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Short communication

An antisense oligodeoxynucleotide to μ-opioid receptors inhibits μ-opioid receptor agonist-induced analgesia in rats

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Abstract

We examined effects of an antisense oligodeoxynucleotide against the μ-opioid receptor on μ-opioid receptor agonist-induced antinociception in the cold water (−3°C) tail-flick test in rats. Rats were injected intracerebroventricularly (i.c.v.) with an antisense, sense or missense oligodeoxynucleotide or artificial cerebrospinal fluid on days 1, 3 and 5. On day 6, antinociceptive effects of opioid agonists were tested. Compared to the artificial cerebrospinal fluid treatment, the cumulative dose-effect curve for subcutaneous (s.c.) morphine was shifted to the right by the antisense oligodeoxynucleotide, but not by the missense oligodeoxynucleotide or the sense oligodeoxynucleotide treatment. Antisense oligodeoxynucleotide treatment reduced the analgesic effect of the μ-opioid receptor agonist PL017 ([*N*-MePhe^3,D-Pro^4]morphiceptin), but not the δ-opioid receptor agonist BW373U86 ((±)-4-((*a*-*R**)-*a*-((2*S**,5*R**)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl)-*N*,*N*-diethyl-benzamide) or the κ-opioid receptor agonist spiradoline ((±)-(5*a*,7*a*,8*b*)-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-(oxaspiro-[4.5]dec-8-yl]benzeneacetamide monohydrochloride). The drugs were given by i.c.v. injection. These findings indicate that i.c.v. administration of a μ antisense oligodeoxynucleotide specifically blocks μ-, but not δ- or κ-opioid receptor-mediated analgesia in the rat cold water tail-flick test.

Keywords: Antisense oligodeoxynucleotide; Analgesia; μ-Opioid receptor; Cold water tail-flick test; (Rat)

1. Introduction

Antisense oligodeoxynucleotides against several neurotransmitter receptors have been demonstrated to reduce receptor numbers and/or receptor functions (for a review, Wahlestedt, 1994). Molecular cloning of μ-, δ- and κ-opioid receptors (for a review, Uhl et al., 1994) has allowed the use of antisense oligodeoxynucleotides to manipulate these receptor systems. Recently, using the radiant heat tail-flick test, Pasternak and co-workers reported that treatment with antisense oligodeoxynucleotides to the rat μ-, mouse δ- or mouse κ-opioid receptor blocked effects of morphine, [D-Pen^{2,5}]enkephalin (DPDPE) or *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] benzeneacetamide (U50,488H), respectively (Rossi et al., 1994; Standifer et al., 1994; Chien et al., 1994). In addition, Tseng et al. (1994) demonstrated that intrathecal (i.t.) injections of an antisense oligodeoxynucleotide to the δ-opioid receptor blocked antinociception elicited by i.t. administered [D-Ala^2]deltorphin II and DPDPE, but not [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO) or U50,488H.

Our laboratory has used the cold water tail-flick test in rats and has demonstrated its usefulness in studying antinociceptive effects of μ-, δ-, and κ-opioid receptor agonists as well as mixed agonist-antagonists (Pizziketti et al., 1985). δ- and κ-opioid receptor agonists administered i.c.v. are inactive in the commonly used heat tail-flick test in rats but are effective antinociceptive agents in the cold water tail-flick test. The cold water tail-flick test thus permits investigation of selectivity among agents acting on the three opioid receptor types.

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We have reported that i.c.v. treatment with an antisense oligodeoxynucleotide to κ-opioid receptors inhibited κ-, but not μ- or δ-, opioid receptor agonist-induced analgesia in rats in the cold water tail-flick test (Adams et al., 1995). In the present study, we examined whether the i.c.v. treatment of an antisense oligodeoxynucleotide to the μ-opioid receptor can antagonize the analgesic effect induced by μ-opioid receptor agonists in the rat cold water tail-flick test. In addition, the selectivity of this antisense oligodeoxynucleotide among μ-, δ- and κ-opioid receptors was investigated. The μ-antisense oligodeoxynucleotide used in this study was an 18-mer targeted at the first 18 nucleotides of the coding region of the μ-opioid receptor, which was different from that used by Rossi et al. (1994).

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats, weighing 125–150 g, were housed in groups of 6–7 for at least 1 week in an animal room maintained at 22 ± 2°C and approximately 50% relative humidity. Lighting was on a 12/12 h light/dark cycle (lights on at 7:00 h and off at 19:00 h). Cannulae were implanted into the lateral ventricle according to standard procedures in our laboratory.

2.2. Oligodeoxynucleotide synthesis

Oligodeoxynucleotides were synthesized on a ABI 394 synthesizer using 15 min tetraethylthiuram disulfide oxidation, then were lyophilized and resuspended in sterile saline. The sequences were as follows: μ-antisense oligodeoxynucleotide, 5′-GCCGGTGCTGCT-GTCCAT-3′; μ-sense oligodeoxynucleotide, 5′-ATG-GACAGCAGCACCGGC-3′; μ-missense oligodeoxynucleotide, 5′-GCGGCTGGTCCTCTCGAT-3′. The μ-antisense oligodeoxynucleotide was directed against the first 18 nucleotides of the coding region of the rat μ-opioid receptor (Chen et al., 1993). This sequence is unique to the μ-opioid receptor since it has no homology to δ- or κ-opioid receptors or any other cloned proteins in the GeneBank database.

2.3. Drugs

Drugs were dissolved in 0.9% saline. Artificial cerebrospinal fluid (pH 7.3–7.4) contained (in mM): 128.5 NaCl, 3.0 KCl, 1.15 $CaCl_2$, 0.80 $MgCl_2$, 21.0 $NaHCO_3$, 0.25 Na_2HPO_4, and 3.4 glucose. PL017 was purchased from Peninsula Labs, Belmont, CA, USA. BW373U86 and spiradoline were obtained from Burroughs Wellcome Co. and Upjohn Co., respectively.

2.4. Injections

Unrestrained rats received i.c.v. treatment of artificial cerebrospinal fluid or an oligodeoxynucleotide on days 1, 3 and 5. Either artificial cerebrospinal fluid or 20 μg oligodeoxynucleotide was injected in a volume of 5 μl followed by 3-μl artificial cerebrospinal fluid flush over 30 s. On day 6, we examined the analgesic effect induced by i.c.v. injection of PL017, BW373U86 or spiradoline, or cumulative s.c. injections of morphine. For i.c.v. drugs, post-drug latencies were taken at 15 and 30 min after drug injection. For s.c. morphine, the drug was injected at 30-min intervals in the following doses: 2, 2, 4, 8, 16 mg/kg. Latency to tail flick was measured 30 min after each injection.

2.5. Nociceptive test

The latency to flick the tail from a −3°C solution of ethylene glycol and water (1:1) maintained with a circulating water bath was used as the analgesic index, according to a standard procedure in our laboratory (Pizziketti et al., 1985). The percent of maximal possible analgesia (MPA) for each animal at each time was calculated using the formula: MPA% = [(test latency − baseline latency)/(60 − baseline latency)] × 100. A cut-off limit of 60 s was set to avoid damage to the tail.

2.6. Statistical analysis

The data are expressed as the mean and standard error. Statistical analysis of difference between groups was assessed with a two-way analysis of variance (ANOVA) followed by Duncan's test and with a grouped t-test. $P \leq 0.05$ was taken as the significant level of difference.

3. Results

Rats were randomly divided into 4 groups, each receiving an i.c.v. injection of artificial cerebrospinal fluid, μ-antisense, μ-sense or μ-missense oligodeoxynucleotide and tested for the analgesic effect of cumulative s.c. administration of morphine (2–32 mg/kg) in the cold water tail-flick test. The results are shown in Fig. 1. The cumulative dose-effect curve of morphine was shifted to the right by μ-antisense oligodeoxynucleotide treatment compared with that of artificial cerebrospinal fluid (ANOVA followed by Duncan's test, $P < 0.01$), μ-sense oligodeoxynucleotide (ANOVA followed by Duncan's test, $P < 0.01$) or μ-missense oligodeoxynucleotide (ANOVA followed by Duncan's test, $P < 0.01$) treatment. There were no significant differences among the groups treated with artificial cerebrospinal fluid, μ-sense oligodeoxynucleotide and

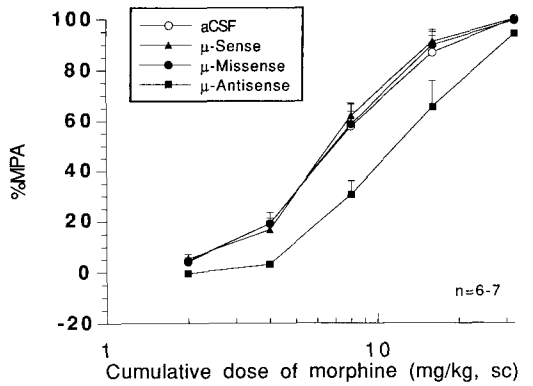

Fig. 1. Dose-effect curves for cumulative s.c. morphine (2–32 mg/kg) in the cold water tail-flick test after treatment with artificial cerebrospinal fluid (aCSF), sense oligodeoxynucleotide, missense oligodeoxynucleotide and antisense oligodeoxynucleotide. Each point represents the mean ± SE of 6–7 rats.

μ-missense oligodeoxynucleotide. Baseline latencies were not significantly different among rats treated with μ-sense oligodeoxynucleotide, μ-missense oligodeoxynucleotide, μ-antisense oligodeoxynucleotide or artificial cerebrospinal fluid.

We then examined the selectivity of this μ-antisense oligodeoxynucleotide among the three types of opioid receptors. Rats were randomly divided into 2 groups, each receiving an i.c.v. injection of artificial cerebrospinal fluid or μ-antisense oligodeoxynucleotide. Animals were then tested for analgesic effects of an i.c.v. injection of the μ-opioid receptor agonist PL017 (2 μg), the δ-opioid receptor agonist BW373U86 (100 μg) or the κ-opioid receptor agonist spiradoline (200 μg). These doses have been shown previously to elicit similar degrees of analgesia in the cold water tail-flick test. The μ-antisense treatment markedly reduced PL017-induced analgesia (t-test, $P < 0.01$, compared to the corresponding artificial cerebrospinal fluid group),

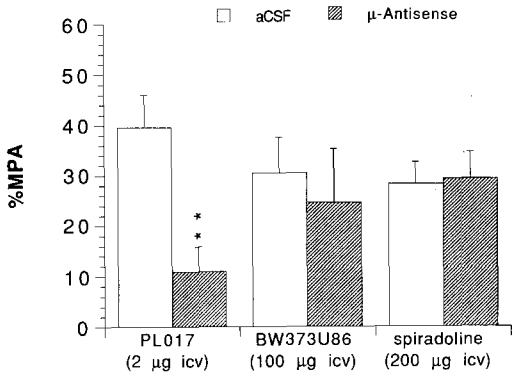

Fig. 2. Maximal analgesic effects over 30 min after i.c.v. injection of PL017 (2 μg), BW373U86 (100 μg) or spiradoline (200 μg) in rats treated with artificial cerebrospinal fluid (aCSF) or antisense oligodeoxynucleotide. Each bar represents the mean ± SE of 6–8 rats. $^{**}P < 0.01$ (grouped t-test), as compared to the corresponding artificial cerebrospinal fluid group.

but did not reduce BW373U86- or spiradoline-elicited analgesia (grouped t-test, both $P > 0.05$) (Fig. 2).

4. Discussion

In this study, we demonstrated that i.c.v. administration of a μ-antisense, but not a μ-missense or μ-sense, oligodeoxynucleotide significantly reduced s.c. morphine-induced analgesia in the rat cold water tail-flick test. This μ-antisense oligodeoxynucleotide inhibited selective μ-, but not δ- or κ-, opioid receptor agonist-induced analgesia. PL017 (Chang et al., 1983) and BW373U86 (Wild et al., 1993) are highly selective for μ- and δ-opioid receptors, respectively. We have shown previously that in the cold water tail-flick test, spiradoline (Meecham et al., 1989) acts on the κ-opioid receptor since its action is blocked by the κ-, but not μ- or δ-, opioid receptor antagonist (Adams et al., 1995).

Using the radiant heat tail-flick test in rats, Rossi et al. (1994) reported similar results, showing that a μ-antisense oligodeoxynucleotide completely blocked the analgesic effects of morphine administered into the periaqueductal grey. There are some notable differences in our study. The μ-antisense oligodeoxynucleotide used by Rossi et al. (1994) was directed against the 5′-untranslated region (bases −87 to −69 upstream from the initiation codon ATG), whereas ours was an 18-mer targeted at the first 18 nucleotides of the coding region of the μ-opioid receptor. In addition, we administered oligodeoxynucleotides by i.c.v. injections whereas they injected directly into the periaqueductal grey. Despite these differences, the observation that these antisense oligodeoxynucleotides reduced μ-opioid receptor function implies a general applicability of this approach for μ-opioid receptors. In the present study, we were able to show the μ selectivity of the μ-antisense oligodeoxynucleotide since μ-, δ- and κ-opioid receptor agonists are all active in the cold water tail-flick test. In the study by Rossi et al. (1994), the issue of selectivity of the antisense oligodeoxynucleotide among opioid receptor types was not addressed.

Antisense oligodeoxynucleotides against δ- or κ-opioid receptors have also been reported to block δ- or κ-opioid receptor function. Recently, we reported that i.c.v. treatment with an antisense oligodeoxynucleotide to κ-opioid receptors blocked spiradoline-, but not PL017- or DPDPE-, induced analgesia in the cold water tail-flick test (Adams et al., 1995). I.t. administration of an antisense oligodeoxynucleotide against the κ-opioid receptor selectively blocked U50,488H-, but not DPDPE- or DAMGO-, induced analgesia in the heat tail-flick test in mice (Chien et al., 1994). Antisense oligodeoxynucleotides to the δ-opioid receptor lowered δ binding in NG108-15 cells by 40–50%

(Standifer et al., 1994). An antisense oligodeoxynucleotide against the δ-opioid receptor given i.t. in mice attenuated i.t. DPDPE- and deltorphin II- (both δ_2-opioid receptor-mediated in the spinal cord), but not DAMGO- or U50,488H-, induced analgesia (Standifer et al., 1994; Tseng et al., 1994). On the other hand, i.c.v. δ-antisense oligodeoxynucleotide selectively inhibited antinociception induced by i.c.v. [D-Ala^2,Glu^4]deltorphin (δ_2), but not by i.c.v. DPDPE (δ_1), DAMGO or U69,593, suggesting the existence of subtypes of the δ-opioid receptor (Bilsky et al., 1994). In addition, an antisense oligodeoxynucleotide to the δ-opioid receptor given i.t. in mice blocked antinociception induced by i.c.v. β-endorphin, but not DAMGO or U50,488H (Tseng and Collins, 1994). These studies underscore the high degree of selectivity among opioid receptors that one can obtain with properly designed antisense oligodeoxynucleotides. Taking advantage of the high degree of selectivity, Pan et al. (1994) have used antisense oligodeoxynucleotides to evaluate a novel opioid receptor as the κ_3 receptor.

The treatment schedule used in this study, i.e., three injections over 5 days, was based on the report that the turnover rate of opioid receptors is 3–5 days (Standifer et al., 1994). The same parameters of antisense treatment have been used successfully in blocking the functions of NPY-Y1 and μ-, δ- and κ-opioid receptors (Wahlestedt, 1994; Chien et al., 1994; Rossi et al., 1994; Standifer et al., 1994).

Mechanisms of action of antisense oligodeoxynucleotides are not well understood. Potential interactions of an antisense oligodeoxynucleotide with the target nucleic acids are complex. Two possible mechanisms are to inhibit the translation process and to provide substrate for RNase H, which degrades the RNA strand of an RNA-DNA duplex (Wahlestedt, 1994). The sense oligodeoxynucleotide has the same sequence as the receptor mRNA. The missense oligodeoxynucleotide has the same base composition as the antisense oligodeoxynucleotide, but contains 6 nucleotide mismatches. The finding that a μ-antisense oligodeoxynucleotide, but not a μ-sense or μ-missense oligodeoxynucleotide, attenuated the analgesic effect of morphine indicates that specific complementary base-pairing is essential for the action of the antisense oligodeoxynucleotide. Whether this μ-antisense oligodeoxynucleotide reduces receptor number or receptor mRNA level is currently under investigation.

In conclusion, an antisense oligodeoxynucleotide against the μ-opioid receptor specifically and selectively blocked μ-, but not δ- or κ-, opioid receptor agonist-induced analgesia in the cold water tail-flick test in rats. This study, taken together with several recent reports (Bilsky et al., 1994; Rossi et al., 1994; Standifer et al., 1994; Chien et al., 1994), indicates that antisense oligodeoxynucleotides against opioid receptors provide new and highly selective tools for opioid pharmacology.

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