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(−)-Pentazocine analgesia in mice: interactions with a σ receptor system

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Abstract

(−)-Pentazocine is active in the tailflick assay in CD-1 mice, although it shows a biphasic dose-response curve with a peak effect of only 30%. Co-administration of haloperidol shifts the dose-response curve to the left and elevates the maximal response to 70% through a blockade of σ_1 receptors, but the curve remains biphasic. (+)-Pentazocine is inactive in all antinociceptive assays, either alone or with haloperidol. The analgesic actions of (−)-pentazocine are readily reversed by nor-binaltorphimine, but not by the μ-selective opioid receptor antagonist β-funaltrexamine, implying a κ_1-opioid receptor mechanism of action. This conclusion is supported by the ability of antisense oligodeoxynucleotides directed against the KOR-1 clone, which encodes the κ_1-opioid receptor, to block (−)-pentazocine analgesia.

Keywords: κ-Opioid analgesia; Opioid receptor; σ Receptor; Haloperidol; Antisense; KOR-1; κ-Opioid receptor

1. Introduction

Pentazocine is widely used clinically in the treatment of mild to moderate pain (Reisine and Pasternak, 1995). Despite its wide-spread clinical utility, pentazocine analgesia has been difficult to demonstrate in thermal nociceptive assays in animals, underscoring the problem with screening κ-opioid analgesics with these assays. In addition, the opioid receptor mechanisms responsible for pentazocine analgesia are controversial. Previous work from our group has established that a tonically active anti-opioid σ receptor system markedly influences the sensitivity of mice toward opioid analgesics, particularly κ-opioid drugs (Chien and Pasternak, 1993, 1994, 1995). The analgesic activity of opioid analgesics can be downregulated by the tonic activity of σ receptor systems or through the administration of σ receptor agonists. Conversely, σ receptor antagonists such as haloperidol significantly enhance opioid analgesia. This increased analgesic activity is mediated through σ receptors and not dopamine receptors even though haloperidol is a potent dopamine D_2 and σ receptor antagonist. The selective dopamine D_2 receptor antagonist (−)-sulpiride, which has no appreciable affinity for σ receptors, is inactive. These studies help explain earlier observations regarding the enhanced activity of morphine co-administered with haloperidol, which had assumed a dopaminergic interaction (Head et al., 1979; Eidelberg and Erspamer, 1975). In the current study, we have addressed the opioid receptor mechanisms of (−)-pentazocine and its modulation by this anti-opioid σ receptor system.

2. Materials and methods

2.1. Materials

Male CD-1 mice (25–35 g; Charles River Breeding Laboratories, Wilmington, MA, USA) were maintained on a 12 h light/12 h dark cycle with Purina rodent chow and water available ad libitum. Mice were housed in groups of 5 until testing. Pentazocine was a generous gift from Sanofi-Winthrop (New York, NY, USA). Morphine sulfate was obtained from Mallinckrodt (St.

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Louis, MO, USA) and U50,488H (*trans*-3,4-dichloro-*N*-[2-(1-pyrrolindinyl)-cyclohexyl]-benzeneacetamide, methane sulfonate hydrate) was a generous gift from Upjohn Pharmaceutics (Kalamazoo, MI, USA). Haloperidol and (−)-sulpiride were purchased from Sigma (St. Louis, MO, USA). β-Funaltrexamine and [D-Pen^2,D-Pen^5]enkephalin were gifts from NIDA Research Technology Branch. Nor-binaltorphimine was purchased from Research Biochemicals International (Natick, MA, USA) and halothane was purchased from Halocarbon Laboratories (Hackensack, NJ, USA). Radioligands were purchased from New England Nuclear Corp. (Boston, MA, USA). Peptides were purchased from Peninsula Laboratories (Belmont, CA, USA).

2.2. Antisense treatment

Oligodeoxynucleotides were synthesized by The Midland Certified Reagent Co. (Midland, TX, USA). The antisense oligodeoxynucleotide corresponds to bases 761–782 of KOR-1 (5′-GGTGCCTCCAAGGACTATCGC-3′) (Minami et al., 1993; Meng et al., 1993; Takeshima et al., 1993; Yasuda et al., 1993). Four bases had been switched to make the mismatch oligodeoxynucleotide (5′-GGAGCCTGCAAGGTCTATGGC-3′). Oligodeoxynucleotides were dissolved in saline and 2 μg (2.5 μg/ml) was injected intrathecally (Hylden and Wilcox, 1980) under light halothane into groups of mice on day 1, 3, and 5. Mice were tested for analgesia on day 6. The light halothane anesthesia is inactive in analgesia testing.

2.3. Analgesia testing

Analgesia was determined using the radiant heat tailflick technique (D'Amour and Smith, 1941) as reported previously (Chien and Pasternak, 1993, 1994; Paul et al., 1990, 1991; Gistrak et al., 1989). For the tailflick assay, the latency to withdraw the tail from a focused light stimulus was measured electronically using a photocell. Baseline latencies (2.0–3.0 s) were determined before experimental treatment for all animals as the mean of two trials. Post-treatment tailflick latencies were determined at 30 min after subcutaneous injection (s.c.) injection. A maximal latency of 10 s was used to minimize tissue damage. Analgesia was defined quantally as a doubling or greater of baseline value for each mouse. The irreversible antagonist β-funaltrexamine was administered 24 h prior to agonist testing. All studies employed groups of at least 10 naive mice and each mouse was exposed to only one experimental treatment. In vivo analgesia data were analyzed by using Fisher's exact test. All animal studies were approved by our IACUC and adhere to NIH guidelines.

2.4. Opioid receptor binding

μ-, δ- and κ-opioid receptor binding was performed as reported previously (Clark et al., 1988; 1989). μ_1-Opioid receptor binding was determined in calf thalamus membranes with [^{3}H][D-Ala^2,D-Leu^5]enkephalin in the presence of unlabeled [D-Pen^2,D-Pen^5]enkephalin to block δ-opioid receptors while μ_2-opioid receptor binding was measured with [^{3}H][D-Ala^2,MePhe^4, Gly(ol)^5]enkephalin in the presence of unlabeled [D-Ala^2,D-Leu^5]enkephalin to block μ_1-opioid sites in calf striatum. δ-Opioid receptor binding was determined in calf frontal cortex with [^{3}H][D-Pen^2,D-Pen^5]enkephalin, κ_1-opioid receptor binding in guinea pig cerebellum with [^{3}H]U69,593 and κ_3-opioid receptor binding in calf striatum with [^{3}H]naloxone benzoylhydrazone. Specific binding was determined by subtracting nonspecific binding determined with levallorphan (1 μM) from total binding. All determinations were performed in triplicate and replicated three times. K_i values were determined by the following equation: $K_i = (IC_{50})/(1 + S)$ where S = (concentration of radioligand)/(K_D of radioligand) (Cheng and Prusoff, 1973; Chou, 1974).

3. Results

When examined in CD-1 mice, a strain sensitive to κ-opioid receptor drugs (Pick et al., 1991a; Chien and Pasternak, 1994) (−)-pentazocine produces a biphasic dose-response curve, with a peak effect of only 30% at 20 mg/kg (Fig. 1). Further dose increases are counterproductive, lowering the response back to baseline levels by 50 mg/kg. However, this response is sensitive to haloperidol, a potent σ receptor antagonist. At a fixed (−)-pentazocine dose of 5 mg/kg, haloperidol

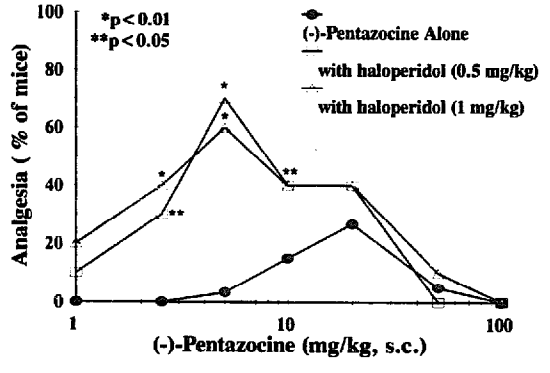

Fig. 1. Dose-response curves of (−)-pentazocine analgesia. Groups of mice ($n \geq 20$) received the stated doses of (−)-pentazocine with or without haloperidol (0.5 or 1 mg/kg). The peak effects seen with either haloperidol dose and (−)-pentazocine at 5 mg/kg are significantly different from the response observed with (−)-pentazocine alone ($P < 0.01$).

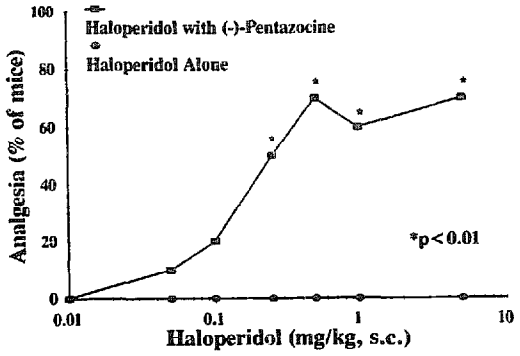

Fig. 2. Dose-response relationship of haloperidol on (−)-pentazocine analgesia. Groups of mice ($n \geq 20$) received a fixed dose of (−)-pentazocine (5 mg/kg s.c.) along with the indicated dose of haloperidol or haloperidol alone. Haloperidol alone is without effect at any dose tested. Haloperidol at doses of 0.25 mg/kg and higher significantly increase the analgesic actions of (−)-pentazocine ($P < 0.01$).

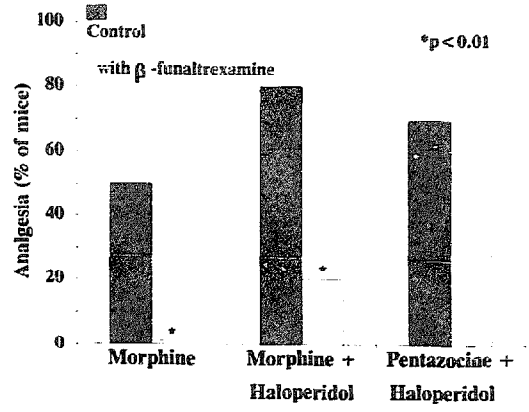

Fig. 3. Effect of β-funaltrexamine on morphine and (−)-pentazocine analgesia. Groups of mice ($n \geq 20$) received vehicle or β-funaltrexamine (40 mg/kg s.c.) 24 h prior to testing with morphine (5 mg/kg s.c.) or (−)-pentazocine (5 mg/kg s.c.) with or without haloperidol (0.5 mg/kg s.c.). β-Funaltrexamine significantly lowers morphine analgesia with or without haloperidol ($P < 0.01$) but not (−)-pentazocine.

markedly increases the analgesic response in a dose-dependent manner from 0% to 70%, with a maximal response seen at doses of 0.5 mg/kg or greater (Fig. 2). Repeating the (−)-pentazocine dose-response studies with fixed haloperidol doses of either 0.5 or 1 mg/kg shifts the (−)-pentazocine curves to the left and increases the maximal response (Fig. 1). With the addition of haloperidol, peak effects are now seen at 5 mg/kg and analgesic responses are produced by (−)-pentazocine doses which are otherwise inactive. However, the response remains biphasic and further dose increases lower the response down to baseline levels, yielding a bell-shaped curve.

Having established an assay in which (−)-pentazocine shows a robust analgesic action, we next explored the receptor subtypes responsible for mediating the analgesic activity. (−)-Pentazocine labels μ- and κ_1-opioid receptors with high affinity (Table 1), suggesting that it acts through one of these. β-Funaltrexamine, a selective μ-opioid receptor antagonist (Ward et al., 1982; Pick et al., 1991b), effectively blocks morphine analgesia, regardless of whether haloperidol is added or not (Fig. 3). However, β-funaltrexamine does not antagonize (−)-pentazocine analgesia. To explore the possibility of a κ_1-opioid receptor mechanism of action, we examined a potent

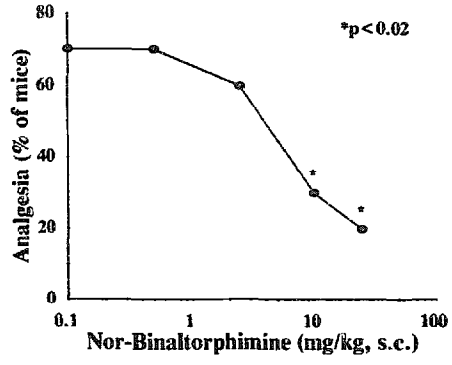

Fig. 4. Effect of nor-binaltorphimine on (−)-pentazocine analgesia. Groups of mice ($n \geq 20$) received (−)-pentazocine (5 mg/kg s.c.) and haloperidol (0.5 mg/kg s.c.) with the indicated dose of nor-binaltorphimine (s.c.). Nor-binaltorphimine at doses of 10 and 25 mg/kg significantly lowers (−)-pentazocine analgesia ($P < 0.02$).

and selective κ_1-opioid receptor antagonist, nor-binaltorphimine (Takemori et al., 1988). Nor-binaltorphimine antagonizes (−)-pentazocine analgesia in a dose-dependent manner, with a potency similar to that observed with other κ_1-opioid receptor agents (Fig. 4; Chien and Pasternak, 1994; Paul et al., 1991).

Table 1
Binding affinity of pentazocine for opioid receptors

Compound	K_i value (nM)				
	μ_1	μ_2	δ	κ_1	κ_3
(−)-Pentazocine	4.8 ± 0.5	21.3 ± 3.1	205 ± 63	10.8 ± 3.5	274 ± 132
(+)-Pentazocine	> 500	> 500	> 500	> 500	> 400
(±)-Pentazocine	7.5 ± 1.4	43.5 ± 7.7	421 ± 103	60.4 ± 33	> 400

Competition assays with the pentazocine isomers were conducted against the indicated receptor binding assay as previously reported (Clark et al., 1988, 1989). IC_{50} values were converted to K_i values as previously described (Cheng and Prusoff, 1973; Chou, 1974). Results are the means of three independent determinations.

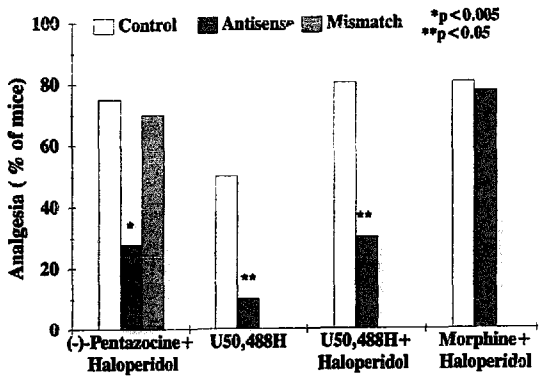

Fig. 5. Effect of a KOR-1 antisense oligodeoxynucleotide on (−)-pentazocine, U50,488H and morphine analgesia. Groups of mice ($n \geq 10$) received intrathecal injections (2 μl) of saline, an antisense oligodeoxynucleotide directed against KOR-1 (5 μg) or a mismatch oligodeoxynucleotide (5 μg) on days 1, 3 and 5, and analgesia was tested on day 6 with (−)-pentazocine (5 mg/kg s.c.) and haloperidol (0.5 mg/kg s.c.), U50,488H (7 mg/kg s.c.), U50,488H (7 mg/kg s.c.) with haloperidol (0.5 mg/kg s.c.) or morphine (5 mg/kg s.c.) with haloperidol (0.5 mg/kg s.c.). The antisense treatment lowers the analgesic response to (−)-pentazocine ($P < 0.005$), U50,488H ($P < 0.05$) and U50,488H with haloperidol ($P < 0.05$).

An antisense oligodeoxynucleotide directed against KOR-1, which encodes the κ_1-opioid receptor (Minami et al., 1993; Meng et al., 1993; Takeshima et al., 1993; Yasuda et al., 1993), which effectively blocks κ_1-opioid analgesia (Chien et al., 1994) also antagonizes systemic U50,488H analgesia in combination with haloperidol, but it is inactive against morphine (Fig. 5). The same oligodeoxynucleotide administered at the spinal level effectively blocks systemic (−)-pentazocine analgesia, indicating a role for κ_1-opioid receptors. Haloperidol is needed in the (−)-pentazocine studies to obtain a sufficiently robust analgesic response to look for its blockade. A mismatch antisense in which several pairs of bases have been switched is inactive, establishing the specificity of the treatment.

4. Discussion

(−)-Pentazocine alone is a weak analgesic in the mouse tailflick assay due to the tonic activity of the anti-opioid σ receptor system. Blockade of this anti-opioid system through the co-administration of a σ receptor antagonist, such as haloperidol, uncovers the full analgesic potential of (−)-pentazocine. The inactivity of (−)-sulpiride (Chien and Pasternak, 1994), a potent dopamine D_2 receptor antagonist (O'Connor and Brown, 1982; Seeman, 1981; Zahniser and Dubocovich, 1983), confirms that the haloperidol-sensitive activity in this system is mediated through σ receptors (Walker et al., 1990; Largent et al., 1987; Quirion et al., 1992; Chavkin, 1990).

The sensitivity of (−)-pentazocine analgesia to the selective κ_1-opioid receptor antagonist nor-binaltorphimine implicates κ_1-opioid receptors in (−)-pentazocine analgesia, a conclusion supported by antisense studies. Antisense approaches have established the importance of the cloned opioid receptors in mediating opioid analgesia and the remarkable selectivity of this approach (Standifer et al., 1994; Rossi et al., 1994). The sequence utilized in our original KOR-1 studies blocks U50,488H analgesia without influencing μ- or δ-opioid analgesics (Chien et al., 1994). In the current studies the KOR-1 antisense oligodeoxynucleotide prevents the analgesic actions of the prototypical κ_1-opioid analgesic U50,488H with or without the concurrent administration of haloperidol. Thus, the inclusion of haloperidol does not change the importance of κ_1-opioid receptor activation in the production of U50,488H analgesia. This is important since the (−)-pentazocine studies require haloperidol to obtain a sufficient level of analgesia. When tested against (−)-pentazocine, the same KOR-1 antisense treatment blocks analgesia, confirming the importance of the κ_1-opioid receptor in (−)-pentazocine analgesia and localizing its actions to the spinal cord.

Although (−)-pentazocine labels μ receptors with high affinity, μ-opioid receptors do not play a significant role in its (−)-pentazocine analgesia. First, the selective μ-opioid receptor antagonist β-funaltrexamine is inactive against (−)-pentazocine analgesia. Second, the antisense studies imply that (−)-pentazocine analgesia is mediated spinally, which contrasts with the supraspinal site of action for systemic morphine. However, (−)-pentazocine might still be a partial agonist at μ-opioid receptors as well as a κ_1-opioid agonist (Zimmerman et al., 1987). This still would be consistent with its blockade of morphine analgesia (Chien and Pasternak, 1993). The antisense studies and the site of action for (−)-pentazocine analgesia also argues against a κ_3-opioid receptor mechanism of action, distinguishing (−)-pentazocine from the mixed agonist/antagonist nalorphine which acts primarily through κ_3-opioid receptors (Paul et al., 1991; Clark et al., 1989).

The biphasic (−)-pentazocine dose-response curve remains a significant question. The progressive loss of analgesia at higher doses might be due to interactions with another receptor. (−)-Pentazocine labels σ receptors approximately 10-fold less potently than its (+)-isomer. The higher (−)-pentazocine dose might progressively activate σ receptor systems, leading to the eventual loss of analgesia. In the presence of haloperidol, increasing (−)-pentazocine doses still yield the same biphasic response, possibly reflecting the ability of higher (−)-pentazocine doses to overcome the actions of a fixed haloperidol dose. If this biphasic response applies to patients, increasing the dose of

pentazocine may eventually be counterproductive and lead to a diminished response, particularly since the drug is supplied clinically as the racemate. Since (+)-pentazocine is devoid of any analgesic actions itself and actually antagonizes opioid analgesia through its activation of σ receptor systems, the (−)-isomer alone might be a superior analgesic.

In conclusion, anti-opioid σ receptor activity may help explain the low ceiling effects of κ-opioid analgesics seen in the animal models. Blockade of this σ activity may provide a more sensitive approach to the investigation of analgesics. If the presence of anti-opioid σ receptor systems extends to man, the analgesic activity of agents such as pentazocine might be potentiated by the co-administration of σ antagonists.

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