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NMDA-R1 antisense oligonucleotide attenuates withdrawal signs from morphine

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Abstract

To test the involvement of NMDA receptor in the development of physical dependence on opioid, the effects of an antisense oligonucleotide against NMDA receptors on the naloxone precipitated withdrawal from morphine were studied. Antisense oligonucleotide (15 nmol/5 μl) corresponding to the nucleotides 4–21 of rat NMDA-R1 subunit, sense oligonucleotide, or saline was injected into the lateral ventricle of rats every 12 h for 6 days. On day 4, the rats were intracerebroventricularly (i.c.v.) infused with morphine (26 nmol μl^{-1} h^{-1}) through osmotic minipumps. Rats then received simultaneous treatment with morphine and oligonucleotides or saline for 3 days. Antisense oligonucleotide, but not saline or sense oligonucleotide, significantly attenuated naloxone precipitated withdrawal signs including jumping, rearing, stretching, teeth chattering, vocalization, and penis licking. Treatment with antisense oligonucleotide, but not sense oligonucleotide, significantly reduced the B_{max} of [^{3}H]MK801 {[3-^{3}H](+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cycloheptene-5,10-imine} binding without significant changes in K_d. These results support the hypothesis that NMDA receptors are involved in the physical dependence on opioid.

Keywords: Morphine; Physical dependence; Antisense oligonucleotide; NMDA receptor

1. Introduction

Opioid drugs are widely used in the clinical management of pain. One of major unwanted properties of opioids is their ability to produce physical dependence upon frequent and chronic administration. Recently, the excitatory amino acid system has been implicated in opioid dependence and withdrawal. Microdialysis studies have shown a dramatic increased release of glutamate within the pontine locus coeruleus during withdrawal from morphine (Aghajanian et al., 1994; Zhang et al., 1994) or butorphanol (Feng et al., 1995; Hoshi et al., 1996). Furthermore, direct intracerebroventricular (i.c.v.) or locus coeruleus injection of glutamate dose-dependently induced withdrawal signs in opioid-dependent animals (Tokuyama et al., 1996, 1998). The *N*-methyl-D-aspartate (NMDA) subtype glutamate receptors are widely distributed in the mammalian central nerve system and are important in a variety of synaptic plasticity phenomena, including long-term potentiation (Collingridge and Bliss, 1987). Numerous behavioral studies have shown that NMDA receptor antagonists can attenuate the expression and development of physical dependence on opioids. Ketamine, dextromethorphan (Koyuncuoglu et al., 1990) and MK801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine] (Rasmussen et al., 1991; Tanganelli et al., 1991; Koyuncuoglu et al., 1992; Cappendijk et al., 1993; Tokuyama et al., 1996), all noncompetitive NMDA receptor antagonists, and LY274614 ((+)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid) (Rasmussen et al., 1991), a competitive NMDA receptor antagonist, have been reported to attenuate signs of naloxone precipitated withdrawal syndrome in morphine dependent animals when they were administered immediately before naloxone. It has also been shown that MK801 inhibited signs of naloxone precipitated opioid withdrawal when co-administered with morphine during the development of physical dependence (Trujillo and Akil, 1991; Fundytus and Coderre, 1994). However, these NMDA receptor antagonists have side-effects. MK801 has been reported to produce a phencyclidine (PCP)-like behavioral syndrome at moderate doses, and stereotypy and ataxia at high doses (Hiramatsu et al., 1989; Tricklebank et al., 1989). Competitive NMDA

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receptor antagonists such as LY274614 induce mild to moderate sedation (Rasmussen et al., 1991). These side effects are suspected to overshadow the signs of opioid withdrawal (Trujillo and Akil, 1995).

Studies based on molecular cloning have demonstrated that NMDA receptor complexes are made up of various combinations of subunits. All NMDA receptors contain at least one obligatory NMDA-R1 (NR1) subunit which is combined with one or more kinds of NMDA-R2 subunits (NR2A-D) (Monyer et al., 1992; Nakanishi, 1992). In the in vitro experiments, NR1 subunits can form a homo-oligomeric structure which has channel activity, whereas NR2 subunits produce functional receptors only when they are coexpressed with NR1 (Monyer et al., 1992; Nakanishi, 1992; Kutsuwada et al., 1992). An 18-mer antisense oligodeoxynucleotide corresponding to nucleotides 4–21 of the NR1 subunit mRNA, which directly follows the translation initiation codon, has been reported to effectively downregulate the NMDA receptors in the rat brain (Wahlestedt et al., 1993). Using this antisense oligonucleotide strategy, the involvement of NMDA receptors in the development of morphine dependence was investigated in this paper.

2. Materials and methods

2.1. Surgical procedures

Adult male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN, USA) weighing 250–275 g were anesthetized with Equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4 \cdot 7H_2O$, 0.972 g sodium pentobarbital, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to make a final volume of 100 ml), 0.3 ml/100 g body weight, i.p., and then fixed in a stereotaxic apparatus. A stainless steel guide cannula (21 gauge, 1 cm long) was implanted into the right lateral cerebral ventricle (AP: −0.5 mm, LAT: +1.3 mm, and DV: −4.5 mm) (Paxinos and Watson, 1986). The presence of cerebrospinal fluid in the guide cannula was examined as verification of proper placement. Dental acrylic cement (Lang Dental MFG, Wheeling, IL, USA) was applied to the surface of the skull, and a protective aluminum cap was placed around the cannula and anchored by three screws. A stylet (26 gauge stainless steel tubing) was placed into the guide cannula to allow the cannula to maintain patent. After surgery, rats were given 300 000 units of procaine penicillin G, s.c., to prevent infection, and were allowed at least a week to recover.

2.2. Administration schedule

NMDA-R1 antisense oligonucleotide and sense oligonucleotide, corresponding to nucleotides 4–21 of NR1 mRNA, were synthesized by Midland Certified Reagent (Midland, TX, USA). The 18-mer NMDA-R1 antisense oligonucleotide has the sequence: 5′-CAGCAGGTG-CATGGTGCT-3′, with the phosphorothioate substitution on the two end base pairs (Soltesz et al., 1994).

After 7 days of recovery, a solution of the NMDA-R1 antisense oligonucleotide or corresponding sense oligonucleotide or vehicle (saline) was injected i.c.v. through a hand-held microliter syringe in a dose of 15 nmol/5 μl. Injections were made at 09:00 and 21:00 for 6 days. In the morning of day 4, under halothane anesthesia, rats were implanted s.c. with osmotic minipumps (Alzet 2001, Alza, Palo Alto, CA, USA) filling with morphine sulfate solution (26 nmol μl^{-1} h^{-1}) between the scapulae. Before introduction into the pump, morphine solution was passed through 0.2 μm sterile Acrodisk filters (Gelman Science, Ann Arbor, MI, USA). The minipumps were primed overnight at 35°C in sterile saline so that the nominal flow rate (1 $\mu l\ h^{-1}$) was attained. An 'L'-shaped stainless steel injector tubing (26-gauge, 2 cm long) was placed into the i.c.v. guide cannula. The external end of the injector tubing was connected with a 2-cm length of Tygon tubing (0.38 mm inner diameter). Oligonucleotides or saline were injected i.c.v. through this Tygon tubing. Another piece of 2-cm Tygon tubing was connected with the outlet of the minipump. The two pieces of Tygon tubing were connected by a 26-gauge steel wire, which was unplugged when drug was to be injected. Rats then received simultaneous treatment with morphine and oligonucleotides or saline from day 4 to day 6. According to previous studies (Horan and Ho, 1991), the dose and period of morphine infusion can successfully produce physical dependence on morphine.

2.3. Behavioral assessment

In the morning of day 7, the connecting tube between the i.c.v. cannula and the outlet of the minipump was disconnected. Two hours following termination of morphine infusion, naloxone (48 nmol/5 μl) was injected i.c.v. by means of a hand-held microliter syringe. Nine behaviors identified as characteristics of the rat opioid withdrawal syndrome were assessed in this study. The absolute frequency of six episodic behaviors was recorded and scored based on multiples of five incidents (0 = no incidents; 1 = 1–5 incidents; 2 = 6–10 incidents; and 3 = > 10 incidents). Behaviors scored in this manner included: jumping (escape behaviors), rearing, wet dog shakes, teeth chattering, scratching, and stretching. Three behaviors (diarrhea, penis-licking, and vocalization) could not be defined in discrete episodes, and were recorded in an all-or-none manner. These withdrawal signs were recorded during a 30-min period after naloxone injection. The reactions of each animal were videotaped and evaluated by an independent observer who did not have prior

knowledge of the nature of the treatment received by the animal.

2.4. Tissue preparations and [^{3}H]MK801 binding assay

After the behavioral tests, the rats were sacrificed by decapitation. The frontal cortex was isolated from the brain. Neuronal membranes were prepared as described by Ebert et al. (1991). Briefly, tissue was homogenized in 10 ml of ice-cold 0.32 M sucrose using a Kinematica Polytron (Brinkmann, Luzern, Switzerland) at low speed. The homogenate was centrifuged at $1000 \times g$ for 10 min at 4°C. The pellets were discarded and the supernatants were centrifuged at 4°C for 20 min at $20\,000 \times g$. The pellets were suspended in ice-cold distilled water and centrifuged at $8000 \times g$ for 20 min at 4°C. The supernatants and 'buffy coats' were collected and centrifuged at $48\,000 \times g$ for 20 min at 4°C. The last step was repeated twice in order to wash out endogenous glutamate. The final pellets were suspended in 5 mM Tris–HCl buffer (pH 7.4) and stored at −70°C until used.

[^{3}H]MK801 (specific activity 23.9 Ci mmol^{-1}, DuPont-NEN, Boston, MA, USA) binding to extensively washed neuronal membranes was performed as described by Ebert et al. (1991). The membranes were thawed at room temperature and suspended in 15 ml of 5 mM Tris–HCl buffer with a sonicator and centrifuged at $48\,000 \times g$ for 20 min. This washing procedure was repeated twice. [^{3}H]MK801 binding was carried out in a final volume of 1 ml 5 mM Tris–HCl buffer containing 200 μg of membrane protein, 30 μM glutamate and 1 μM glycine. Six different concentrations of [^{3}H]MK801 ranging from 1 to 20 nM were used. Nonspecific binding of [^{3}H]MK801 to the NMDA receptors was determined in the presence of 100 μM unlabeled MK801. Following 4 h of incubation at 25°C, the samples were filtered through Whatman GF/B filters presoaked in 0.1% polyethylenimine for 1 h using a 24-well Brandel cell harvester. The filters were then washed twice with 5 ml cold Tris–HCl buffer and transferred to scintillation vials. Radioactivity was measured by a liquid scintillation counter, using 10 ml Ultima Gold liquid scintillation cocktail, at a counting efficiency of approximately 56%. Dissociation constants (K_d) and maximal numbers of binding sites (B_{max}) were obtained from LIGAND computer program. Protein concentrations were determined as described by Lowry et al. (1951).

2.5. Statistics

Scores of behaviors defined in discrete episodes were analyzed by the nonparametric Kruskal–Wallis and Dunn test. Quantal (all-or-none) behavioral data were analyzed by the chi-square test and the Bonferroni inequality to adjust the P values. For the receptor binding study, the K_d

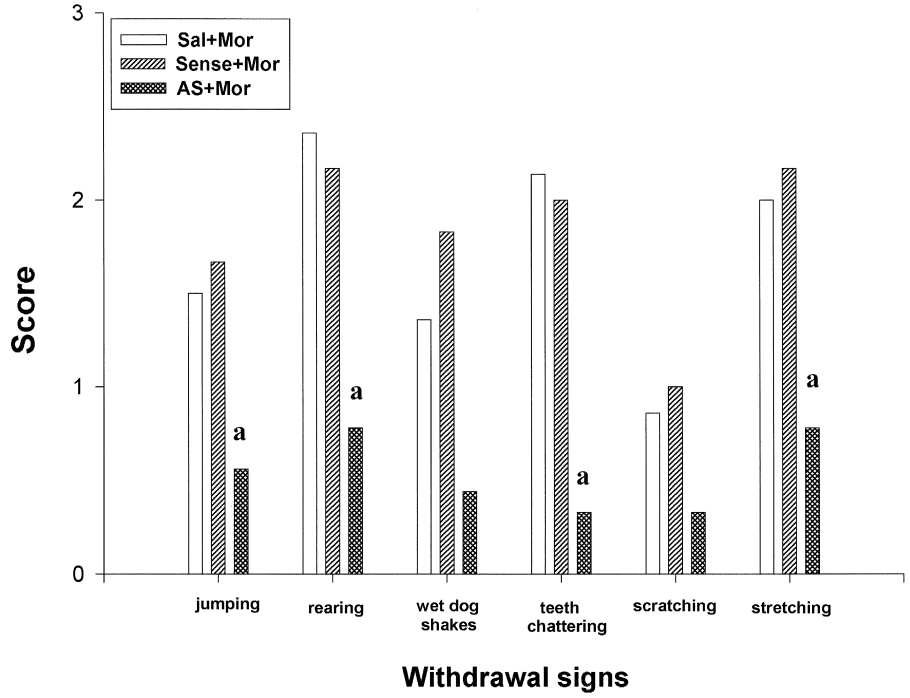

Fig. 1. Effects of i.c.v. administration of NMDA-R1 antisense oligonucleotide on the naloxone precipitated withdrawal signs from morphine. Rats received i.c.v. injection of NMDA-R1 antisense oligonucleotide (AS), saline (Sal), or sense oligonucleotide (Sense) from day 1 to day 6 and received i.c.v. infusion of morphine (Mor, 26 nmol μl^{-1} h^{-1}) from day 4 to day 6. On day 7, rats received i.c.v. injection of naloxone (48 nmol/5 μl) 2 h following termination of morphine infusion. Withdrawal behaviors were scored during a 30-min period after naloxone injection. Data are presented as mean. NMDA-R1 antisense oligonucleotide significantly decreased the intensity of jumping, rearing, teeth chattering, and stretching over that of saline and sense controls. [a] $P < 0.05$, Kruskal–Wallis test and Dunn test.

Table 1
Effects of i.c.v. administration of NMDA-R1 antisense oligonucleotide on the naloxone precipitated withdrawal behaviors from morphine

	Saline ($n=14$)	Antisense ($n=9$)	Sense ($n=6$)
Penis licking	11/14	1/9[a]	5/6
Vocalization	10/14	1/9[b]	4/6
Diarrhea	3/14	0/9	1/6

Quantal data from the behavioral studies. Rats received i.c.v. injection of NMDA-R1 antisense oligonucleotide, saline or sense oligonucleotide from day 1 to day 6 and received i.c.v. infusion of morphine (26 nmol μl^{-1} h^{-1}) from day 4 to day 6. On day 7, rats received i.c.v. injection of naloxone (48 nmol/5 μl) 2 h following termination of morphine infusion. Withdrawal behaviors were recorded during a 30-min period after naloxone injection.
Data are presented as fractions (the number of rats showing positive signs over the total number of rats tested). [a]$P < 0.05$, [b]$P < 0.01$, significantly different from both saline and sense groups as analyzed by chi-square tests.

and B_{max} were analyzed by one-way analysis of variance (ANOVA) and Bonferroni *t*-tests. Significance was set at $P < 0.05$.

3. Results

Fig. 1 and Table 1 show the effects of treatment of NMDA-R1 antisense oligonucleotide on the naloxone precipitated withdrawal signs from morphine. Pretreatment with the NMDA-R1 antisense oligonucleotide attenuated, but not abolished certain behavioral signs. In detail, NMDA-R1 antisense oligonucleotide significantly decreased the intensity of jumping, rearing, teeth chattering, and stretching over the saline treated morphine group (Fig. 1). Although the results did not reach statistical significance, fewer wet dog shakes and scratching behaviors occurred in the antisense oligonuceotide treated rats compared to the controls. There was no significant difference between the saline treated morphine group and the sense oligonucleotide treated morphine group. Penis licking ($\chi^2 = 7.471$, $P < 0.01$) and vocalization ($\chi^2 = 6.565$, $P < 0.05$) were also significantly inhibited by NMDA-R1 antisense oligonucleotide but not sense oligonucleotide (Table 1).

To examine whether the NMDA receptors were down-regulated by the NMDA-R1 antisense oligonucleotide treatment, [^{3}H]MK801 binding to the membranes of the frontal cortex was performed. The receptor density (B_{max}) for NMDA-R1 antisense treated rats was significantly reduced ($F(2,28) = 3.66$, $P < 0.05$, ANOVA) compared with that from either saline or sense controls. There were no significant differences in K_d of [^{3}H]MK801 binding among the groups (Table 2).

Table 2
Effects of i.c.v. administration of NMDA-R1 antisense oligonucleotide on [^{3}H]MK801 binding to the membranes of the frontal cortex

	Antisense ($n=9$)	Saline ($n=14$)	Sense ($n=6$)
B_{max} (pmol mg^{-1} protein)	1.51 ± 0.05[a]	1.74 ± 0.06	1.75 ± 0.10
K_d (nM)	1.30 ± 0.09	1.43 ± 0.10	1.21 ± 0.09

Rats received i.c.v. injection of NMDA-R1 antisense oligonucleotide in a dose of 15 nmol/5 μl every 12 h for 6 days. [^{3}H]MK801 binding to membranes of the frontal cortex was performed as described in Section 2. Dissociation constant (K_d) and maximal number of binding sites (B_{max}) were obtained from Scatchard analysis. Data represent mean ± S.E.M.
[a]Significantly different from both saline and sense groups, $P < 0.05$.

4. Discussion

The NMDA receptors have been proposed to play a role in physical dependence on opioids. Systemic administration of NMDA receptor antagonists have been shown to inhibit the expression and development of opioid dependence (for review see Trujillo and Akil, 1995). However, behavioral side effects such as sedation and ataxia have been found with several classes of NMDA receptor antagonists (Hiramatsu et al., 1989; Tricklebank et al., 1989; Rasmussen et al., 1991). Therefore, the inhibitory effects of the NMDA receptor antagonists on the opioid dependence are suspected to be due to 'behavioral competition' (Trujillo and Akil, 1995).

In this study we used antisense oligonucleotide as a pharmacological tool to selectively inhibit the expression of the NMDA receptors. Partial phosphorothioate modified oligonucleotides (phosphorothioates on the end, phosphodiesters in the middle) were used in this study in order to resist nuclease degradation and reduce nonspecific neurotoxicity (Krieg, 1993; Thierry and Dritschilo, 1992; Hooper et al., 1994). The sequence of the NMDA-R1 antisense oligonucleotide was identical with the NMDA-R1/c of the Wahlestedt et al. (1993). Both in vivo and in vitro experiments by Wahlestedt et al. have shown that treatment with the NMDA receptor antisense oligonucleotide did not affect the binding sites of ligands that are unrelated to the NMDA receptor, indicating the specificity of the antisense for the NMDA receptor. Moreover, it has been reported that administration of similar NMDA-R1 antisense oligodeoxynucleotide did not alter motor functions of the rats (Sun and Faden, 1995). In the present study, we observed that i.c.v. administration of the NMDA receptor antisense oligonucleotide, but not sense oligonucleotide, significantly attenuated certain morphine withdrawal signs including jumping, rearing, teeth chattering, stretching, vocalization, and penis licking. These results support the hypothesis that the NMDA receptors are involved in the development of morphine dependence.

The effects of NMDA-R1 antisense oligonucleotide on the NMDA receptor protein synthesis were examined by

[^{3}H]MK801 binding. MK801 has high affinity for the activated state of the NMDA receptor ion channel (Kemp et al., 1987; Ransom and Stec, 1988) and the NR1 subunit is believed to be essential for channel activity (Monyer et al., 1992; Nakanishi, 1992; Kutsuwada et al., 1992). The reason why the frontal cortex was selected is to make our results comparable with the previous studies (Wahlestedt et al., 1993; Zapata et al., 1997). The binding study showed that treatment with NMDA-R1 antisense oligonucleotide, but not sense oligonucleotide, resulted in a significant reduction in the B_{max} of [^{3}H]MK801 binding without significant change in K_d. These results are consistent with our conclusion that the behavioral effects of the NMDA receptor antisense oligonucleotide do result from the downregulation of the NMDA receptors by antisense oligonucleotide. The disproportion between dramatic change in behavior and modest reduction in receptor binding has been reported in several studies using antisense oligonucleotides (Standaert et al., 1996; Zhou et al., 1994). It is possible that antisense oligonucleotide may inhibit the synthesis of a small pool of functional receptors at synaptic sites which has rapid turn over rate, but not affects the pool of extrasynaptic receptors which turns over more slowly (Standaert et al., 1996; Zhou et al., 1994).

The mechanism underlying the inhibitory effects of NMDA receptor antisense oligonucleotide and NMDA receptor antagonists on the opioid dependence is unknown. Activation of NMDA receptors leads to the opening of the receptor-gated ion channels, which allows calcium to enter the neuron. The entry of calcium then has the ability to participate in numerous intracellular processes, including activation of protein kinase (for review see Wroblewski and Danysz, 1989). It has been reported that chronic opioid treatment increased the activity of cAMP-dependent protein kinase A (Nestler, 1992) and protein kinase C (Narita et al., 1994; Tokuyama et al., 1995), and increased synaptosomal calcium accumulation (Harris et al., 1977; Yamamoto et al., 1978; Guerrero-Munoz et al., 1979). Thus, the possible mechanisms underlying the effects of NMDA receptor antagonists and NMDA receptor antisense are that they prevent changes in the second messenger systems during the development of opioid dependence.

In conclusion, i.c.v. injection of NMDA-R1 antisense oligonucleotide attenuated several withdrawal signs from morphine. Repeated antisense administration significantly downregulated the NMDA receptors. These results supported the hypothesis that the brain NMDA receptors are involved in the development of opioid dependence.

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References

Aghajanian, G.K., Kogan, J.H., Moghaddam, B., 1994. Opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus: an in vivo microdialysis study. Brain Res. 636, 126–130.

Cappendijk, S.L.T., de Vries, R., Dzoljic, M.R., 1993. Excitatory amino acid receptor antagonists and naloxone-precipitated syndrome in morphine-dependent mice. Eur. Neuropsychopharmacol. 3, 111–116.

Collingridge, G.L., Bliss, T.V.P., 1987. NMDA receptors—their role in long term potentiation. Trends Neurosci. 10, 288–293.

Ebert, B., Wong, E.H.F., Krogsgaard-Larsen, P., 1991. Identification of a novel NMDA receptor in rat cerebellum. Eur. J. Pharmacol. 208, 49–52.

Feng, Y.Z., Zhang, T., Rockhold, R.W., Ho, I.K., 1995. Increased locus coeruleus glutamate levels are associated with naloxone-precipitated withdrawal from butorphanol in the rat. Neurochem. Res. 20, 745–751.

Fundytus, M.E., Coderre, T.J., 1994. Effect of activity at metabotropic, as well as ionotropic (NMDA), glutamate receptors on morphine dependence. Br. J. Pharmacol. 113, 1215–1220.

Guerrero-Munoz, F., Cerreta, K.V., Guerrero, M.L., Way, E.L., 1979. Effects of morphine on synaptosomal Ca^{2+} uptake. J. Pharmacol. Exp. Ther. 209, 132–136.

Harris, R.A., Yamamoto, H., Loh, H.H., Way, E.L., 1977. Discrete changes in brain calcium with morphine analgesia, tolerance-dependence, and abstinence. Life Sci. 20, 501–505.

Hiramatsu, M., Cho, A.K., Nabeshima, T., 1989. Comparison of the behavioral and biochemical effects of the NMDA receptor antagonists, MK801 and phencyclidine. Eur. J. Pharmacol. 166, 359–366.

Hooper, M.L., Chiasson, B.J., Robertson, H.A., 1994. Infusion into the brain of an antisense oligonucleotide to the immediate-early gene c-*fos* suppresses production of FOS and produces a behavioral effect. Neurosci. 63, 917–924.

Horan, P., Ho, I.K., 1991. The physical dependence liability of butorphanol: a comparative study with morphine. Eur. J. Pharmacol. 203, 387–391.

Hoshi, K., Ma, T.G., Ho, I.K., 1996. Naloxone-precipitated κ-opioid receptor agonist withdrawal increases glutamate levels in rat locus coeruleus. Eur. J. Pharmacol. 314, 301–306.

Kemp, J.A., Foster, A.C., Wong, E.H.F., 1987. Non-competitive antagonists of excitatory amino acid receptors. Trends Neurosci. 10, 265–298.

Koyuncuoglu, H., Gungor, M., Sagduyu, H., Aricioglu, F., 1990. Suppression by ketamine and dextromethorphan of precipitated abstinence syndrome in rats. Pharmacol. Biochem. Behav. 35, 829–832.

Koyuncuoglu, H., Dizdar, Y., Aricioglu, F., Sayin, U., 1992. Effects of MK801 on morphine physical dependence: attenuation and intensification. Pharmacol. Biochem. Behav. 43, 487–490.

Krieg, A.M., 1993. Uptake and efficacy of phophodiester and modified antisense oligonucleotides in primary cell cultures. Clin. Chem. 39, 710–712.

Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., Meguro, H., Masak, H., Kumanishi, T., Arakawa, M., Mishina, M., 1992. Molecular density of the NMDA receptor channel. Nature 358, 36–41.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., Seeburg, P.H., 1992. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 256, 1217–1221.

Nakanishi, S., 1992. Molecular diversity of glutamate receptors and implications for brain functions. Science 258, 597–603.

Narita, M., Makimura, M., Feng, Y.Z., Hoskins, B., Ho, I.K., 1994. Influence of chronic morphine treatment on protein kinase C activity: comparison with butorphanol and implication for opioid tolerance. Brain Res. 650, 175–179.

Nestler, E.J., 1992. Molecular mechanisms of drug addiction. J. Neurosci. 12, 2439–2450.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd edn. Academic Press, Orlando, FL.

Ransom, R.W., Stec, N.L., 1988. Cooperative modulation of [^{3}H]-MK801 binding to the NMDA receptor-ion channel complex by L-glutamate, glycine, and polyamines. J. Neurochem. 51, 830–836.

Rasmussen, K., Fuller, R.W., Stockton, M.E., Perry, K.W., Swinford, R.M., Ornstein, P.L., 1991. NMDA receptor antagonists suppress behaviors but not norepinephrine turnover or locus coeruleus unit activity induced by opiate withdrawal. Eur. J. Pharmacol. 117, 9–16.

Soltesz, I., Zhou, Z., Smith, G.M., Mody, I., 1994. Rapid turnover rate of the hippocampal synaptic NMDA-R1 receptor subunits. Neurosci. Lett. 181, 5–8.

Standaert, D.G., Testa, C.M., Rudolf, G.D., Hollingsworth, Z.R., 1996. Inhibition of *N*-methyl-D-aspartate glutamate receptor subunit expression by antisense oligonucleotides reveals their role in striatal motor regulation. J. Pharmacol. Exp. Ther. 276, 342–352.

Sun, F.-Y., Faden, A.I., 1995. Pretreatment with antisense oligodeoxynucleotides directed against the NMDA-R1 receptor enhances survival and behavioral recovery following traumatic brain injury in rats. Brain Res. 693, 163–168.

Tanganelli, S., Antonelli, T., Morari, M., Bianchi, C., Beani, L., 1991. Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs. Neurosci. Lett. 122, 270–272.

Thierry, A.R., Dritschilo, A., 1992. Intracellular availability of unmodified, phosphorothioated and liposomally encapsulated oligodeoxynucleotides for antisense activity. Nucleic Acids Res. 20, 5691–5698.

Tokuyama, S., Feng, Y.Z., Wakabayashi, H., Ho, I.K., 1995. Ca^{2+} channel blocker, diltiazem, prevents physical dependence and the enhancement of protein kinase C activity by opioid infusion in rats. Eur. J. Pharmacol. 279, 93–98.

Tokuyama, S., Wakabayashi, H., Ho, I.K., 1996. Direct evidence for a role of glutamate in the expression of opioid withdrawal syndrome. Eur. J. Pharmacol. 295, 123–129.

Tokuyama, S., Zhu, H., Wakabayashi, H., Feng, Y.Z., Ho, I.K., 1998. Role of glutamate in the locus coeruleus during opiate withdrawal and effect of H-7, protein kinase inhibitor, on the action of glutamate in rats. J. Biomed. Sci. 5, 45–53.

Tricklebank, M.D., Singh, L., Oles, R.J., Preston, C., Iversen, S.D., 1989. The behavioral effects of MK801: a comparison with antagonists acting non-competitively and competitively at the NMDA receptor. Eur. J. Pharmacol. 167, 127–135.

Trujillo, K.A., Akil, H., 1991. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK801. Science 251, 85–87.

Trujillo, K.A., Akil, H., 1995. Excitatory amino acids and drug abuse: a role for *N*-methyl-D-aspartate receptors in drug tolerance, sensitization and physical dependence. Drug Alcohol Dependence 38, 139–154.

Wahlestedt, C., Golanov, E., Yamamoto, F., Yee, F., Ericson, H., Yoo, H., 1993. Antisense oligodeoxynucleotides to NMDA-R1 receptor channel protect cortical neurons from excitotoxicity and reduce focal ischaemic infarction. Nature 363, 260–263.

Wroblewski, J.T., Danysz, W., 1989. Modulation of glutamate receptors: molecular mechanisms and functional implications. Annu. Rev. Pharmacol. Toxicol. 29, 441–447.

Yamamoto, H., Harris, R.A., Loh, H.H., Way, E.L., 1978. Effects of acute and chronic morphine treatments on calcium localization and binding in brain. J. Pharmacol. Exp. Ther. 205, 255–264.

Zapata, A., Capdevila, J.L., Tarrason, G., Adan, J., Martinez, J.M., Piulats, J., Trullas, R., 1997. Effects of NMDA-R1 antisense oligodeoxynucleotide administration: behavioral and radioligand binding studies. Brain Res. 745, 114–120.

Zhang, T., Feng, Y.Z., Rockhold, R.W., Ho, I.K., 1994. Naloxone-precipitated morphine withdrawal increases pontine glutamate levels in the rat. Life Sci. 55, PL25–PL31.

Zhou, L.-W., Zhang, S.-P., Qin, Z.-H., Weiss, B., 1994. In vivo administration of an oligodeoxynucleotide antisense to the D_2 dopamine receptor messenger RNA inhibits D_2 dopamine receptor-mediated behavior and the expression of D_2 dopamine receptors in mouse striatum. J. Pharmacol. Exp. Ther. 268, 1015–1023.