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The effect of pretreatment with a δ_2-opioid receptor antisense oligodeoxynucleotide on the recovery from acute antinociceptive tolerance to δ_2-opioid receptor agonist in the mouse spinal cord

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1 An intrathecal (i.t.) injection of a selective δ_2-opioid receptor agonist, [D-Ala^2]deltorphin II, produced an acute antinociceptive tolerance to the antinociceptive effect of a subsequent i.t. challenge of [D-Ala^2]deltorphin II. This acute tolerance lasted 3 to 9 h and completely subsided by 12 h. The experiments were designed to examine the effect of pretreatment with an antisense oligodeoxynucleotide to δ_2-opioid receptor mRNA (δ-AS oligo) on the recovery from tolerance to [D-Ala^2]deltorphin II-induced antinociception in male ICR mice.

2 Pretreatment with δ-AS oligo (1.63 to 163 pmol, i.t.), but not mismatched oligo (MM oligo) (163 pmol), prevented the recovery from acute tolerance to [D-Ala^2]deltorphin II-induced antinociception in a dose-dependent manner. However, treatment with δ-AS oligo (163 pmol) did not prevent the recovery from tolerance to either the μ-opioid receptor agonist [D-Ala^2,NMePhe^4,Gly(ol)^5]enkephalin (DAMGO) or the κ-opioid receptor agonist U50,488H, indicating subtype specificity in the mechanism by which δ-AS oligo inhibits recovery from δ_2-opioid tolerance.

3 Treatment with [D-Ala^2]deltorphin II (i.t.) significantly reduced the binding of [tyrosyl-3,5-^{3}H(N)]-Tyr-D-Ser-Gly-Phe-Leu-Thr ([^{3}H]-DSLET), a δ_2-opioid receptor agonist ligand, in the spinal cord 3 h after treatment, but binding returned to control levels by 24 h after treatment. However, [^{3}H]-DSLET binding in the spinal cord remained significantly reduced at 24 h if δ-AS oligo (163 pmol) was coadministered with [D-Ala^2]deltorphin II (6.4 nmol).

4 Based on these findings, it is concluded that a single stimulation of spinal cord δ_2-opioid receptors by intrathecally-administered [D-Ala^2]deltorphin II induces a long-lasting desensitization of δ_2-opioid receptors to [D-Ala^2]deltorphin II. Recovery from δ_2-opioid receptor-mediated antinociceptive tolerance apparently depends on replenishment by newly synthesized δ_2-opioid receptor protein rather than immediate reversal of δ_2-opioid receptors.

Keywords: Acute tolerance; antisense oligodeoxynucleotide; δ_2-opioid receptor; desensitization; receptor turnover; spinal cord; antinociception

Introduction

The δ-opioid receptors have been further classified into δ_1 and δ_2-opioid receptors (see review by Porreca *et al*., 1995). The δ_2-receptor has been cloned and well characterized by pharmacological studies, whereas the δ_1-receptor has yet to be cloned (Evans *et al*., 1992; see review by Reisin, 1996). The cloning of δ_2-opioid receptors has provided a powerful new approach for using antisense oligodeoxynucleotides to δ_2-opioid receptors mRNA (δ-AS oligo) to study the regulation of δ_2-opioid receptor function. A δ-AS oligo is a short piece of synthetic DNA with a nucleotide sequence that is the reverse of and complementary to a portion of the δ_2-opioid receptor mRNA. It, therefore, hybridizes to δ_2-opioid receptor mRNA and inhibits the synthesis of the encoded δ_2-opioid receptor protein. We previously pretreated mice intrathecally (i.t.) with a δ-AS oligo that was complementary to bases 25 to 44 of the cloned mouse δ_2-opioid receptors (Evans *et al*., 1992). Daily i.t. treatment with δ-AS oligo for 3 days selectively attenuated the antinociception induced by intrathecally-administered δ-opioid receptor agonists (Tseng *et al*., 1994) and significantly decreased specific δ_2-opioid receptor bindings in the mouse spinal cord (unpublished observation). These findings suggest that by this route the δ-AS oligo could access the target neurone and be taken up into cells and survive long enough to act. Similar results were also obtained with a different nucleotide sequence of δ-AS oligo (Standifer *et al*., 1994; Lai *et al*., 1994; Bilsky *et al*., 1996).

Tolerance, or reduced drug effect following chronic drug administration, develops to many opioid effects after prolonged treatment (Cox, 1991). For instance, chronic intraventricular or i.t. infusion of morphine or [D-Ala^2,D-Leu^5]enkephalin produces tolerance to its antinociceptive effects (Tseng, 1982; 1983). However, it is believed that antinociceptive tolerance also develops after a single intracerebroventricular (i.c.v.) or i.t. administration. It has been shown that a single i.c.v. injection of β-endorphin, morphine or [D-Pen^{2,5}]enkephalin (DPDPE) could cause attenuation of the antinociceptive response to a subsequent i.c.v. challenge with β-endorphin, morphine or DPDPE, respectively (Suh & Tseng, 1990). A single i.t. injection of [D-Ala^2,NMe-Phe^4,Gly(ol)^5]enkephalin (DAMGO), a selective μ-opioid receptor agonist, likewise produces an acute antinociceptive tolerance to a second i.t. injection of DAMGO in mice (Narita *et al*., 1995). The tolerance to antinociception induced by i.c.v. or i.t. injection of opioid receptor agonists occurs rapidly; the effect becomes apparent within 3 h after the first injection.

In the present study we found that i.t. treatment of mice with [D-Ala^2]deltorphin II, a highly selective δ_2-opioid receptor agonist (Eraspamer *et al*., 1989; Raynor *et al*., 1994), alone produced acute antinociceptive tolerance to the subsequent i.t. challenge of [D-Ala^2]deltorphin II. This acute tolerance to [D-Ala^2]deltorphin II-induced antinociception was reversible and completely dissipated by 12 h. However, a concomitant treatment with both [D-Ala^2]deltorphin II and δ-AS oligo prevents the recovery from the antinociceptive tolerance to δ_2-opioid receptor agonist.

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Methods

Animals

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

Antinociceptive test

Antinociception was determined by the tail-flick test (D'Amour & Smith, 1941). 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) 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 to 5 s. Antinociception was expressed as % maximum 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 agonist and T_2 was the cutoff time, which was set at 10 s for the test to avoid injury to the tail.

Membrane preparation for receptor binding

Animals were killed by decapitation and their spinal cords were quickly excised on an ice-cold petri-dish. The spinal cord was homogenized in 15 volumes (w/v) of ice-cold 0.32 M sucrose with a Potter-Elvehjem tissue grinder. The homogenates were centrifuged at 4°C for 10 min at $1,000 \times g$. The pellets were discarded and the supernatants were centrifuged at 4°C for 20 min at $20,000 \times g$ to obtain crude mitochondrial pellets. The mitochondrial pellets were resuspended in double-distilled deionized water and dispersed with a Potter-Elvehjem tissue grinder. The suspensions were centrifuged at 4°C for 20 min at $8,000 \times g$. The pellets were discarded and the supernatant, including the soft buffy layer, was collected from each tube, incubated at 25°C for 2 h to degrade endogenous ligands, and centrifuged at 4°C for 30 min at $40,000 \times g$ to obtain crude synaptosomal pellets. The crude synaptosomal pellets were resuspended in 50 mM Tris-HCl buffer (pH 7.4) and centrifuged at 4°C for 30 min at $40,000 \times g$. The final pellets were stored at −70°C until experiments.

δ_2-Opioid receptor binding assay

Just before the binding experiment, the stocked pellets were resuspended in 50 mM Tris-HCl buffer (pH 7.4) and centrifuged at 4°C for 30 min at $40,000 \times g$. The pellets were resuspended in 50 mM Tris-HCl buffer (pH 7.4) and used for the binding assay. Binding assays for the δ_2-opioid receptor agonist-sensitive site were carried out in triplicate with [tyrosyl-3,5-^{3}H(N)]-Tyr-D-Ser-Gly-Phe-Leu-Thr ([^{3}H]-DSLET;57.0 - Ci mmol^{-1}; NEN, Boston, MA) at 4 nM in a final volume of 1.0 ml which contained 50 mM Tris-HCl buffer (pH 7.4) and 0.1 ml of the homogenated membrane fraction. The test tubes were incubated for 120 min at 25°C. The specific binding was defined as the difference in binding observed in the absence and presence of 10^{-5} M[D-Ala^2]deltorphin II. Unlabelled [D-Ala^2,NMePhe^4,Gly(ol)^5]enkephalin (DAMGO; 100 nM) was included in incubation medium containing [^{3}H]-DSLET to block binding of this radioligand to μ-opioid receptors. The incubations were terminated by collecting the membranes on Whatman GF/B filters with a Brandel cell harvester (Model M-24, Brandel, MD). The filters were then washed three times with 5 ml of 50 mM Tris-HCl buffer (pH 7.4) at 4°C and transferred to scintillation vials. Then, 0.5 ml of Soluene-350 (Packard Instrument Company, Inc., Meriden, CT) and 5 ml of Hionic Fluor Cocktail (Packard Instrument Company) were added to the vials. After a 12 h equilibration period, the radioactivity in the samples was determined in liquid scintillation analyser (Model 1600 CA, Packard Instrument Company). The amount of membrane protein used in each assay was in the range of 200 to 250 μg, as determined by the method of Lowry *et al*. (1951). The amount of specific binding of the ligand was expressed as fmol mg^{-1} protein.

Drugs and intrathecal treatment

[D-Ala^2-NMePhe^4, Gly(ol)^5]enkephalin (DAMGO) was purchased from Peninsula Laboratory (Belmont, CA) and U50,488H (trans-(1S,2S-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide hydrochloride) from Research Biochemicals (Natick, MA). The δ-AS oligo, mismatched (MM) oligo and [D-Ala^2]deltorphin II (Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2) were synthesized by Dr John Richard (Molecular Research Laboratories, Durham, NC). A computer program which analysed δ_2-opioid receptor mRNA secondary structure was used to ensure optimum hybridization potential. Database searches (GENBANK and EMBL) ensured specificity of hybridization of the δ-AS oligo and the lack of specificity for any known gene for the mismatched sequence oligo. The optimum δ-AS oligo corresponding to bases 25 to 44 of DOR-1 of mouse δ_2-opioid receptor (Evans *et al*., 1992) consists of a phosphorothioate of the following sequence: 5′-AGG GCA CCA GCT CCA TGG CG-3′. The MM oligo, which has the following sequence: 5′-GGC GTC GAC CTA CTT CGG CG-3′, served as a control.

I.t. administration was performed following the method described by Hylden and Wilcox (1980) with a 10 μl Hamilton syringe with a 30 gauge needle. Injection volumes were 5 μl for i.t.

Statistical data analyses

The data are expressed as the mean ±s.e.mean. Statistical analysis of differences between groups was assessed with Student's t test (comparisons of two groups) or analysis of variance (ANOVA) followed by Newman-Keuls test (comparison between multiple groups). $P < 0.05$ was considered significant.

Results

Inhibition of the tail-flick response after i.t. injection of [D-Ala^2]deltorphin II

Groups of mice were injected i.t. with [D-Ala^2]deltorphin II (6.4 nmol) and the tail-flick response was measured at different times after the injection. As shown in Figure 1, the inhibition of the tail-flick response developed in 10 min and returned to the control level in 60 min.

Effects of i.t. pretreatment with [D-Ala^2]deltorphin II or a combination of [D-Ala^2]deltorphin II and δ-AS oligo on the antinociception induced by i.t. administered [D-Ala^2]deltorphin II

Time course study Different groups of mice were pretreated i.t. with [D-Ala^2]deltorphin II (6.4 nmol) or a combination of [D-Ala^2]deltorphin II and δ-AS oligo and then they were challenged i.t. with [D-Ala^2]deltorphin II (6.4 nmol) at 3, 6, 9, 12, 18 or 24 h after the pretreatment. A single injection (pretreatment) was made at the time points. The tail-flick response was measured 10 min after the second injection challenge. As shown in Figure 2, i.t. pretreatment with [D-Ala^2]deltorphin II attenuated the antinociception induced by i.t. challenged [D-Ala^2]deltorphin II. The attenuation of [D-Ala^2]deltorphin II-induced antinociception developed in 3 h, lasted 3–9 h and subsided to control levels by 12–24 h after the [D-Ala^2]deltorphin II pretreatment. Pretreatment of δ-AS oligo (49.9 and 163 pmol) with [D-Ala^2]deltorphin II did not affect the attenuation of i.t.-administered [D-Ala^2]deltorphin II-induced antinociception, but it blocked dose-dependently the recovery from the at-

tenuation of [D-Ala^2]deltorphin II-induced antinociception. The [D-Ala^2]deltorphin II-induced antinociception remained attenuated in mice at 12, 18, and 24 h after i.t. pretreatment with a combination of δ-AS oligo and [D-Ala^2]deltorphin II.

Dose-response of δ-AS oligo pretreatment study

Groups of mice were pretreated i.t. with [D-Ala^2]deltorphin II (6.4 nmol) 24 h before an i.t. challenge with [D-Ala^2]deltorphin II (6.4 nmol) and the tail-flick response was measured 10 min after [D-Ala^2]deltorphin II challenge. Pretreatment with [D-Ala^2]deltorphin II or δ-AS oligo alone for 24 h did not have any effect on [D-Ala^2]deltorphin II-induced antinociception. However, pretreatment with different doses (1.63 – 163 pmol) of δ-AS oligo with [D-Ala^2]deltorphin II caused a dose-dependent attenuation of [D-Ala^2]deltorphin II-induced antinociception. Pretreatment of MM oligo in combination with [D-Ala^2]deltorphin II did not affect [D-Ala^2]deltorphin II-induced antinociception (Figure 3).

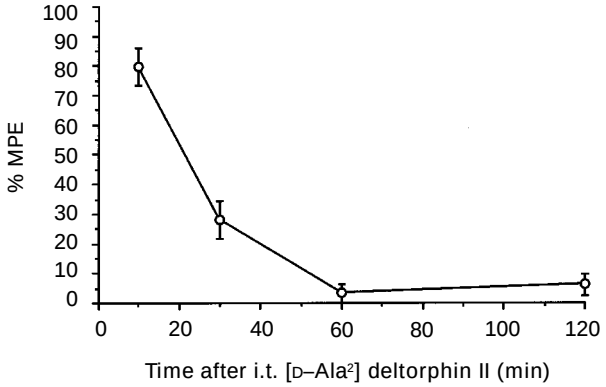

Figure 1 Effect of i.t. [D-Ala^2]deltorphin II on the inhibition of the tail-flick response. The tail-flick response was measured at 10, 30, 60 and 120 min after i.t. injection of [D-Ala^2]deltorphin II (6.4 nmol). The vertical lines indicate s.e.mean; $n=10$ mice for each group.

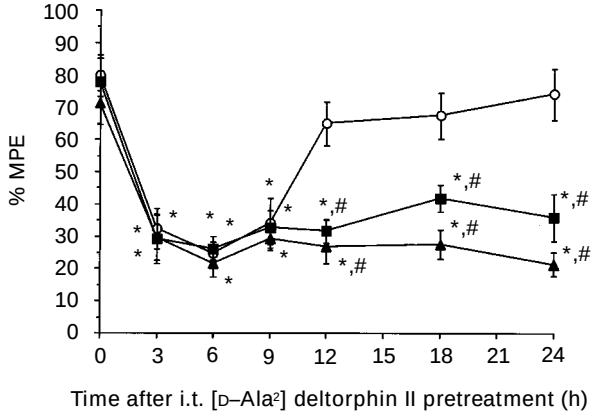

Figure 2 Time course of the change of i.t.-administered [D-Ala^2]deltorphin II-induced tail-flick inhibition in mice pretreated i.t. with [D-Ala^2]deltorphin II or a combination of [D-Ala^2]deltorphin II and antisense oligodeoxynucleotide to δ_2-opioid receptor mRNA (δ-AS oligo). Groups of mice were pretreated i.t. with [D-Ala^2]deltorphin II (6.4 nmol) alone (○) or in combination with [D-Ala^2]deltorphin II and δ-AS oligo (49.9 pmol: ■ or 163 pmol: ▲). Mice were then challenged i.t. with [D-Ala^2]deltorphin II (6.4 nmol) at different times after the first injection. The tail-flick response was measured 10 min after the [D-Ala^2]deltorphin II treatment at 0 h or 10 min after the second [D-Ala^2]deltorphin II injection at 3, 6, 9, 12, 15, 18, 21 or 24 h. A single injection was made at the time points. The vertical lines represent s.e.mean; $n=9-20$ mice for each point. The point '0 h' indicates the value of a single injection of [D-Ala^2]deltorphin II or a combination of [D-Ala^2]deltorphin II and δ-AS oligo. $^*P<0.05$, vs 0 h; $\#P<0.05$, vs [D-Ala^2]deltorphin II alone.

Effects of i.t. pretreatment with μ- or κ-opioid receptor agonist and δ-AS oligo for 24 h on antinociception induced by respective i.t.-challenged μ- or κ-opioid receptor agonist

Groups of mice were pretreated i.t. with a selective μ-opioid receptor agonist, DAMGO (19.5 pmol), or a κ-opioid receptor agonist, U50,488H (107 nmol), alone or with a combination of the respective opioid and δ-AS oligo 3 or 24 h before the i.t. challenge of [D-Ala^2]deltorphin II. The tail-flick responses were measured 10 min after respective DAMGO or U50,488H injection. Pretreatment with DAMGO or U50,488H alone caused an attenuation of DAMGO- and U50,488H-induced antinociception, respectively, 3 h after the pretreatment. The attenuation of opioid-induced antinociception recovered to the control level 24 h after the pretreatment. Pretreatment of δ-AS oligo in addition to DAMGO or U50,488H did not affect the recovery from the attenuation of DAMGO- and U50,488H-induced antinociception (Figure 4a and b).

Effects of i.t. treatment with δ-AS oligo and/or [D-Ala^2]deltorphin II on [^{3}H]-DSLET binding in the spinal cord

Groups of mice were injected i.t. with saline, δ-AS oligo (163 pmol), [D-Ala^2]deltorphin II (6.4 nmol) alone or a combination of [D-Ala^2]deltorphin II and δ-AS oligo and were killed 3 or 24 h after injection. The spinal cord was dissected and used for radioligand binding. An i.t. treatment with [D-Ala^2]deltorphin II 6.4 nmol significantly decreased specific [^{3}H]-DSLET binding 3 h after the treatment (12.06 ± 0.47 fmol mg^{-1} protein; 20% inhibition; $P<0.01$), as compared to saline treatment (15.13 ± 0.47 fmol mg^{-1} protein), but the level had returned to control levels by 24 h after injection (Figure 5). An i.t. treatment

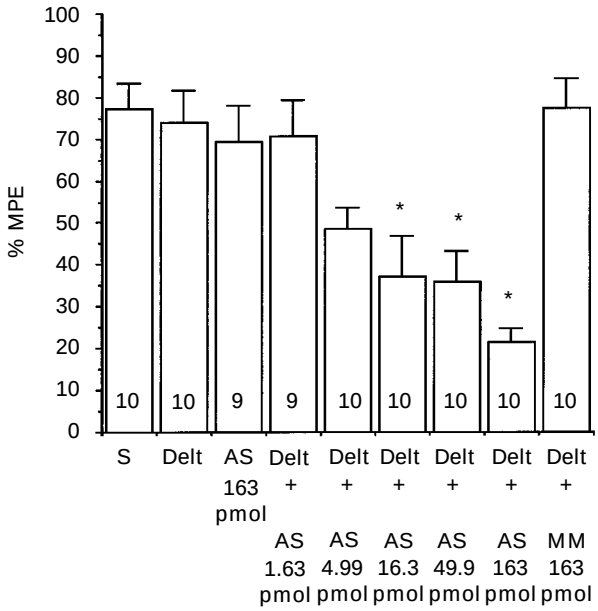

Figure 3 Dose-dependent inhibition of the recovery from acute tolerance to [D-Ala^2]deltorphin II antinociception by pretreatment with the antisense oligodeoxynucleotide to δ_2-opioid receptor mRNA (δ-AS oligo). Groups of mice were pretreated i.t. with saline (S ;5 μl), [D-Ala^2]deltorphin II (Delt; 6.4 nmol) alone, δ-AS oligo (AS; 163 pmol) alone or a combination of [D-Ala^2]deltorphin II (6.4 nmol) and δ-AS oligo (AS; 1.63 to 163 pmol) or mismatched oligo (MM; 163 pmol). Mice were then challenged i.t. with [D-Ala^2]deltorphin II (6.4 nmol) at 24 h after the first injection. The tail-flick response was measured 10 min after the second ([D-Ala^2]deltorphin II) injection. The number within the column indicates the number of mice used and the vertical line indicates the s.e.mean. $^*P<0.05$, compared with mice pretreated with i.t. [D-Ala^2]deltorphin II alone.

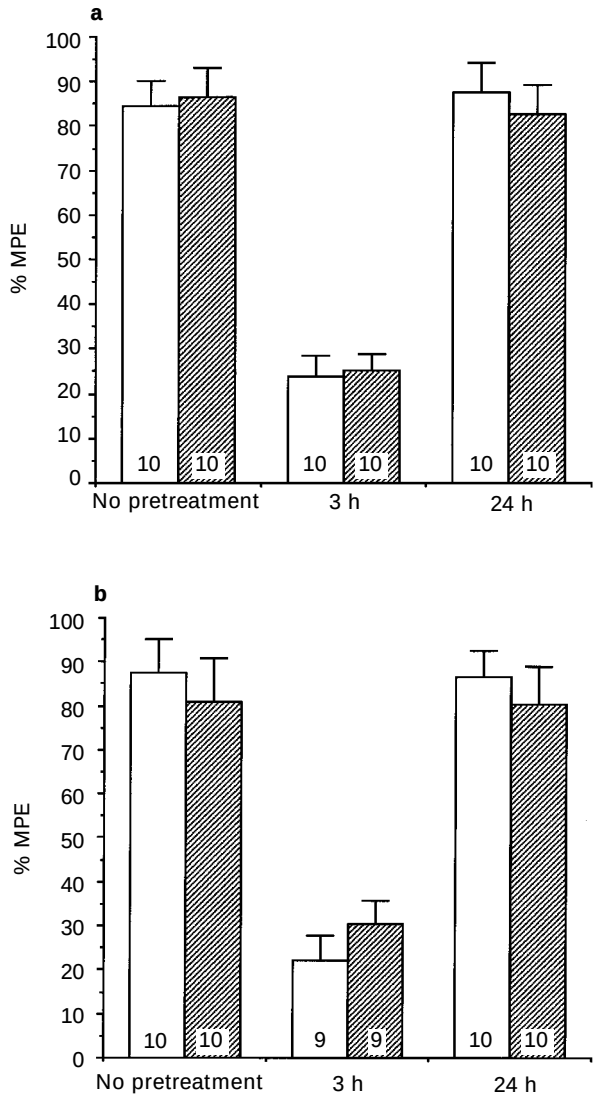

Figure 4 The lack of effect of treatment with the antisense oligodeoxynucleotide to δ_2-opioid receptor mRNA (δ-AS oligo) on the recovery from acute antinociceptive tolerance induced by a selective (a) μ-opioid receptor agonist DAMGO or (b) κ-opioid receptor agonist U50,488H. Groups of mice were pretreated i.t. with DAMGO (19.5 pmol) or U50,488H (107 nmol) alone (open columns), or a combination of DAMGO or U50,488H and δ-AS oligo (163 pmol) (hatched columns). Mice were then challenged i.t. with DAMGO or U50,488H at 3 or 24 h after the first injection. The tail-flick response was measured 10 min after the first (no pretreatment) or second injection. The number within the column indicates the number of mice used and the vertical lines indicate s.e.mean. No pretreatment column represents the value of a single injection of DAMGO or U50,488H (open columns), or a combination of each opioid and δ-AS oligo (hatched columns).

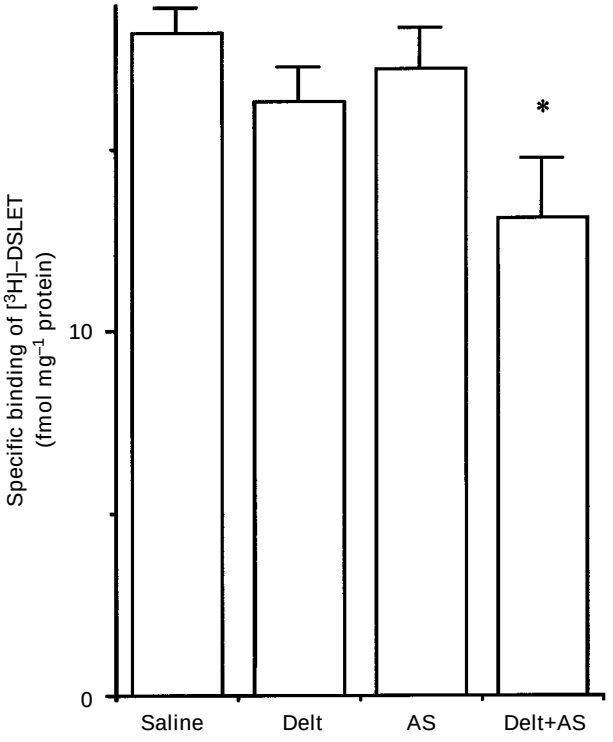

Figure 5 Effect of a single i.t. injection with an antisense oligodeoxynucleotide to δ_2-opioid receptor mRNA (δ-AS oligo), [D-Ala^2]deltorphin II or a combination of δ-AS oligo and [D-Ala^2]deltorphin II on [^{3}H]-DSLET binding to crude synaptic membranes in the spinal cord. Groups of mice ($n=5$) were injected i.t. with saline (5 μl), δ-AS oligo (AS; 163 pmol), [D-Ala^2]deltorphin II (Delt; 6.4 nmol) or a combination of δ-AS oligo (AS; 163 pmol) and [D-Ala^2]deltorphin II (Delt; 6.4 nmol). The spinal cord was removed 24 h after the i.t. injection. The binding of [^{3}H]-DSLET to membranes in the spinal cord was carried out at a concentration of 4 nM in the presence of 100 nM DAMGO. The specific binding of [^{3}H]-DSLET was defined as the difference in binding in the absence and presence of 10^{-5} M of unlabelled [D-Ala^2]deltorphin II. [^{3}H]-DSLET binding was determined in each in 4 to 5 independent sets. Each set contained more than 5 mice. $^*P<0.05$, compared to mice treated with i.t. saline.

with δ-AS oligo for 24 h did not affect specific [^{3}H]-DSLET binding (Figure 5). However, treatment with δ-AS oligo in addition to [D-Ala^2]deltorphin II for 24 h significantly attenuated [^{3}H]-DSLET binding (Figure 5).

Discussion

An i.t. injection of a highly selective δ_2-opioid receptor agonist, [D-Ala^2]deltorphin II (Eraspamer *et al*., 1989; Raynor *et al*., 1994), produced profound antinociception which lasted 10–30 min after the injection and disappeared in 1 h. In the present acute tolerance studies, we found significant attenuation of i.t.-administered [D-Ala^2]deltorphin II-induced antinociception if the challenge was preceded by an earlier i.t. injection of [D-Ala^2]deltorphin II. This antinociceptive tolerance 3–9 h, with normal sensitivity to [D-Ala^2]deltorphin II antinociception recovered in 12–24 h. However, the dissipation of this acute tolerant state was blocked if δ-AS oligo was co-administered with the earlier [D-Ala^2]deltorphin II pretreatment; the effect of the i.t. [D-Ala^2]deltorphin II challenge remained attenuated even after 12–24 h later. These findings suggest that the pretreatment with δ-AS oligo prevents recovery from the acute antinociceptive tolerance to δ_2-opioid receptor agonist which is disclosed by the δ-AS oligo pretreatment.

A similar time course was also found in the δ_2-opioid receptor binding studies. Pretreatment of mice i.t. with [D-Ala^2]deltorphin II significantly decreased δ_2-opioid receptor agonist-sensitive binding sites 3 h after treatment, but levels had returned to control by 24 h after injection. However, treatment with both δ-AS oligo and [D-Ala^2]deltorphin II caused a significant reduction in δ_2-opioid receptor agonist-sensitive binding sites at 24 h.

Based on these findings, we propose that the stimulation of δ_2-opioid receptors by i.t.-administered [D-Ala^2]deltorphin II can cause a long-lasting change of δ_2-opioid receptors, rendering them insensitive to δ_2-opioid receptor agonists. Tolerance to δ_2-opioid receptor agonists develops because the concentration of resting δ_2-opioid receptors is decreased. Recovery from δ_2-opioid receptor-mediated antinociceptive tol-

erance after acute [D-Ala^2]deltorphin II treatment depends on the replenishment of newly synthesized δ_2-opioid receptor protein. Recovery from the acute antinociceptive tolerant state is inhibited in mice treated with δ-AS oligo because the synthesis of δ_2-opioid receptor protein is inhibited. It is most likely that this state of tolerance is mediated by the phosphorylation of δ_2-opioid receptors. We have previously demonstrated that concomitant i.t. pretreatment with a highly selective protein kinase C (PKC) inhibitor, calphostin C, markedly prevented the development of acute tolerance to the i.t.-administered [D-Ala^2]deltorphin II-induced antinociception (Narita *et al*., 1996a). On the other hand, a selective protein kinase A (PKA) inhibitor, KT5720, did not have any effect on the development of acute tolerance to [D-Ala^2]deltorphin II-induced antinociception (Narita *et al*., 1996a), indicating that PKC, but not PKA, plays an important role in the process of homologous desensitization of the spinal δ_2-opioid receptor-mediated antinociception. Furthermore, the receptor phosphorylation by activated PKC is the possible mechanism for the uncoupling of the δ_2-opioid receptor from G-proteins, leading to a decrease in receptor responsiveness. This contention is supported by the findings that a PKC activator, phorbol ester, blocked the increase of a high affinity GTPase activity induced by [D-Ala^2]deltorphin II in the mouse spinal cord (Narita *et al*., 1996b). Our findings support the hypothesis proposed by Sadëe *et al*. (1994) that antinociceptive tolerance to opioid receptor agonists is mediated by the conversion of opioid receptors to phosphorylated receptors which are not sensitive to opioid agonists.

The long-lasting conversion of δ_2-opioid receptors is specific to the stimulation by a δ_2-opioid receptor agonist. Pretreatment with a μ-opioid receptor agonist, DAMGO, or κ-opioid receptor agonist, U50,488H, was found to be unable to prevent the recovery from δ_2-opioid receptor-mediated tolerance in mice receiving δ-AS oligo.

This new concept that stimulation of δ_2-opioid receptors by agonists can cause a long lasting change of the receptor is supported by other experiments in which δ_2-opioid receptors are activated pharmacologically and physiologically. The antinociception induced by i.c.v.-administered β-endorphin is mediated by the release of Met-enkephalin which then acts on δ_2-opioid receptors in the spinal cord (Tseng *et al*., 1985). I.c.v. pretreatment of mice with β-endorphin 24 h earlier did not have any effect on δ_2-opioid receptor-mediated activity in the spinal cord. However, concomitant pretreatment with δ-AS oligo given i.t. and β-endorphin given i.c.v. attenuated i.t. [D-Ala^2]deltorphin II-induced antinociception 24 h later (Tseng *et al*., 1995). The antinociception induced by cold water swimming (CWS) is mediated by the release of Met-enkephalin which then acts on δ-opioid receptors in the spinal cord (Mizoguchi *et al*., 1995). Pretreatment with either CWS or i.t.-δ-AS oligo did not affect [D-Ala^2]deltorphin II-induced antinociception 24 h later. However, concomitant pretreatment with both CWS and δ-AS oligo markedly attenuated [D-Ala^2]deltorphin II-induced antinociception (Mizoguchi *et al*., 1996). The results of these experiments support the contention that stimulation of δ_2-opioid receptors causes a long-lasting conversion of δ_2-opioid receptors.

In conclusion, we have demonstrated that the inhibition of biosynthesis of δ_2-opioid receptor protein by δ-AS oligo selectively prevents the recovery from acute antinociceptive tolerance to δ_2- but not μ- or κ- opioid receptor agonist. We propose that stimulation of the δ_2-opioid receptor causes a conversion of the receptor to a constitutively inactive δ_2-opioid receptor. The recovery from spinal δ_2-opioid receptor-mediated acute antinociceptive tolerance does not depend on immediate reversal of the converted opioid receptors but rather on the replenishment by newly synthesized δ_2-opioid receptor protein.

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