using U50,488H as
an agonist in the present assay could account for this
discrepancy. Because not all dose-effect curves for the naltrex-
ome/U50,488H interaction were parallel, apparent \( \alpha \) anal-
ysis for this case was considered inappropriate. Analysis of
\( \alpha \) values, however, indicate that naltrexone was \(-10\) times
less potent in antagonizing the antinociceptive effect of
U50,488H than of morphine, fentanyl and butorphanol. That
naltrexone produced rightward shifts in the U50,488H dose-
effect curve at doses that were \(-10\) times the doses required
to antagonize the antinociceptive effects of morphine and
fentanyl suggests that U50,488H antinociception was not
due to activity at \( \mu \) receptors. Previous \textit{in vivo} and \textit{in vitro}
studies show that naltrexone is much less potent in antago-
nizing the effects of U50,488H (Dykstra, 1990; Takemori and
Portoghese, 1984).

Previous studies show that \( \beta \)-FNA is a highly specific for
\( \mu \) receptors in both \textit{in vitro} and \textit{in vivo} assays (Takemori et al.,
1981; Zimmerman et al., 1987). In the present study,
\( \beta \)-FNA produced a dose-related antagonism of the analgesic
activity of butorphanol. Pretreatment with (0.1 mg/kg) of
\( \beta \)-FNA produced a 2.6-fold rightward shift in the dose-effect
curve for butorphanol, although it was not parallel to control.
This dose of \( \beta \)-FNA caused a “flattening” of the dose-effect
curve, decreasing the maximum level of analgesia from
84\%MPE to 63\%MPE. Theoretically, this lack of parallelism
would be expected for the interaction of full or partial ago-
nists in combination with irreversible or insurmountable an-
tagonists (Ruffolo, 1982). Furthermore, the lack of parallel-
ism of the \( \beta \)-FNA-produced shift in the dose-effect curve of
butorphanol is consistent with a previous study in which
\( \beta \)-FNA antagonized the antinociceptive effects of various
mixed agonist/antagonists, including butorphanol, in the
mouse abdominal stretch test (Zimmerman et al., 1987). Re-
results differ, however, in terms of the magnitude of the shift
produced. This discrepancy can probably be explained by the
much larger pretreatment dose of \( \beta \)-FNA (i.e., 80 mg/kg) that
was used in the previous study, as well as species (rat vs.
mouse) or procedural (abdominal stretch test vs. radiant-heat
tail-flick test) differences. In the report by Zimmerman et al.,
(1987), 80 mg/kg \( \beta \)-FNA produces a 72.4-fold shift in the
butorphanol dose-effect curve. Although the dose-effect curve
generated by pretreatment with 80 mg/kg \( \beta \)-FNA was shifted in
a nonparallel fashion, the antagonism was surmountable.

Limitations imposed by the lethal effects of butorphanol in
the present assay prevented the assessment of higher doses
of butorphanol. The lethal effect was not prevented by a dose
of 10 mg/kg naltrexone. Therefore, in the present study, it is
not clear whether the antagonism of the antinociceptive ef-
fects of butorphanol by \( \beta \)-FNA was surmountable. Previ-
iously, nor-BNI has been reported to be a specific antagonist
at \( \kappa \) receptors in \textit{in vitro} and \textit{in vivo} studies (Broadbear et al.,
1994; Portoghese et al., 1987). In the present study, pretreatment
with 32 mg/kg nor-BNI failed to antagonize the analgesic
effects of butorphanol; however, there was a signifi-
cant rightward shift produced by pretreatment with a lower
dose, 10 mg/kg nor-BNI. The reason for this inconsistent
finding is presently unclear, although it suggests that some
components of the analgesic effects of butorphanol are medi-
atred through \( \kappa \) receptors. Unlike \( \beta \)-FNA, pretreatment
with nor-BNI did not cause a decrease in the maximum level
of analgesia produced by butorphanol, suggesting that nor-
BNI was not an irreversible or insurmountable antagonist of
the analgesic effects of butorphanol. Previous studies using
nor-BNI as an antagonist of the antinociceptive effects of \( \mu 
\) agonists such as morphine have shown that nor-BNI does not
cause a shift in the dose-effect curves for these agonists and
does not decrease the level of effect obtained under control
conditions (Broadbear et al., 1994; Horan et al., 1991). Fi-
nally, pretreatment with the \textit{delta}-specific antagonist nal-
trindole (20 and 32 mg/kg) failed to antagonize the antino-
icceptive effects of butorphanol. In mice, naltrindole (20 mg/kg
s.c.) antagonizes the antinociceptive effects of the \textit{delta}-se-
lective agonist DSLET without affecting the analgesic effects
of morphine or U50,488H (Portoghese et al., 1988). Because
naltrindole did not antagonize the effects of butorphanol in
the present assay, it is unlikely that \( \delta \) receptors play a
significant role in its analgesic effects.

Low doses of butorphanol in combination with morphine
produced antagonist-like effects. Pretreatment with butor-
phanol (1.0, 1.8 or 3.2 mg/kg) caused parallel, rightward
shifts in the dose-effect curve of morphine alone. This is
consistent with the expectation that a low efficacy agonist
would antagonize the effects of a higher efficacy agonist
when given in combination. These findings are similar to
those reported in a previous study in which butorphanol
antagonizes the antinociceptive effects of the \( \mu \) agonist
l-methadone in monkeys (Dykstra, 1990). In contrast, low
doses of butorphanol in combination with U50,488H pro-
duced leftward shifts in the dose-effect curve of U50,488H
alone, suggesting that the antinociceptive effects of butor-
phanol “add to” those of U50,488H. This finding is consistent
with a previous report by Butelman et al., (1995) in which
butorphanol produced a nonparallel leftward shift in the
antinociceptive effects of U50,488H; however, it contrasts
with the Dykstra (1990) study in which butorphanol also
antagonizes the analgesic effects of U50,488H, with the same
potency that it antagonizes the effects of l-methadone. Bu-
torphanol has also antagonized the effects of both \( \mu \) and
\( \kappa \) agonists in the \textit{in vitro} mouse vas deferens assay
(Miller et al., 1986). The contrast in results may reflect dif-
fences in species (mouse vs. squirrel monkey) and/or exper-
imental conditions used (thermal antinociception vs. shock
titration vs. \textit{in vitro} vas deferens assay). Either \( \mu \)- or
\( \kappa \)-mediated antinociceptive effects of butorphanol could
explain the leftward shifts of the U50,488 dose-effect curve
when combined with low doses of butorphanol. For example,
the data are consistent with butorphanol being an interme-
diate efficacy \( \mu \) agonist that potentiates the antinociceptive
effects of U50,488H. Alternatively, these data may reflect a
summation of \( \kappa \)-mediated antinociceptive effects of both
drugs.

In summary, most of the results presented here suggest
that butorphanol acts as a partial \( \mu \) agonist in producing its
antinociceptive actions in the mouse radiant-heat tail-flick
test. This evidence comes from \( \alpha \) analysis of naltrexone
antagonism data; experiments with selective antagonists for
\( \mu \), \( \kappa \) and \( \delta \) receptors; and the evaluation of butor-
phanol as an antagonist of higher-efficacy agonists. Some of
the evidence also suggests that butorphanol has a \( \kappa \)
component to its analgesic actions in the mouse radiant-heat
tail-flick test: The \( \kappa \)-selective antagonist nor-BNI antag-
onized butorphanol analgesia, and combinations of butorpha-
nol with U50,488H produces greater antinociception than U50,488H alone, suggesting a coagonist action. That butorphanol may have kappa activity in this assay was not surprising given that numerous studies (some of which are reviewed herein) have reported a kappa component to the actions of butorphanol. What was surprising was that the data supporting a kappa-antinociceptive effect is somewhat equivocal. In the selective antagonist study, the antagonism of butorphanol analgesia by nor-BNI was not dose dependent. Furthermore, one could interpret the coagonist effects of butorphanol in combination with U50,488H as the summed actions of a mu (butorphanol) and kappa (U50,488H) agonist. However, a common theme throughout the butorphanol literature is that results often seem to depend on species and the particular effect being measured. Taken together, the results of the present study suggest that butorphanol acts as a partial agonist in the mouse radiant heat tail-flick test and that activity at mu receptors accounts for the majority of its antinociceptive effects.

Acknowledgments

The authors thank Wen-Lin Sun and Greg Davis for technical assistance and Dr. Scott Baron for helpful advice in preparation of the manuscript.

References


Send reprint requests to: Dr. William D. Wessinger, University of Arkansas for Medical Sciences, Department of Pharmacology and Toxicology, Slot 611, 4301 W. Markham Street, Little Rock, AR 72205. E-mail: wdwessinger@life.uams.edu