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Short communication

Stimulation of spinal δ-opioid receptors in mice selectively enhances the attenuation of δ-opioid receptor-mediated antinociception by antisense oligodeoxynucleotide

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Abstract

Intrathecal (i.t.) pretreatment of male ICR mice with antisense oligodeoxynucleotide to δ-opioid receptor mRNA once a day for 1–3 days caused a time-dependent attenuation of i.t. administered [D-Ala^2]deltorphin II-induced antinociception as measured by the tail-flick test. The attenuation of the antinociception induced by i.t. administered [D-Ala^2]deltorphin II, a δ-opioid receptor agonist, was enhanced by i.t. pretreatment for 1 day with [D-Ala^2]deltorphin II, but not [D-Ala^2,*N*MePhe^4,Gly(ol)^5]enkephalin (DAMGO), a μ-opioid receptor agonist, or U50,488H, a κ-opioid receptor agonist, given together with antisense oligodeoxynucleotide to δ-opioid receptor mRNA. The present results indicate that stimulation of spinal δ-opioid receptors by i.t. injection of [D-Ala^2]deltorphin II selectively causes a loss of δ-opioid receptor-mediated antinociception in mice pretreated with antisense oligodeoxynucleotide to δ-opioid receptor mRNA.

Keywords: Antisense oligodeoxynucleotide; δ-Opioid receptor; Desensitization; Antinociception

1. Introduction

The importance of δ-opioid receptors as mediators for antinociception in the spinal cord of mice and rats is recognized (Mattia et al., 1992; Tseng and Collins, 1992; Stewart and Hammond, 1993; Tseng et al., 1993). Intrathecal (i.t.) injection of a selective δ-opioid receptor agonist, [D-Pen^2,D-Pen^5]enkephalin (DPDPE) and [D-Ala^2]deltorphin II produces antinociception. The effects are selectively blocked by selective δ-opioid receptor antagonists, naltrindole, ICI174,864 ((Allyl)2-Tyr-Aib-Aib-Phe-Leu-OH) and naltriben (Mattia et al., 1992; Stewart and Hammond, 1993; Tseng et al., 1993). The δ-opioid receptors in the spinal cord also play an important role in mediating the antinociception induced by β-endorphin given supraspinally (Tseng and Collins, 1993; Tseng et al., 1993). β-Endorphin given supraspinally releases endogenous [Met^5]enkephalin which subsequently stimulates δ-opioid receptors in the spinal cord to produce antinociception (Tseng et al., 1985; Tseng and Collins, 1993). Blockade of δ-opioid receptors by i.t. injection of δ-opioid receptor antagonists blocks the antinociception induced by supraspinally administered β-endorphin (Tseng et al., 1993).

A δ-opioid receptor has been cloned from NG108-15 cell. The product of the cloned receptor cDNA, when expressed in COS cells, stereoselectively binds δ ligands with high affinities (Evans et al., 1992). An antisense oligodeoxynucleotide is a short piece of synthetic DNA with a nucleotide sequence that is the reverse of and complementary to a part of mRNA. It therefore hybridizes to mRNA and inhibits the synthesis of the encoded protein. Using an antisense oligodeoxynucleotide to δ-opioid receptor mRNA, we have previously demonstrated that the blockade of the synthesis of δ-opioid receptors in the spinal cord by intrathecal (i.t.) pretreatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA once a day for 3 days selectively blocks the antinociception induced by i.t. administered δ-, but not μ- or κ-opioid

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receptor agonists (Tseng et al., 1994). Similar results were obtained by Standifer et al. (1994) using a different nucleotide sequence of antisense oligodeoxynucleotide to δ-opioid receptor mRNA. These studies demonstrate the utility of antisense oligodeoxynucleotide approaches in the study of δ-opioid receptor functions. The finding that repeated treatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA causes a decrease of δ-opioid receptor-mediated antinociception seems to indicate that there is a turnover of δ-opioid receptors. Therefore, the present study was designed to both analyze the time course of the decline of the δ-opioid receptor mediated-antinociception in the spinal cord following the treatment of mice with antisense oligodeoxynucleotide to δ-opioid receptor mRNA, and determine if the decrease of δ-opioid receptor activity can be facilitated by selective stimulation of δ-opioid receptors by a potent δ-opioid receptor agonist, [D-Ala^2]deltorphin II (Stewart and Hammond, 1993).

2. Materials and methods

2.1. Animals

Male ICR mice weighing 25–30 g (Sasco, Omaha, NE, USA) were used. The animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light-dark cycle. Food and water were available ad libitum. The animals were used only once.

2.2. Experimental procedure

Intrathecal administration was performed following the method described by Hylden and Wilcox (1980) using a 10-μl Hamilton syringe with a 30-gauge needle. The injection volume for i.t. injection was 5 μl. Groups of mice were injected i.t. with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (163 pmol, 1 μg) or saline once a day for 1, 2 or 3 days. Other groups of mice were injected i.t. with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (163 pmol, 1 μg) or saline and, 10 min later, with [D-Ala^2]deltorphin II (6.4 nmol, 5 μg), naltriben (δ-opioid receptor antagonist, 20 nmol), DAMGO (μ-opioid receptor agonist, 9.7 pmol), U50,488H (*trans* (±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide methane sulfonate; κ-opioid receptor agonist, 85.9 nmol) or saline given i.t. 10 min after antisense oligodeoxynucleotide to δ-opioid receptor mRNA. The doses of agonists or antagonist were chosen based on previous reports that they selectively block the respective opioid receptors (Tseng and Collins, 1992; Tseng et al., 1993). [D-Ala^2]Deltorphin II (6.4 nmol, 5 μg) was administered i.t. 24 h after the last injection and antinociceptive testing was performed with the tail-flick method 10 min after i.t. injection of [D-Ala^2]deltorphin II. The mismatch oligo (163 pmol, 1 μg) served as a control. For measurement of the latency of the tail-flick response, the mice were gently held by hand with their tail positioned in the apparatus (Model TF6, EMDIE Instrument Co., Maidens, VA, USA) for radiant heat stimulation on the dorsal surface of the tail. The intensity of heat stimulus was adjusted so that the animal flicked its tail after 3–5 s. Antinociception was expressed as percent of the maximal possible effect, '%MPE', which was calculated as: $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where T_0 and T_1 were the tail-flick latencies before and after the injection of the opioid receptor agonist and T_2 was the cutoff time, which was set at 10 s for the test to avoid injury to the tail.

2.3. Drugs

The drugs used were DAMGO ([D-Ala^2,*N*-MePhe^4,Gly(ol)^5]enkephalin, Peninsula Laboratory, Belmont, CA, USA), naltriben methanesulfonate (Research Biochemicals International, Natick, MA, USA) and U50,488H (Research Biochemicals International). The antisense oligodeoxynucleotide to δ-opioid receptor mRNA, mismatch oligodeoxynucleotide and [D-Ala^2]deltorphin II were synthesized by Dr. John Richard (Molecular Research Laboratories, Durham, NC, USA). The opimum antisense oligodeoxynucleotide corresponding to bases 25–44 of DOR-1 of mouse δ-opioid receptor (Evans et al., 1992) consists of a phosphorothioate with the following sequence: 5′-AGG GCA CCA GCT CCA TGG GG-3′. The mismatch oligodeoxynucleotide, which has the following sequence: 5′-GGC GTC GAC CTA CTT CGG CG-3′, served as a control.

2.4. Statistics

The data are expressed as the means and S.E.M. The statistical significance of differences between groups was assessed with a one-way analysis of variance (ANOVA) followed by the Newman-Keuls test.

3. Results

3.1. Time course of the decline of i.t. [D-Ala^2]deltorphin II-induced antinociception following i.t. treatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA

Daily i.t. pretreatment of mice with saline for 1–3 days did not have any effect on [D-Ala^2]deltorphin II-induced antinociception (%MPE ± S.E.M. in mice treated with saline for 1, 2 and 3 days were 77.3 ± 6.1; 77.3 ± 8.2 and 73.9 ± 8.3, respectively). On the other

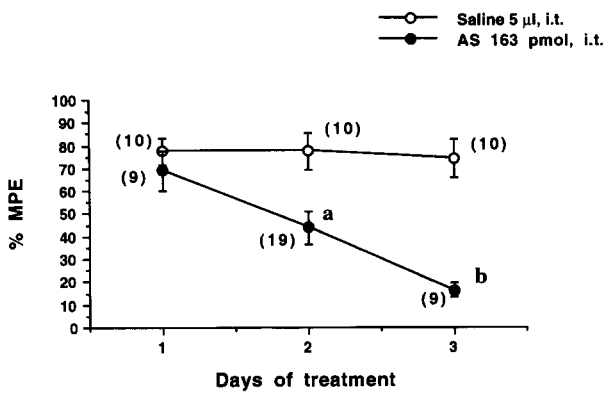

Fig. 1. Time course of the decline of [D-Ala^2]deltorphin II-induced antinociception following i.t. treatment with the antisense oligodeoxynucleotide (AS oligo) to δ-opioid receptor mRNA. Groups of mice were injected i.t. with saline (5 μl) or AS oligo (163 pmol) once a day for 1, 2 and 3 days and were injected i.t. with [D-Ala^2]deltorphin II the next day. Tail-flick response was measured 10 min after [D-Ala^2]deltorphin II injection at 6.4 nmol. The vertical bar indicates the S.E.M.; $n = 9$–19 mice for each point. $^aP < 0.05$, $^bP < 0.01$, compared to mice injected with i.t. saline.

hand, i.t. pretreatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (163 pmol) once a day for 1–3 days caused a time-dependent decline of [D-Ala^2]deltorphin II-induced antinociception. The antinociception induced by i.t. injection of [D-Ala^2]deltorphin II did not change significantly 1 day after i.t. treatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (%MPE ± S.E.M. = 69.1 ± 8.8), but declined progressively after 2 and 3 days of treatment (%MPE ± S.E.M. = 43.9 ± 7.2 and 16.3 ± 3.0, respectively; Fig. 1). The time for the decline of the [D-Ala^2]deltorphin II-induced antinociception to reach 50% was roughly estimated to be 2.3 days. Intrathecal pretreatment of mice with mismatch oligodeoxynucleotide once a day for 3 days also had no effect on [D-Ala^2]deltorphin II-induced antinociception (%MPE ± S.E.M. = 79.3 ± 8.7%, not significantly different from saline control).

3.2. Effects of i.t. pretreatment with [D-Ala^2]deltorphin II, naltriben, DAMGO, U50,488H on i.t. [D-Ala^2]deltorphin II-induced antinociception in mice pretreated for 1 day with antisense oligodeoxynucleotide to δ-opioid receptor mRNA

Pretreatment of mice with [D-Ala^2]deltorphin II or antisense oligodeoxynucleotide to δ-opioid receptor mRNA alone for 1 day did not have any significant effect on i.t. administered [D-Ala^2]deltorphin II-induced antinociception in mice pretreated with saline. However, pretreatment with [D-Ala^2]deltorphin II (1.3 and 6.4 nmol) given i.t. together with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (163 pmol) for 1 day caused a dose-dependent attenuation of the i.t. [D-Ala^2]deltorphin II-induced antinociception (Table 1). Naltriben alone as pretreatment i.t. did not have any effect on [D-Ala^2]deltorphin II-induced antinociception in mice pretreated with antisense oligodeoxynucleotide to δ-opioid receptor mRNA, but blocked its attenuation by [D-Ala^2]deltorphin II pretreatment in mice pretreated with antisense oligodeoxynucleotide to δ-opioid receptor mRNA. Intrathecal pretreatment of mice with the μ-opioid receptor agonist, DAMGO, or the κ-opioid receptor agonist, U50,488H, for 1 day did not have any significant effect on the i.t. administered [D-Ala^2]deltorphin II-induced antinociception in mice pretreated with antisense oligodeoxynucleotide to δ-opioid receptor mRNA. In-

Table 1
Effects of i.t. pretreatment with [D-Ala^2]deltorphin II, naltriben, DAMGO, U50,488H on i.t. [D-Ala^2]deltorphin II-induced antinociception in mice pretreated for 1 day with antisense oligodeoxynucleotide to δ-opioid receptor mRNA

Pretreatment	[D-Ala^2]Deltorphin II-induced antinociception (%MPE ± S.E.M.)	Number of animals
Saline + saline	74.7 ± 5.2	18
Saline + [D-Ala^2]deltorphin II (6.4)	71.2 ± 8.2	10
δ-AS oligo + saline	67.0 ± 5.7	24
δ-AS oligo + [D-Ala^2]deltorphin II (1.3)	50.7 ± 7.0	9
δ-AS oligo + [D-Ala^2]deltorphin II (6.4)	18.3 ± 3.8 [a]	10
δ-AS oligo + naltriben	68.2 ± 7.8	10
δ-AS oligo + naltriben + [D-Ala^2]deltorphin II (6.4)	68.5 ± 7.9	14
δ-AS oligo + DAMGO	62.1 ± 8.3	15
δ-AS oligo + U50,488H	68.1 ± 6.7	19
MM oligo + [D-Ala^2]deltorphin II (6.4)	72.4 ± 6.1	10

Groups of mice were injected i.t. with saline, [D-Ala^2]deltorphin II (1.3 or 6.4 nmol), naltriben, [D-Ala^2]deltorphin II (6.4 nmol) in combination with naltriben (20 nmol), DAMGO (9.7 nmol) or U50,488H (85.9 nmol) 10 min after i.t. injection of antisense oligodeoxynucleotide to δ-opioid receptor mRNA (δ-AS oligo; 163 pmol), mismatch oligodeoxynucleotide (MM oligo; 163 pmol) or saline. [D-Ala^2]Deltorphin II (6.4 nmol) was then injected i.t. on the 2nd day and the tail-flick response was measured 10 min after the injection. [a] $P < 0.01$, compared to mice pretreated with δ-AS oligo + saline.

trathecal pretreatment of mice for 1 day with mismatch oligodeoxynucleotide had no effect on [D-Ala^2]deltorphin II-induced antinociception in mice pretreated i.t. with [D-Ala^2]deltorphin II.

4. Discussion

We now found that the inhibition of the biosynthesis of δ-opioid receptor protein by i.t. pretreatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA once a day for 1–3 days caused a time-dependent decline of [D-Ala^2]deltorphin II-induced antinociception, indicating that the δ-opioid receptor function for antinociception in the spinal cord is blocked by pretreatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA. The inhibition of the tail-flick response induced by [D-Ala^2]deltorphin II given i.t. remaining unchanged for 1 day, indicating that initially there are enough δ-opioid receptors remaining in the cellular membrane to maintain its normal antinociception. However, the capacity of δ-opioid receptor activity was reduced after 2 or 3 days of antisense oligodeoxynucleotide to δ-opioid receptor mRNA treatment. The time for the decline of [D-Ala^2]deltorphin II-induced antinociception to 50% was roughly estimated to be 2.3 days. This value may reflect the $t_{1/2}$ of the hypothesized turnover for δ-opioid receptor function in the spinal cord.

The present results indicated that there exists a turnover of δ-opioid receptors in the spinal cord. It is most likely that the functional receptors located in the cell membrane are constantly replenished by newly synthesized receptor protein and the inhibition of the biosynthesis of the receptor protein by antisense oligodeoxynucleotide results in depletion of the functional receptors. Although the exact mechanism causing the turnover of δ-opioid receptors in the spinal cord is not clear, the results we now obtained suggest that continuous stimulation of the δ-opioid receptors by the spontaneously released endogenous [Met^5]enkephalin (Tseng et al., 1985; Höllt, 1986; Tseng and Collins, 1993) may be responsible for the loss of functional δ-opioid receptors. This contention is supported by the finding that blockade of δ-opioid receptors by i.t. injection of naltriben completely prevents the decline of spinal δ-opioid receptor-mediated antinociception in mice treated i.t. with antisense oligodeoxynucleotide to δ-opioid receptor mRNA. Concomitant i.t. treatment with bestatin or thiorphan, which inhibits the degradation of the released [Met^5]enkephalin, therefore, potentiates the [Met^5]enkephalin effect, while antisense oligodeoxynucleotide to δ-opioid receptor mRNA enhanced the attenuation of δ-opioid receptor mediated-antinociception (Narita and Tseng, manuscript in preparation). Intraventricular injection of β-endorphin releases [Met^5]enkephalin, which subsequently stimulates the δ-opioid receptors in the spinal cord, producing antinociception (Tseng et al., 1985). We found that i.c.v. injection of β-endorphin enhanced the attenuation of the δ-opioid receptor agonist-induced antinociception in mice treated with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (Tseng and Narita, unpublished observation).

To obtain direct evidence that the loss of δ-opioid receptor function in mice treated with antisense oligodeoxynucleotide is due to stimulation of δ-opioid receptors, the effect of pretreatment of mice with [D-Ala^2]deltorphin II on the [D-Ala^2]deltorphin II-induced antinociception in mice pretreated with antisense oligodeoxynucleotide to δ-opioid receptor mRNA was studied. We found that the stimulation of δ-opioid receptors by i.t. injection of [D-Ala^2]deltorphin II blocked δ-opioid receptor-mediated antinociception in mice pretreated for 1 day with antisense oligodeoxynucleotide to δ-opioid receptor mRNA, but not in mice pretreated with saline. The effect is specifically mediated by the stimulation of δ-opioid receptors because naltriben, which selectively blocks the δ-opioid receptor, prevented the effect. Furthermore, stimulation of μ- and κ-opioid receptors by i.t. injection of DAMGO and U50,488H, respectively, did not cause any attenuation of [D-Ala^2]deltorphin II-induced antinociception in mice pretreated for 1 day with antisense oligodeoxynucleotide to δ-opioid receptor mRNA. The findings support the contention that the stimulation of δ-opioid receptors by a single injection of a δ-opioid agonist specifically causes a loss of δ-opioid receptor function in mice in which the synthesis of δ-opioid receptor protein is inhibited by antisense oligodeoxynucleotide.

It is concluded that stimulation of spinal δ-opioid receptors by a δ-opioid agonist selectively enhances the attenuation of the δ-opioid receptor-mediated antinociception by antisense oligodeoxynucleotide to δ-opioid receptor mRNA in the mouse.

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