Research report

Pharmacological characterization of buprenorphine, a mixed agonist–antagonist with \( \kappa \) analgesia

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Abstract

Buprenorphine is a mixed opioid agonist/antagonist analgesic. This study was designed to determine the role of opioid receptor subtypes, especially \( \kappa \), in buprenorphine-induced analgesia in mice. Buprenorphine, when injected systemically, revealed a potent analgesic effect by tailflick assay, with a biphasic dose–response curve, which was reversed by naloxone. The presence of analgesic cross-tolerance between buprenorphine and naloxone benzoylhydrazone (NalBzoH) and morphine indicated a role for \( \kappa \) and \( \mu \) receptor subtypes in buprenorphine analgesia. Additional studies with selective opioid antagonists indicated \( \kappa \) mechanisms of action. We did not detect any involvement of the \( \delta \) receptor subtype. Low doses of buprenorphine antagonized morphine analgesia, while high doses of buprenorphine coadministered with morphine elicited increasing analgesia in a dose-dependent manner. These findings suggest that buprenorphine elicits analgesia through an interaction with \( \kappa \) receptors and to a lesser extent with \( \mu \) receptors as well as its activity as partial \( \mu \) receptor agonist.

Keywords: Analgesia; Buprenorphine; Naloxone benzoylhydrazone; Opiate receptor mixed agonist–antagonist

1. Introduction

Like most neurotransmitters, the opiates and opioid peptides interact with many classes of receptors. Each has a unique profile of ligand selectivity, regional distribution and pharmacological actions, and each is independently capable of modulating the perception of pain. The first receptor system to be implicated in opiate analgesia was the \( \mu \) (\( \mu \)) subtype followed by the delta (\( \delta \)) subtype, in response to DPDPE. The highly \( \kappa \) (\( \kappa \))-selective agonist U50,488 has been shown to elicit selective \( \kappa \) analgesia, and NalBzoH is accepted as an effective tool for examining \( \kappa \) (\( \kappa \)) analgesia [10]. By testing highly selective opioid drugs with minimal side effects, we can clarify the differences between the specific receptor subtypes and thereby enable more successful pain treatment.

Buprenorphine is a semi-synthetic, highly lipophilic opioid derivative of the baine, closely related in chemical structure to etorphine. Studies in animals and humans have shown that buprenorphine has a spectrum of opioid activities, very similar to morphine. It is, however, almost devoid of morphine’s side effects of dependence, tolerance and constipation; it also has a lower abuse potential, and its duration of action is longer. Buprenorphine is more potent than morphine [4] and has been proven effective in alleviating postoperative pain and chronic pain in the elderly [8]. One of the clinical advantages of buprenorphine is its availability for sublingual administration.

Buprenorphine has a similar potency and high affinity for both the \( \mu \) and \( \kappa \) opioid receptor subtypes [15]. According to some studies, buprenorphine is a partial \( \mu \) receptor agonist and \( \kappa \)-receptor antagonist [6]. Others, however, have shown that buprenorphine can antagonize the antinociceptive actions of morphine in mice and rats, indicating antagonistic activity at the \( \mu \) receptor [2]. Tyers [18], for example, found that buprenorphine induced analgesia via agonistic activity at the \( \kappa \) receptor; he claimed, therefore, that buprenorphine is similar in analgesic action to nalorphine and not to morphine [18]. In a previous study we showed that nalorphine produces analgesia mainly via
a \( \kappa \) mechanism [12]; this is the same receptor subtype found in the present study to mediate buprenorphine analgesia.

In this study we sought to clarify the role of the different opioid receptor subtypes in buprenorphine-induced analgesia, with particular emphasis on \( \kappa_3 \).

2. Materials and methods

2.1. Subjects

Male ICR mice (25–35 g) were purchased from the Levinstein colony (Yokneam, Israel). The mice were maintained on a 12:12 h light/dark cycle with Purina rodent chow and water ad libitum. The animals were housed in groups of 20 under standard conditions and were divided into groups of five 1 day before testing.

2.2. Method

The study was broken down into four experiments.

2.2.1. Experiment 1

In the first stage of the study, groups of mice (\( \geq 10 \)) were injected with low (\( < 0.25 \) mg/kg), intermediate (0.5–5.0 mg/kg) and high (\( \geq 10.0 \) mg/kg) subcutaneous (s.c.) doses of buprenorphine to determine the effect of the drug in eliciting analgesia.

2.2.2. Experiment 2

The sensitivity of buprenorphine to four selective antagonists was examined. Five groups of mice (\( \geq 10 \)) were treated with \( \beta \)-FNA 40 mg/kg s.c. 24 h before buprenorphine challenge, or with one of the following drugs: naloxone 10 mg/kg s.c., naltrindole 20 mg/kg s.c., nor-BNI 10 mg/kg s.c. or saline immediately before buprenorphine was injected. For comparison, \( \beta \)-FNA was tested against morphine, nor-BNI against U50,488H mediation and naltrindole against DPDPE, in separate groups of mice.

2.2.3. Experiment 3

The action of buprenorphine on selective opioid receptor subtype agonists was tested, as follows: (a) groups of mice (\( \geq 10 \)) were given increasing doses of buprenorphine with a fixed dose of morphine, a \( \mu \) receptor agonist (5 mg/kg s.c.); (b) DPDPE, a selective \( \delta \)-receptor agonist, was injected intrathecally (i.t.) alone or with an inactive dose of buprenorphine (2.5 mg/kg s.c.); (c) U50,488H, a selective \( \kappa_1 \) receptor agonist, was injected s.c. alone or with an inactive dose of buprenorphine (2.5 mg/kg s.c.); (d) NarBzoH a \( \kappa_1 \) receptor agonist, was injected s.c. alone or with an inactive dose of buprenorphine (2.5 mg/kg s.c.).

2.2.4. Experiment 4

Cross-tolerance of buprenorphine and NarBzoH (\( \kappa \), agonists): Cross-tolerance can be a useful approach in investigating the role of receptor subtypes. Groups of mice (\( \geq 10 \)) were injected with buprenorphine 20 mg/kg or NarBzoH 50 mg/kg or saline 10 mg/kg daily for 4 days. The mice (in the treated groups) revealed marked tolerance when they were challenged again with the same drug on the fifth day. In other groups (\( \geq 10 \)) we then administered the various agents for 4 days and challenged with a different compound on the fifth day to examine cross-tolerance. Mice tolerant to buprenorphine were challenged with NarBzoH and mice tolerant to NarBzoH were challenged with buprenorphine.

Intrathecal (i.t.) injections were made under light ethane anesthesia, using a Hamilton 10-\( \mu \)l syringe fitted to a 30-gauge needle with \( V_1 \) tubing. The i.t. injections were introduced by lumbar puncture [5]. The volume for central injections was 1 \( \mu \)l/mouse.

2.3. Agents

Several agents were generously donated as follows: buprenorphine by CTS Chemical Industries (Petah Tiqva, Israel); morphine sulfate by Tева (Jerusalem, Israel); U50,488-H (\{trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolindinyl)-cyclohexyl]benzeneacetamide\} by Upjohn Pharmaceuticals (West Sussex, England); and naloxone benzoyl-hydrazone (NalBzoH) by Dr. G.W. Pasternak of Memorial Sloan-Kettering Cancer Center, New York, NY. Ethane (Enflurane) was purchased from Abbott (Campoverde, Italy), and D-Pen2,D-Pen5-enkephalin (DPDPE), \( \beta \)-FNA, nor-BNI, naltrindole and naloxone HCl were obtained from the Research Technology Branch of the National Institute of Drug Abuse (Rockville, MD). All other compounds were purchased from commercial sources.

2.4. Nociceptive tests

Animals were tested for analgesia by using the hot water tailflick technique [14] 15 min after i.t. injections or 30 min after each s.c. injection. In this test, the latency to withdrawal of the tail from hot water (50–52°C) is measured manually, using a stopwatch. Baseline latencies (2.0–3.6 s) were determined before experimental treatment for all animals. Post-treatment latencies were determined as indicated for each experiment, and a maximal latency of 10 s was used to minimize tissue damage. Analgesia was defined quantitatively as a doubling or more of baseline values for each mouse. For each dose at least 10 different mice were checked, and their scores were summarized to determine the percentage of animals with an analgesic response. Each mouse was checked once.
2.5. Data analysis

Dose–response curves were analyzed using a modification of the SPSS program. This program maximizes the log-likelihood function to fit a parallel set of Gaussian normal sigmoid curves to the dose–response data. Single-dose antagonist studies were analyzed using the Fisher exact test.

3. Results

3.1. Buprenorphine analgesia

Buprenorphine administered s.c. elicited analgesia in a biphasic dose–response curve (Fig. 1). Very low doses of buprenorphine produced a dose-related effect, reaching a ceiling at 0.25 mg/kg. For intermediate doses (from 0.5 to 5.0 mg/kg), the effects were more or less stable (plateau). High doses (from 10.0 mg/kg) elicited analgesia in a dose–response manner.

3.2. Sensitivity of buprenorphine analgesia to selective antagonists

The analgesia induced by buprenorphine was antagonized by naloxone (10 mg/kg s.c.; \( P < 0.005 \)), implying as expected, an opioid mechanism of action in buprenorphine analgesia (Fig. 2).

Regarding the potential involvement of \( \mu, \delta \) and \( k_1 \) receptor subtypes in buprenorphine analgesia, using selective antagonists (Fig. 2), we found that \( \beta \)-FNA reversed buprenorphine analgesia at the same dose that it antagonized morphine analgesia (\( P < 0.005 \)), suggesting a role for \( \mu \) receptor in buprenorphine analgesia. NorBNI only partially reversed buprenorphine analgesia at the same dose that it antagonized \( k_1 \) analgesia, mediated by U50,488H (\( P < 0.005 \)). The dose of naltrindole that reversed DPDPE analgesia did not affect buprenorphine analgesia. The activity of each of the antagonists was confirmed with its prototypic agonists (data not shown). None of the antagonists mediated analgesia by themselves, nor did they change the baseline latencies of the pretreated animals.

3.3. Buprenorphine action on selective opioid receptor subtypes

3.3.1. Buprenorphine–morphine (\( \mu \) receptor interactions)

The interaction between morphine and buprenorphine was bidirectional (Fig. 3). Morphine alone (5 mg/kg s.c.) produced analgesia in 60% of the mice. Low doses of buprenorphine antagonized morphine analgesia in a dose-
Table 1

<table>
<thead>
<tr>
<th>Opioid receptor subtypes</th>
<th>Without buprenorphine</th>
<th>With buprenorphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPDPE (δ subtype)</td>
<td>723 ng (451, 1023)</td>
<td>520 ng (380, 870)</td>
</tr>
<tr>
<td>U50,488H (κ₁ subtype)</td>
<td>5.5 mg/kg (3.1, 11.3)</td>
<td>4.6 mg/kg (2.2, 9.2)</td>
</tr>
<tr>
<td>Nalorphine (κ₂ subtype)</td>
<td>48.4 mg/kg (30.7, 95.2)</td>
<td>18.8 mg/kg (10.0, 29.4)</td>
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The numbers in parentheses are the 95% confidence limits of the ED₅₀. About 10 mice were used for each test. *P < 0.05, compared with the group without buprenorphine.

dependent manner, reaching a maximum effect at 15 mg/kg (17.5%; n = 40 mice). Although the analgesic response was significantly lower than that with morphine alone (P < 0.005), at no dose did buprenorphine antagonize morphine analgesia completely. The antagonistic effect of buprenorphine was achieved at a dose below that occurring with buprenorphine alone (40% after 10 mg/kg, Fig. 1). Further increases in the dose of buprenorphine, coadministered with morphine, elicited increasing analgesia in a dose-dependent manner, similar to that of buprenorphine alone (P < 0.01).

3.3.2. Buprenorphine–DPDPE (δ receptor interactions)
We found no differences between the dose–response curves in these groups. ED₅₀ of DPDPE without buprenorphine was 723 ng (451, 1023; 95% confidence limit (CL)) and with buprenorphine, 520 ng (380, 870; 95% CL) (Table 1).

3.3.3. Buprenorphine–U50-488H (κ₁ receptor interactions)
No differences were found between the dose–response curves. ED₅₀ of U50,488H without buprenorphine was 5.5 mg/kg s.c. (3.1, 11.3; 95% CL) and with buprenorphine, 4.6 mg/kg s.c. (2.2, 9.2) (Table 1).

3.3.4. Buprenorphine–NalBzoH (κ₃ subtype interactions)
We found a significant shift in the dose–response curve when buprenorphine was given in addition to NalBzoH (P < 0.005). ED₅₀ of NalBzoH without buprenorphine was 48.4 mg/kg s.c. (30.7, 95.2; 95% CL) and when buprenorphine was added, 18.8 mg/kg s.c. (10.0, 30.1) (Table 1).

3.4. Cross-tolerance of buprenorphine with NalBzoH (κ₃ subtype) and morphine (μ subtype) interactions
Mice tolerant to buprenorphine showed cross-tolerance to buprenorphine, NalBzoH and morphine, while mice tolerant to NalBzoH showed cross-tolerance to buprenorphine and NalBzoH (P < 0.05, Fig. 4). Mice tolerant to morphine expressed cross-tolerance to buprenorphine and morphine (P < 0.05, Fig. 4). These results suggest that buprenorphine analgesia might involve κ₃ and μ agonistic activity.

Fig. 4. Analgesia cross-tolerance studies.
4. Discussion

In the present study we found that low buprenorphine doses reversed morphine (μ receptor subtype) analgesia, whereas high doses produced analgesia and cross-tolerance to morphine. In other experiments we found that buprenorphine analgesia implied a role for κ₁ mechanisms of action, and to a lesser extent it implied κ₁ mechanisms of action. We could not detect a δ involvement in buprenorphine-induced analgesia.

Buprenorphine has a very complex pharmacological profile. In receptor-binding assays it has been found to have affinity to κ₁ and μ subtypes but not to δ [15]. The general agreement is that buprenorphine has a mediatory analgesic effect as a partial μ agonist and κ₁ antagonist.

The relationship between buprenorphine and morphine is dose-dependent. This interaction, also found in previous studies between morphine and other drugs, reveals κ₁ action. The same is true for NalBzoH [3,11,2], nalbuphine [13] and levorphanol [17]. It was suggested that buprenorphine is a partial agonist of μ receptors [6]. This assumption is consistent with our results showing that high doses of buprenorphine enhance morphine-induced analgesia, while low doses reverse morphine analgesia. These findings are in agreement with Lizasoain et al. [7], who found that low doses of buprenorphine given to morphine-dependent mice caused an ‘abstinence syndrome’. The same effect was found with naloxone. In addition, in the same model, when high doses of buprenorphine were administered, the ‘abstinence syndrome’ could not be detected. Leander [6], in a ‘shock titration assay’ with monkeys, found that buprenorphine did antagonize μ receptor effects, but this action was inconsistent and dependent on the individual animals [9]. We found that the μ antagonist β-FNA blocked buprenorphine analgesia. The same effect has been also demonstrated by the ‘abdominal writhing test’ [6].

The ability of low buprenorphine doses to lower morphine analgesia (Fig. 3) is consistent with a partial agonist and it is more likely that it is a partial μ agonist at the lower doses than at the higher doses. This possibility is supported by the observation that buprenorphine tolerant animals are also cross tolerant to morphine (Fig. 2).

Cross-tolerance between buprenorphine and NalBzoH suggested that κ₁ receptors played a significant role in buprenorphine-induced analgesia. In NalBzoH-tolerant mice, buprenorphine induced almost complete reduction of analgesia. This finding highlights the major importance of κ₁ receptors in buprenorphine analgesia and the lesser importance of κ₁ receptors. NalBzoH is a κ₁ agonist with antagonist actions at μ and κ₁ receptors. Thus, chronic NalBzoH treatment should elicit tolerance only at κ₁ receptor subtype, and not at μ or κ₁ receptors [17]. In addition, according to Tyers [18], the antinociceptive action of nalorphine and buprenorphine is mediated by κ subtype analgesia. As mentioned previously, the antinociceptive action of nalorphine was mediated mainly by κ₁ [12]. According to our present findings, buprenorphine produces its effect via κ₁ subtypes as well. Studies, using the κ₁ selective antagonist nor-BNI, suggest a spinal site of action [11,16]. In contrast, κ₃ analgesia is mediated supraspinally [11], at the same site at which buprenorphine exerts its analgesic effect [1].

The importance of κ₁ receptor subtypes in buprenorphine analgesia has already been demonstrated by different analgesic assays. Buprenorphine has also been found to have a high affinity for κ₁ by in vitro receptor binding assay [15]. In the present study, buprenorphine analgesia was blocked by nor-BNI, indicating κ₁ analgesia. A shift to the right has been found previously in monkeys [9], mice or pigeons [6], followed by coinjection of buprenorphine and U50,488H. In contrast, we could not detect any potentiation of buprenorphine-induced analgesia when buprenorphine was given in addition to U50,488H. In another study it was shown that buprenorphine is predominantly a κ antagonist and produces its analgesia in the same way that nalorphine does [18]. Thus, it appears from our study that there is an important role of the κ subtype in buprenorphine analgesia, mainly for κ₁ and to a lesser extent for κ₃.

Selected opioid antagonists are used to clarify the receptor subtype(s) involvement in drug actions. However, it is possible that the antagonist are not selected as generally believed. It is unclear whether β-FNA blocks NalBzoH analgesia in the same way it blocks buprenorphine. It is still possible that there are μ/κ₃ interactions which are responsible for buprenorphine analgesia. Such interactions might also help explain the action of β-FNA.

In view of the complexity of buprenorphine’s analgesia actions, it is now necessary to further characterize the receptor mechanisms responsible for its effects. Our results confirm the importance of κ₁ and κ₃ mechanisms in buprenorphine analgesia and suggest a partial agonistic activity at the μ receptors, but not at the δ receptors. Buprenorphine is widely used clinically and does not have an exceptionally high incidence of psychotomimetic or dysphoric effect, which are often associated with κ activity. This raises the possibility that it is possible to develop κ₃ analgesic agents that are not associated with adverse side effects.

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References


