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Short communication

Pretreatment with β-endorphin facilitates the attenuation of δ-opioid receptor-mediated antinociception caused by δ-opioid receptor antisense oligodeoxynucleotide

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Abstract

Intracerebroventricular (i.c.v.) pretreatment of male ICR mice with β-endorphin (0.6 nmol) or intrathecal (i.t.) pretreatment with antisense oligodeoxynucleotide to δ-opioid receptor mRNA (163 pmol) alone given 24 h earlier did not have any effect on i.t. administered δ-opioid receptor agonist [D-Ala^2]deltorphin II (6.4 nmol)-induced antinociception. However, a concomitant i.c.v. pretreatments with β-endorphin (0.08–0.6 nmol) and i.t. pretreatment with δ-opioid receptor antisense oligodeoxynucleotide (163 pmol) for 24 h dose-dependently attenuated i.t. challenged [D-Ala^2]deltorphin II-induced antinociception. A concomitant i.c.v. pretreatment with μ-opioid receptor agonist [D-Ala^2,*N*MePhe^4,Gly(ol)^5]enkephalin (DAMGO) or κ-opioid receptor agonist U50,488H and i.t. pretreatment with δ-opioid receptor antisense oligodeoxynucleotide for 24 h did not affect i.t. challenged [D-Ala^2]deltorphin II-induced antinociception. β-Endorphin given supraspinally has been documented to release [Met^5]enkephalin acting on δ-opioid receptors in the spinal cord. Our results indicate that supraspinal pretreatment with β-endorphin selectively causes a loss of spinal δ-opioid receptor-mediated antinociception in mice receiving δ-opioid receptor antisense oligodeoxynucleotide.

Keywords: Antisense oligodeoxynucleotide; β-Endorphin; δ-Opioid receptor; Antinociception

1. Introduction

The cloning of opioid receptors has provided a powerful new approach of using antisense oligodeoxynucleotides to study the regulation of opioid receptors (Wahlestedt, 1994). We have previously demonstrated that blockade of the synthesis of δ-opioid receptors in the spinal cord by intrathecal (i.t.) pretreatment with antisense oligodeoxynucleotide corresponding to bases 25–44 of the cloned mouse δ-opioid receptor mRNA reported by Evans et al. (1992) once a day for 3 days selectively attenuates the antinociception induced by i.t. administered δ-, but not μ- or κ-opioid receptor agonists (Tseng et al., 1994).

The δ-opioid receptor-mediated antinociception was not significantly attenuated 1 day after δ-opioid receptor antisense oligodeoxynucleotide treatment but declined progressively after 2 and 3 days of treatments (Narita and Tseng, 1995). The finding that the inhibition of the biosynthesis of δ-opioid receptor protein produced by chronic pretreatment with δ-opioid receptor antisense oligodeoxynucleotide causes a delayed attenuation of δ-opioid receptor-mediated antinociception seems to suggest that there exists a slow turnover of δ-opioid receptors which may be regulated by the release of the endogenous opioid peptide, [Met^5]enkephalin. This contention is supported by our recent findings that the attenuation of i.t. challenged [D-Ala^2]deltorphin II-induced antinociception caused by i.t. pretreatment 24 h earlier with δ-opioid receptor antisense oligodeoxynucleotide was prevented by the concomitant pretreatment with naltriben, a δ-opioid receptor antagonist, but was markedly enhanced by concomitant i.t. pretreatment with thiorphan or bestatin, which inhibits the degradation of endogenously released [Met^5]enkephalin (Tseng and Narita, 1995). Further, the antinociception induced by cold water swimming, which is mediated by the release of [Met^5]enkephalin acting on δ-opioid receptors in the

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spinal cord (Mizoguchi et al., 1995a,b), was blocked by a concomitant i.t. pretreatment 24 h earlier with [D-Ala^2]deltorphin II and δ-opioid receptor antisense oligodeoxynucleotide (Mizoguchi et al., 1995c). Likewise, the antinociception induced by i.t. challenged [D-Ala^2]deltorphin II was attenuated by a concomitant pretreatment 24 h earlier with cold water swimming and i.t. injection of δ-opioid receptor antisense oligodeoxynucleotide (Mizoguchi et al., 1995c). These findings indicate that stimulation of δ-opioid receptors by exogenously applied [D-Ala^2]deltorphin II or endogenously released [Met^5]enkephalin causes a loss of the δ-opioid receptor function in mice receiving δ-opioid receptor antisense oligodeoxynucleotide.

The antinociception induced by β-endorphin given supraspinally is mediated by the release of [Met^5]enkephalin which then acts on δ-opioid receptors in the spinal cord (Tseng, 1995). We have previously demonstrated that chronic i.t. treatment of mice with δ-opioid receptor antisense oligodeoxynucleotide for 3 days selectively attenuates intracerebroventricularly (i.c.v.) administered β-endorphin-induced antinociception without any effect on the antinociception induced by i.c.v. administered μ- or κ-opioid receptor agonists (Tseng and Collins, 1994), indicating that the β-endorphin-induced antinociception is selectively mediated by the stimulation of δ-opioid receptors in the spinal cord. [Met^5]Enkephalin is an endogenous ligand which stimulates δ-opioid receptors for performing physiological functions (Tseng, 1995). Inasmuch as β-endorphin given supraspinally releases [Met^5]enkephalin and subsequently stimulates δ-opioid receptors in the spinal cord, β-endorphin given i.c.v. will be expected to facilitate the turnover of spinal δ-opioid receptors in mice receiving i.t. δ-opioid receptor antisense oligodeoxynucleotide. We report here that an i.c.v. pretreatment with β-endorphin for 24 h facilitates the attenuation of i.t. challenged [D-Ala^2]deltorphin II-induced antinociception induced by i.t. pretreatment with δ-opioid receptor antisense oligodeoxynucleotide.

2. Material and methods

Male ICR mice weighing 25–30 g (Sasco, Omaha, NE, USA) were used for the study. 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. Animals were used only once. I.c.v. administration was performed according to the method described by Haley and McCormick (1957) and i.t. administration was performed according to the procedure described by Hylden and Wilcox (1980) using a 10-μl Hamilton syringe with a 30-gauge needle. The injection volume for i.c.v injection was 4 μl and, for i.t., 5 μl. Groups of mice were pretreated i.t. 24 h earlier with δ-opioid receptor antisense oligodeoxynucleotide (163 pmol, 1 μg), mismatched oligodeoxynucleotide (163 pmol, 1 μg) or saline (5 μl) and, 10 min later, i.c.v. with β-endorphin (0.08, 0.15, 0.3 and 0.6 nmol), DAMGO (19.5 pmol), [D-Ala^2]deltorphin II (6.4 nmol), U50,488H (85.9 nmol), β-endorphin plus β-endorphin-(1–27) (2 nmol) or saline (4

Table 1
Effects of i.c.v. pretreatment with β-endorphin, DAMGO, [D-Ala^2]deltorphin II or U50,488H on i.t. challenged [D-Ala^2]deltorphin II-induced antinociception in mice receiving i.t. antisense oligodeoxynucleotide to δ-opioid receptor mRNA

Pretreatment (24 h)	i.t. [D-Ala^2]deltorphin II-induced antinociception (% MPE ± S.E.M.)	Number of animals
i.t. Saline + i.c.v. saline	65.8 ± 9.7	10
i.t. Saline + i.c.v. β-EP (0.6 nmol)	71.4 ± 7.0	9
i.t. DOR AS oligo + i.c.v. saline	60.7 ± 10.4	8
i.t. DOR AS oligo + i.c.v. β-EP (0.08 nmol)	53.0 ± 12.1	8
i.t. DOR AS oligo + i.c.v. β-EP (0.15 nmol)	32.8 ± 4.1 [a]	10
i.t. DOR AS oligo + i.c.v. β-EP (0.3 nmol)	29.2 ± 8.8 [a]	8
i.t. DOR AS oligo + i.c.v. β-EP (0.6 nmol)	13.9 ± 3.1 [b]	10
i.t. DOR AS oligo + i.c.v. β-EP (0.6 nmol) + i.c.v. β-EP-(1–27)	47.1 ± 10.3 [c]	8
i.t. MM oligo + i.c.v. β-EP (0.6 nmol)	64.0 ± 10.2	9
i.t. DOR AS oligo + i.c.v. DAMGO	69.1 ± 10.1	8
i.t. DOR AS oligo + i.c.v. [D-Ala^2]deltorphin II	57.9 ± 10.2	8
i.t. DOR AS oligo + i.c.v. U50,488H	65.4 ± 10.6	8

Groups of mice were pretreated i.t. with δ-opioid receptor antisense oligodeoxynucleotide (DOR AS oligo) (163 pmol), mismatched oligodeoxynucleotide (MM oligo; 163 pmol) or saline and, 10 min later, i.c.v. with β-endorphin (β-EP, 0.08, 0.15, 0.3 or 0.6 nmol), DAMGO (19.5 pmol), [D-Ala^2]deltorphin II (6.4 nmol), U50,488H (85.9 nmol), β-endorphin plus β-endorphin-(1–27) (β-EP-(1–27), 2 nmol) or saline and challenged i.t. with [D-Ala^2]deltorphin II (6.4 nmol) 24 h later. Antinociception was measured by the tail-flick test 10 min after i.t. [D-Ala^2]deltorphin II injection. [a] $P < 0.05$, [b] $P < 0.01$, compared to mice pretreated with i.t. DOR AS oligo plus i.c.v. saline. [c] $P < 0.05$, compared to mice pretreated with i.t. DOR AS oligo plus i.c.v. β-endorphin (0.6 nmol).

μl) and were challenged i.t. with [D-Ala^2]deltorphin II (6.4 nmol, 5 μg). The antinociception was measured by the tail-flick test 10 min after [D-Ala^2]deltorphin II injection. For measurement of the latency of the tail-flick response, 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 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. The data are expressed as the means and S.E.M. The statistical analysis of difference between groups was assessed using a one-way ANOVA followed by Newman-Keuls multiple comparison test.

Drugs used were human β-endorphin (Peninsula Laboratory, Belmont, CA, USA), human β-endorphin-(1–27) (Peninsula Laboratory), DAMGO (D-Ala^2, *N*MePhe^4,Gly(ol)^5]enkephalin, Peninsula Laboratory), U50,488H (*trans*(±3,4-dichloro-*N*-methyl-*N*-[2-(l-pyrrolidinyl)cyclohexyl]benzeneacetamide, Research Biochemicals, Natick, MA, USA) and [D-Ala^2]deltorphin II (Molecular Research Laboratories, Durham, NC, USA). The δ-opioid receptor antisense oligodeoxynucleotide and mismatched oligodeoxynucleotide were synthesized by Dr. John Richard (Molecular Research Laboratories). The optimum δ-opioid receptor antisense oligodeoxynucleotide corresponding to bases 25–44 of mouse δ-opioid receptor mRNA (Evans et al., 1992) consists of a phosphorothioate of the following sequence: 5′-AGG GCA CCA GCT CCA TGG CG-3′. The mismatched oligodeoxynucleotide, which has the following sequence: 5′-GGC GTC GAC CTA CTT CGG CG-3′, served as a control.

3. Results

As shown in Table 1, i.c.v. pretreatment with β-endorphin (0.6 nmol) or i.t. pretreatment with δ-opioid receptor antisense oligodeoxynucleotide (163 pmol) for 24 h did not have any effect on inhibition of the tail-flick response induced by i.t. challenged [D-Ala^2]deltorphin II (6.4 nmol). However, a concomitant i.t. pretreatment with δ-opioid receptor antisense oligodeoxynucleotide (163 pmol) and i.c.v. pretreatment with β-endorphin (0.08, 0.15, 0.3 and 0.6 nmol) for 24 h dose-dependently attenuated i.t. administered [D-Ala^2]deltorphin II-induced antinociception. The attenuation of i.t. [D-Ala^2]deltorphin II-induced antinociception by β-endorphin was blocked by a concomitant i.c.v. pretreatment with β-endorphin-(1–27) (2 nmol). A concomitant i.t. pretreatment with δ-opioid receptor antisense oligodeoxynucleotide (163 pmol) and i.c.v. pretreatment with DAMGO (19.5 pmol), [D-Ala^2]deltorphin II (6.4 nmol) or U50,488H (85.9 nmol) did not affect i.t. administered [D-Ala^2]deltorphin II-induced antinociception challenged 24 h later.

4. Discussion

The antinociception induced by β-endorphin given supraspinally is mediated by the stimulation of ε-opioid receptors (Tseng, 1995). The effect of β-endorphin is mediated specifically by the stimulation of ε-opioid receptors, because it is blocked by the concomitant administration of β-endorphin-(1–27), an ε-opioid receptor antagonist (Tseng and Collins, 1991; Tseng, 1995). The activation of ε-opioid receptors at supraspinal sites results in the release of [Met^5]enkaphalin and subsequent stimulation of δ-opioid receptors (Tseng, 1995). The stimulation of δ-opioid receptors in the spinal cord by i.c.v. administered β-endorphin was then used as the experimental model for studying the effect of δ-opioid receptor antisense oligodeoxynucleotide on δ-opioid receptor function. The results of our study demonstrated that i.c.v. pretreatment with β-endorphin for 24 h attenuated i.t. challenged δ-opioid receptor agonist [D-Ala^2]deltorphin II-induced antinociception in mice receiving δ-opioid receptor antisense oligodeoxynucleotide. The findings of this study indicate that β-endorphin pretreatment causes a loss of δ-opioid receptors in the spinal cord which is exposed by the inhibition of the biosynthesis of δ-opioid receptor protein with δ-opioid receptor antisense oligodeoxynucleotide treatment.

The loss of δ-opioid receptor function in mice receiving δ-opioid receptor antisense oligodeoxynucleotide is therefore caused by the stimulation of δ-opioid receptor by released [Met^5]enkephalin induced by i.c.v. administered β-endorphin. This contention is also supported by our recent studies which demonstrated that a stimulation of δ-opioid receptors by endogenously released [Met^5]enkephalin or exogenously applied [D-Ala^2]deltorphin II causes a loss of δ-opioid receptor functions in mice receiving δ-opioid receptor antisense oligodeoxynucleotide. The antinociception induced by cold water swimming is mediated by the released [Met^5]enkephalin acting on δ-opioid receptors in the spinal cord (Mizoguchi et al., 1995a). Pretreatment 24 h earlier with i.t. administered [D-Ala^2]deltorphin II, cold water swimming or δ-opioid receptor antisense oligodeoxynucleotide alone did not affect antinociception induced by i.t. challenged [D-Ala^2]deltorphin II. However, a concomitant i.t. pretreatment with [D-Ala^2]deltorphin II or cold water swimming and δ-opioid receptor antisense oligodeoxynucleotide for 24 h attenuates i.t. challenged [D-Ala^2]-

deltorphin II-induced antinociception (Narita and Tseng, 1995; Narita et al., 1995; Mizoguchi et al., 1995c). Likewise, a concomitant i.t. pretreatment 24 h earlier with [D-Ala^2]deltorphin II and δ-opioid receptor antisense oligodeoxynucleotide attenuates antinociception induced by cold water swimming (Mizoguchi et al., 1995c). Our results indicate that stimulation of δ-opioid receptors by endogenously released [Met^5]enkephalin by β-endorphin or cold water swimming or by exogenously applied [D-Ala^2]deltorphin II caused a loss of δ-opioid receptors and the recovery of δ-opioid receptor function requires the replenishment of newly synthesized opioid receptor protein. Our finding argues against the possibility that δ-opioid receptors are rapidly reversible or recycled after use.

The descending pain control systems induced by the activation of μ, δ, and κ-opioid receptor agonists applied supraspinally are mediated by the activation of different neurotransmitter systems. The antinociception induced by μ-opioid receptor agonist DAMGO given supraspinally is mediated by the release of norepinephrine and serotonin (5-HT) acting on α_2-adrenoceptors and 5-HT receptors, respectively, while antinociception induced by κ-opioid receptor agonist U50,488H is mediated by the release of 5-HT and dynorphin which subsequently stimulate 5-HT and κ-opioid receptors, respectively (Tseng 1995; Tseng and Collins, 1991, 1993). The antinociception induced by δ-opioid receptor agonist DPDPE given supraspinally is mediated by a descending 5-HT system but not noradrenergic or opioidergic systems (unpublished observation). We found that the stimulation of supraspinal μ- δ- or κ-opioid receptors by i.c.v. pretreatment with DAMGO, [D-Ala^2]deltorphin II or U50,488H did not attenuate i.t. challenged [D-Ala^2]deltorphin II-induced antinociception. These findings provide an additional evidence that descending pain control systems activated by DAMGO, [D-Ala^2]deltorphin II or U50,488H given supraspinally do not activate δ-opioid receptors in the spinal cord.

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Note added in proof

(1) L.F. Tseng, K.A. Collins and J.P. Kampine, 1994, Antisense oligodeoxynucleotide to a δ-opioid receptor selectively blocks the spinal antinociception induced by δ-, but not μ- or κ-opioid receptor agonist in the mouse, Eur. J. Pharmacol. 258, R1–R3.

In this article, page R2, first column, line 12, the nucleotide sequence of antisense oligodeoxynucleotide to δ-opioid receptor mRNA should have read "5′-AGG GCA CCA GCT CCA TGG CG-3′."

(2) M. Narita and L.F. Tseng, 1995, Stimulation of spinal δ-opioid receptors in mice selectively enhances the attenuation of δ-opioid receptor-mediated antinoception by antisense oligodeoxynucleotide, Eur. J. Pharmacol. 284, 185–189.

In this article, page 186, second column, line 30, the nucleotide sequence of antisense oligodeoxynucleotide to δ-opioid receptor mRNA should have read "5′-AGG GCA CCA GCT CCA TGG CG-3′."

The authors regret these errors.

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