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Short communication

Peripheral κ_1-opioid receptor-mediated analgesia in mice

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Abstract

When injected directly into the tail, U50,488H is a potent analgesic in the tailflick assay (ED_{50} 3.1 μg). The analgesic activity is lost if the radiant heat is focused 1 cm away from the site of injection. The κ_1-opioid receptor antagonist nor-binaltorphimine given systemically reverses the local analgesic response of U50,488H, but the antagonist is 100-fold more potent when injected directly into the tail. Intrathecal antisense treatment with a probe targeting the mRNA encoding the κ_1-opioid receptor blocks the local analgesic actions of U50,488H in the tail, suggesting that U50,488H is acting on dorsal ganglia neurons.

Keywords: κ-Opioid receptor; Tail-flick assay; Analgesia, peripheral

1. Introduction

Classically, central opioid receptors in both the brain and spinal cord have been associated with analgesia (for review see Pasternak, 1993), but peripheral analgesia has become increasingly important in understanding the actions of opioid analgesics (for reviews see Stein, 1993, 1995; Junien and Wettstein, 1992; Joris et al., 1987; Barber and Gottschlich, 1992). Opioid receptors have been identified on peripheral nerves (Fields et al., 1980; Hassan et al., 1993) and in the periphery opioids may act on dorsal root ganglia neurons. Traditionally, it has been difficult to demonstrate κ-opioid receptor-mediated analgesia in mice using thermal nociceptive assays. This may be due, in part, to varying sensitivities of different strains to the opioids (Pick et al., 1991). Most animal studies have examined peripheral opioid analgesia in inflammatory and hyperalgesic models while clinical investigations have focused primarily upon postoperative paradigms. We now present a peripheral antinociceptive thermal assay which we have used to explore peripheral κ_1-opioid receptor-mediated analgesia.

2. Materials and methods

Male CD-1 mice (25–30 g) were purchased from Charles River (Wilmington, MA, USA). Oligodeoxynucleotides were purchased from Midland (Midland, TX, USA). U50,488H was obtained from the Research Technology Branch of the National Institute on Drug Abuse (Rockville, MD, USA) and nor-binaltorphimine from RBI (Natick, MA, USA). All drug solutions were made in normal saline.

Antinociception was assessed using the tailflick assay, as previously described (Pasternak, 1993; Chien et al., 1994). Baseline latencies, ranging from 2 to 3 s, were determined for each mouse. Analgesia was defined quantally as a doubling or greater of the baseline latency for an individual mouse and was determined 30, 15 and 5 min after systemic, intrathecal (2 μl) or local injections in the tail (10 μl), respectively. These represent the times to peak effects. Local injections were made subcutaneously in the tail in the region exposed to the light/heat source. Significance was assessed using the Fisher exact test. ED_{50} values with 95% confidence limits were determined from dose-response curves using the Bliss program (Umans and Inturrisi, 1981).

Antisense treatment was similar to that previously reported (Chien et al., 1994). An antisense probe corresponding to bases 761–782 of the mouse KOR-1 clone cDNA (5′GGT GCC TCC AAG GAC TAT CGC3′) or a mismatch (5′GGA GCC TGC AAG GTC CTA TGC3′)

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oligodeoxynucleotide sequence (5 μg) was administered intrathecally (Hylden and Wilcox, 1980) on days 1, 3 and 5, followed by analgesia testing on day 6, as previously reported (Chien et al., 1994). To examine local analgesia, U50,488H was injected subcutaneously in the tail (5 μg in 10 μl) on day 6 and analgesia determined. The radiant heat was focused upon the region in which the drug was injected and the latency determined.

3. Results

U50,488H is a potent, centrally active κ_1-opioid receptor analgesic (Von Voigtlander et al., 1983). When injected directly into the tail, U50,488H produces a robust and reliable analgesia (Fig. 1a), with an ED_{50} of 3.1 μg (95% confidence limits: 1.8, 4.4). Its actions are mediated locally. Control studies in which the heat is focused 1 cm proximally or distally from the site of injection yielded values unchanged from baseline values, confirming that the actions are mediated locally at the site of injection.

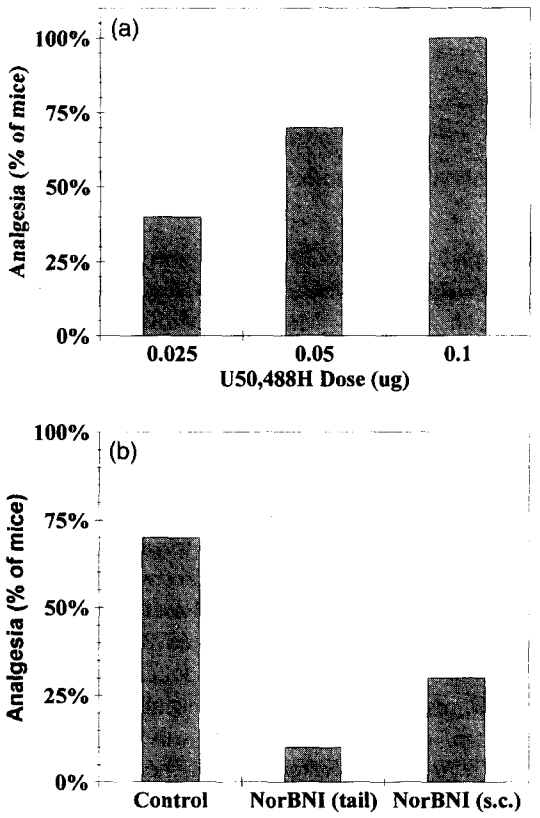

Fig. 1. Potency and antagonists sensitivity of local U50,488H analgesia. (a) Groups of mice ($n \geq 10$) received the stated doses of U50,488H locally in the tail and the mice were tested in the tailflick assay as described in Materials and methods. The ED_{50} value for the dose-response curve is 3.1 μg (95% confidence limits: 1.8, 4.4). (b) Groups of mice ($n \geq 10$) received U50,488H (5 μg) locally in the tail alone or with nor-binaltorphimine given systemically (20 mg/kg s.c.) or locally (5 μg). Nor-binaltorphimine antagonized the analgesic response in both conditions ($P < 0.01$).

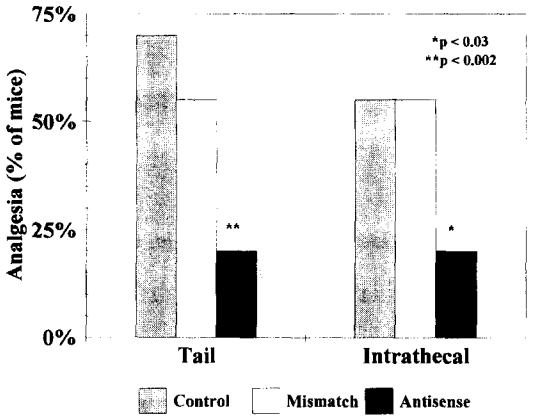

Fig. 2. Effects of antisense treatment on U50,488H analgesia. Groups of mice ($n \geq 10$) were treated with saline or antisense or mismatch oligodeoxynucleotides (5 μg) on days 1, 3 and 5. On day 6, the mice received U50,488H (5 μg) locally in the tail and analgesia was assessed in the tailflick assay. The analgesic response lasted less than 1 h. Approximately 4 h after testing, the mice were tested with intrathecal U50,488H (30 μg i.t.) and analgesia reassessed. Mismatch values are not significantly different from saline controls. Antisense values were significantly lowered for both local ($P < 0.002$) and intrathecal ($P < 0.03$) U50,488H analgesia.

Injection of vehicle has no effect on tailflick latencies. In addition, the selective κ_1-opioid receptor antagonist nor-binaltorphimine potently reverses local U50,488H (Fig. 1b) and is over 100-fold more potent when administered locally than systemically.

We reported the blockade of κ_1-opioid receptor-mediated analgesia in mice following administration of an antisense oligodeoxynucleotide selectively targeting the KOR-1 mRNA which encodes the κ_1-opioid receptor (Chien et al., 1994), a result which was quickly confirmed in rats (Adams et al., 1994). Using the mouse paradigm, we again see the blockade of intrathecal U50,488H analgesia with the antisense probe ($P < 0.03$), but not its mismatch control (Fig. 2). When the animals were retested with U50,488H injected into the tail, the antisense oligodeoxynucleotide treatment again markedly lowered the response to U50,488H ($P < 0.002$). The mismatch treatment was inactive.

4. Discussion

Our results indicate that U50,488H is a potent analgesic peripherally. When administered directly into the tail, its ED_{50} dose (3.1 μg) is approximately 40-fold lower than the corresponding systemic dose. When the focus of the radiant heat is moved 1 cm away from the site of injection, the analgesic action is lost. Finally, the analgesia is blocked by the κ_1-opioid receptor antagonist nor-binaltorphimine far more potently when it is given locally. Together, these results are consistent with a local mechanism of action rather than through systemic absorption.

U50,488H is probably acting through κ_1-opioid receptors localized on the dorsal root ganglia neurons. Although the evidence strongly supports the local activity of the drug, its actions can be blocked by an antisense oligodeoxynucleotide against the cloned κ_1-opioid receptor given intrathecally. The antisense oligodeoxynucleotide would be expected to interfere with the synthesis of κ_1-opioid receptors synthesized in the dorsal root ganglion cells as well as the spinal cord. It is unlikely that the antisense probe has any direct effects in the tail for a variety of reasons. All antisense and mismatch probes were deoxynucleotides lacking stabilizing bonds such as the phosphothioates. Diffusion of the probes within the central nervous system is quite limited (Yee et al., 1994) and any material that is absorbed systemically would be expected to be degraded very rapidly. Thus, the blockade of the local analgesic response in the tail by an intrathecal antisense oligodeoxynucleotide targeting the KOR-1 clone confirms the selectivity of the response for κ_1-opioid receptors and strongly suggests that U50,488H is acting on the sensory peripheral nerves.

Our results indicate the presence of a peripheral κ_1-opioid analgesic system. Additional studies from our laboratory have observed similar peripheral actions of morphine using the same assay systems. In addition, the peripheral μ-opioid receptor system synergistically interacts with central systems, giving it great importance in systemic morphine analgesia. The presence of these peripheral opioid analgesic systems is quite intriguing and may prove to be a valuable pharmacological target in the design of novel analgesics. It also is interesting to speculate that these systems may be a target of the opioid peptides released into the systemic circulation by the adrenal and pituitary glands, particularly in times of stress.

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