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Enhanced κ-opioid receptor-mediated analgesia by antisense targeting the σ_1 receptor

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Abstract

In the current study, we used an antisense oligodeoxynucleotide targeting the recently cloned σ_1 receptor to assess its functions within the nervous system. σ_1 antagonists potentiate the analgesic actions of opioids. Similarly, the antisense probe targeting the σ_1 receptor enhanced the analgesic activity of the κ_1-opioid receptor agonist U50,488H (*trans*-3,4-dichloro-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeacetamidel) and the κ_3-opioid receptor agonist naloxone benzoylhydrazone. A mismatch control was inactive. These results confirm the role of σ_1 receptors in an anti-opioid analgesic system and illustrate the utility of antisense approaches towards the elucidation of σ receptor functions. © 1997 Elsevier Science B.V.

Keywords: Opioid; Analgesia; σ Receptor

Although initially considered a member of the opioid receptor family, σ receptors are now considered to be a distinct group of receptors (Quirion et al., 1992). They are widely distributed both within and outside the nervous system, as well as in a wide variety of tumors, but their functions are only now being uncovered. In addition to other activities, σ_1 receptors comprise a tonically active anti-opioid system (Chien and Pasternak, 1993; Chien and Pasternak, 1994). Blockade of σ_1 receptor-mediated actions greatly enhances the analgesic activity of a variety of opioids, particularly those acting through the kappa receptor systems, while activation of σ_1 receptors functionally antagonizes opioid analgesic actions. The recent cloning of the guinea pig σ_1 receptor provided a major advance in our understanding of σ receptor pharmacology (Hanner et al., 1996). It does not correspond to a traditional G-protein-coupled receptor and it has little structural homology to any established mammalian protein. The report of the guinea pig receptor (Hanner et al., 1996) was quickly followed by the human (Kekuda et al., 1996) and the mouse clones (Y.-X. Pan, J. Mei, M. King, J. Xu, B.-L. Wan, A. Chang and G.W. Pasternak, data not shown). Using the sequence from the mouse homolog of the σ_1 receptor (GenBank accession No. AF004927) which we recently cloned, we now report that targeting the σ_1 receptor with an antisense oligodeoxynucleotide significantly potentiates the analgesic actions of two κ-opioid receptor agonists.

Male CD-1 mice (25–30 g; Charles River Laboratories, Raleigh, NC, USA) were administered the antisense oligodeoxynucleotides intracerebroventricularly (i.c.v.) under halothane anesthesia (Standifer et al., 1994, 1996). The oligodeoxynucleotides (Midland Certified Reagent, Midland, TX, USA) were based upon the mouse sequence (GenBank accession No. AF004927) and were purified in our laboratory (Standifer et al., 1994). Three pairs of bases in Antisense A (GAGTGCCCAGCCACAACCAGG) were switched to generate the mismatch control (GAG**GT**CC-C**GA**CCACA**CA**CAGG). Mice received the oligodeoxynucleotides (10 μg in 2 μl, i.c.v.) or vehicle on days 1, 2 and 4 and were tested on day 5 with either systemic U50,488H (*trans*-3,4-dichloro-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeacetamidel) or naloxone benzoylhydrazone, as previously described (Standifer et al., 1994). Analgesia, assessed in the radiant heat tailflick assay, was defined quantally as a doubling or greater of the baseline latency for each mouse (Standifer et al., 1994) and analyzed by the Fischer exact test. All mice were used only once.

At the doses given, both U50,488H and naloxone benzoylhydrazone produced a limited analgesic response (< 20%) in vehicle-treated animals (Fig. 1). We had specifi-

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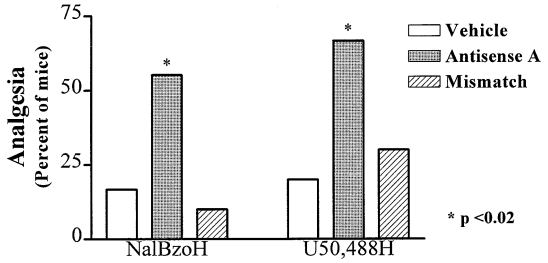

Fig. 1. Antisense effects of U50,488H or naloxone benzoylhydrazone analgesia. Groups of mice ($n \geq 20$) received saline or the indicated oligodeoxynucleotide on days 1, 2 and 4. On day 5 all mice were tested with U50,488H (2 mg/kg; s.c.) or naloxone benzoylhydrazone (30 mg/kg; s.c.) and analgesia assessed quantally. Antisense A significantly potentiated U50,488H or naloxone benzoylhydrazone analgesia ($p < 0.02$).

cally chosen low doses of the drugs to facilitate looking for potentiation. The mismatch oligodeoxynucleotide-treated mice showed similar limited analgesic responses. In contrast, Antisense A significantly facilitated U50,488H and naloxone benzoylhydrazone analgesia ($P < 0.02$; Fig. 1) compared to the vehicle controls.

Antisense approaches provide a sensitive and highly selective approach to evaluate the role of selected proteins within the central nervous system (Pasternak and Standifer, 1995). Prior work from our group had established that blockade of σ_1 receptors with haloperidol markedly potentiated the analgesic actions of opioids, particularly κ-opioids. The inactivity of (−)-sulpiride in control studies ensured that these actions did not reflect dopamine D_2 receptor blockade. The actions of haloperidol contrasted with the ability of (+)-pentazocine, a σ_1 receptor agonist, to functionally antagonize opioid analgesia. The current studies were carried out to ensure that the actions previously observed were, indeed, mediated through σ_1 receptors. Our results reveal that the σ_1 receptor antisense enhanced the analgesic activities of both the κ_1- and κ_3-opioid receptor analgesics, U50,488H and naloxone benzoylhydrazone, respectively. The inactivity of the mismatch sequence is an important control to ensure the specificity of the result. Correlating the behavioral actions of σ receptor drugs with targets at the molecular level will lead to a greater understanding of the mechanisms responsible for their modulation of opioid functions.

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