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Attenuation of Morphine Tolerance and Withdrawal Syndrome by Coadministration of Nalbuphine

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Morphine has been used widely on the treatment of many types of chronic pain. However the development of tolerance to and dependence on morphine by repeat application is a major problem in pain therapy. The purpose of the present study was to investigate whether combined administration of nalbuphine with morphine affects the development of tolerance to and dependence on morphine. We hypothesize that the use of nalbuphine, κ-agonist may prove to be useful adjunct therapy to prevent morphine-induced undesirable effects in the management of some forms of chronic pain. Morphine (10 mg/kg) was injected to rats intraperitoneally for 5 day. The variable dose of nalbuphine (0.1, 1.0 and 5.0 mg/kg) was administered (i.p.) in combination with morphine injection. The development of morphine tolerance was assessed by measuring the antinociceptive effect with the Randall-Selitto apparatus. The development of dependence on morphine was determined by the scoring the precipitated withdrawal signs for 30 min after injection of naloxone (10 mg/kg, i.p.). Nalbuphine did not attenuate antinociceptive effect of morphine in rats. Interestingly, combined administration of morphine with nalbuphine (10:1) significantly attenuated the development of dependence on morphine. The elevation of [^{3}H]MK-801 binding in frontal cortex, dentate gyrus, and cerebellum after chronic morphine infusion was suppressed by the coadministration of nalbuphine. In addition, the elevation of NR1 expression by morphine was decreased by the coadministration of nalbuphine in rat cortex. These results suggest that the coadministration of nalbuphine with morphine in chronic pain treatment can be one of therapies to reduce the development of tolerance to and dependence on morphine.

Key words: Morphine, Nalbuphine, Tolerance, μ receptor, κ receptor, Autoradiography

INTRODUCTION

The analgesic action of opioid is very remarkable. Historically, the discovery of opioid receptors preceded the isolation and characterization of the opioid peptides. Several lines of evidence support the existence of multiple opioid receptors. Most classes of the receptor are composed of multiple subpopulations that mediate varied physiological effects. Such multiple receptor types and subtypes are sufficiently closely related to that they are usually capable of recognizing more than one endogenous ligand of the same class. One of the earliest findings suggests this multiplicity was the demonstration by Martin *et al.* (1976) that different classes of opioid drugs produced distinct behavioral signs and that tolerance to one group of opioids did not result in cross-tolerance to another class of opioids. Martin's work in the spinal dog clearly showed that the actions of morphine and two benzomorphan derivatives (cyclazocine and pentazocine) were attributable to two distinct receptor sites, designated mu (μ) and kappa (κ) where morphine and ketocyclazocine were prototypical agonists, respectively. In addition, the existence of yet another receptor type, referred to delta (δ), was named after the mouse vas deferens bioassay, where enkepalin peptides were found to be particularly potent (Lord *et al.*, 1977). Thus, the heterogeneity of opioid receptor is now well established, con-

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sisting of three distinct receptor types termed μ, δ, and κ. The μ opioid receptor agonists, of which prototype is morphine, are of enormous therapeutic importance in the control of moderate to severe pain. The activation of μ receptor by morphine yields the most consistent and efficacious analgesia. Unfortunately, repeated treatment with morphine produces physical dependence, characterized by withdrawal symptoms and a tolerance. Thus, there must be a continuing search for morphine-type compounds which are devoid of addiction liability and are orally effective antinarcotic agent or preparation with lesser side effects.

The μ and δ receptors mediated effects have some resemblance with the central effects leading not only to analgesia to thermal, chemical and mechanical stimuli but to respiratory depression, dependence and constriction of the pupil (Dickenson, 1989). In contrast, μ and κ agonists are known to have opposite and/or different effects with each other (Pillai and Ross, 1986). Historically, κ receptor agonists have interested clinical pharmacologists because they provide acceptable analgesia, limited actions on respiration and gastrointestinal transit and do not induce morphine-like physical dependence (Millan, 1990). However, κ receptor agonists elicit dysphoric states and possess psychotomimetic properties (Kumor *et al.*, 1986; Pfeiffer *et al.*, 1986).

The analgesic nalbuphine has an interesting pharmacological profile both in animals and in humans. Nalbuphine, an opioid mixed agonist-antagonist, is structurally related to the potent opioid, oxymorphone, and the potent opioid antagonist, naloxone (Walker and Young, 1993; Chen *et al.*, 1992). Nalbuphine analgesia has been classified as κ, but its lower incidence of behavioral side effects distinguishes it from other mixed agonist/antagonist agents such as pentazocine (Schmidt *et al.*, 1985). The nalbuphine has a low dependence profile, possibly related to its ability to an antagonize morphine and other μ opioid drugs (Schmidt *et al.*, 1985). For the prevention of opioid-related side effects, both nalbuphine and naloxone can effectively decrease the incidence of respiratory depression, nausea, vomiting, and pruritus (Penning *et al.*, 1988).

It has been suggested that the development of physical dependence on opioid is closely related to the NMDA receptors, since the noncompetitive NMDA antagonist, MK-801, blocks the naloxone-precipitated behavioral signs of morphine withdrawal (Tokuyama *et al.*, 1996, 2001). The involvement of NMDA receptors in the development of opioid dependence was verified by using antisense oligonucleotides corresponding to the nucleotides 4-21 of the NMDA receptor NR1 subunit cDNA (Zhu and Ho, 1998).

The μ and δ agonists are known to have similar effects on several pharmacological actions, whereas μ and κ agonists appear to have different and/or opposite effect each other. Whereas μ and δ opioid receptors are couples to K^+-channels, κ receptors are linked to voltage-dependent Ca^{2+}-chanels in the mouse dorsal root ganglion neurons (Werz and MacDonald, 1985). It has been reported that μ agonists produce the antidiuretic action, whereas κ agonists increase diuresis (Huidobro-Taro and Parada, 1984). Treatments with μ and κ agonists produce hyperthermia and hypothermia, respectively (Pillai and Ross, 1986). In the place-conditioning paradigm, mice and rats prefer an environment associated with administration of μ and δ agonists but avoid an environment associated with administration of κ agonists (Shippenberg *et al.*, 1988; Suzuki *et al.*, 1991). These findings suggest that although both μ and κ agonists produce the analgesic effects through different mechanisms, κ agonists may suppress some pharmacological actions of μ agonists. Therefore, the effects of κ agonist, nalbuphine, on the morphine-induced antinociception, tolerance and dependence were investigated with variable combination ratio.

MATERIALS AND METHODS

Materials

[^{3}H]MK-801 (20.3 Ci/mmol) was purchased from New England Nuclear (Boston, MA, U.S.A.). Morphine chloride (Myungmun Pharm, Seoul), nalbuphine hydrochloride (Jeil Pharm., Seoul), and naloxone hydrochloride (Sigma) were dissolved in saline and administered to rat intraperitoneally or intracerebroventrically.

Animal treatment protocol and tissue preparation

Male Sprague-Dawley rats from Daehan Animals (Eumsung, Korea) weighing 220-240 g were acclimatized for 1 week with free access to rat chow and tap water. The temperature (24±3°C) and light (12 h dark) of the housing environment were maintained constantly. All procedures involving rats were performed using protocols approved by the Institutional Animal Care and Use Committee of Ewha University. Rats were anesthetized by injection (i.p.) of ketamine (50 mg/kg) and xylazine (1 mg/kg) before standard stereotaxic surgery was performed on a Kopf stereotaxic frame. A stainless steel guide cannula (21 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle (AP: -0.5 mm, LAT: +1.3 mm, and DV: -4.5 mm) of each rat (Franklin and Paxinos, 1997). Rats were allowed 1 week for recovery before implantation of osmotic minipump. Morphine withdrawal was induced by continuous i.c.v infusion with morphine (26 nmol/μL/h) for 3 day through osmotic minipumps (Alzet 2001, Alza, Palo Alto, CA), and abruption of infusion for 7 h. According to previous studies, this dose and period of morphine infusion can successfully produce physical dependence. A control group received an i.c.v. infusion of saline (1 μL/h). Before introduction into the pump, the solution was

passed through 0.2 μm sterile Acrodisk™ filters (Gelman Science, Ann Arbor, MI). The minipumps were primed overnight at 35°C in sterile saline so that the normal flow rate (1 μL/h) was attained prior to implantation. Under ether anesthesia, osmotic minipumps were implanted subcutaneously between the scapulae. The tygon tubing (0.38 μm inner diameter, Cole-Palmer, Chicago, IL) was used to connect the outlet of the minipump to a piece of 'L'-shaped stainless steel injector tubing (26 gauge, 20 mm long), which was placed into the i.c.v. guide cannula. The connecting tube between the i.c.v. cannula and the outlet of the minipump was disconnected. Seven hours following termination of morphine and/or nalbuphine infusion, brains were removed immediately and were frozen in liquid nitrogen for 20 sec. Horizontal sections, 20 μm thickness, were cut on an LEICA microtome at -18°C, thaw-mounted on gelatin-coated microscope slides and stored at -80°C until used.

Antinociceptive testing

Tests were carried out in a quiet room away from the colony room. Prior to the test phase, the rats had no experience of the procedure. Nociceptive thresholds were determined by a modification of the Randall-Selitto method described previously (Kayser and Guilbaud, 1983), where increasing pressure is applied to the hindpaw until the rat squeaks. For each rat, a preliminary threshold or control threshold (mean of two consecutive stable thresholds, expressed in grams) was determined before injection of opioid. Nociceptive pressure thresholds were then measured every 30 min after opioid administration, until they returned to baseline.

Measurement of the inhibition of naloxone-induced withdrawal

The inhibition of naloxone-induced withdrawal syndrome in morphine-dependent rat was estimated by the observation of the withdrawal syndrome by injection of naloxone 10 mg/kg (i.p.) on the seventh day or 7 h after the abruption of morphine infusion. The naloxone-induced behavior withdrawal syndrome was observed after placing animals on each cage for 30 min. The prototype of withdrawal syndrome was as below: wet-dog shake, rearing, escape behavior, penis licking, grooming, ptosis, diarrhea, teeth chattering. In other experimental group, to avoid the peripheral effect of opioid on withdrawal syndrome, morphine and/or nalbuphine was infused into cerebroventricle *via* osmotic minipump with the rate of 26 nmol/μL/h for 3 day.

Autoradiography

Receptor autoradiography of [^{3}H]MK-801 was performed according to the method of Sakurai *et al.* (1993) with modifications (Oh *et al.*, 2000). In brief, tissue sections were thawed and dried at room temperature, and sections were pre-washed in 50 mM Tris-HCl buffer (pH 7.4) to remove endogenous glutamate and glycine for 30 min at 4°C and blown dry under a stream of room-temperature air before the [^{3}H]MK-801 binding. Tissue sections were incubated in 50 mM Tri-HCl buffer containing 10 nM [^{3}H]MK-801 and 30 μM glutamate/10 μM glycine for 120 min at room temperature, rinsed with cold 50 mM Tris-HCl buffer two times for 30 min each, dipped once in ice-cold distilled water, and immediately dried in a stream of cool air. Non-specific binding was determined in the presence of non-radioactive 50 μM MK-801. Dried tissue sections were placed in X-ray cassettes with a set of tritium standards ([^{3}H]Micro-scale RPA 510, Amersham) and juxtaposed to Hyperfilm (Amersham). Following a 4-week exposure period at 4°C, the film was developed and autoradiograms were analyzed as described for in situ hybridization. Non-specific binding was less than 5% of the total binding and was negligible for analyzing the autoradiograms.

Measurement of the level of NR1 and NR2B in opioid withdrawal rat brain

Laemmli loading buffer was added to extracts (60 μg protein) and the samples were boiled for 4 min. Extracts were run on 12% SDS-PAGE gels and transferred electrophoretically to nitrocellulose. Blots were blocked in 5% skim milk and 2% bovine serum albumin in TBST for 3 h and the membrane was probed with anti-NR1, anti-NR2B antibody (Pharmingen) at a dilution of 1:1000 for overnight at 4°C. Blots were rinsed three times for 20 min in TBST, and incubated in horseradish peroxidase-conjugated goat anti-mouse IgG or horse anti-rabbit IgG (Santa Cruz Biotechnology Inc.) at a 1:1000 dilution. The membrane was then rinsed 3 times for 5 min in TBST. Immunoreactivity was visualized using ECL chemiluminescence (Amersham Pharmacia Biotech).

RESULTS

Inhibition of analgesic tolerance to morphine

Acute coadministration of nalbuphine with morphine (1:100, 1:10, 5:10) did not modulate the analgesia (Fig. 1A). Daily treatment of rat with morphine alone for 5 consecutive days resulted in 50% decrease in its antinociceptive effect. However, the morphine-induced analgesic tolerance was suppressed by the coadministration of nalbuphine with the ratio of 1:10 and 5:10 (nalbuphine vs morphine) in a dose-dependent manner (Fig. 1B).

Inhibition of naloxone-induced withdrawal

The inhibitory action of nalbuphine injection on naloxone-

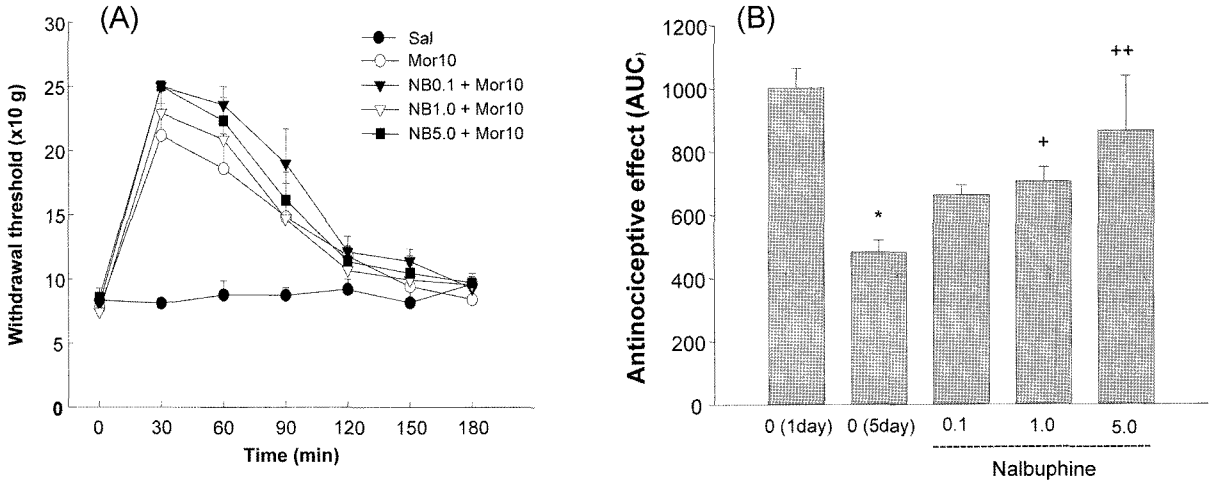

Fig. 1. The effects of various doses of nalbuphine on morphine-induced analgesia. (A) Nalbuphine (0.1, 1 and 5 mg/kg, i.p.) was co-administrated with morphine 10 mg/kg. The threshold of paw withdrawal was determined at before and at every 30 min up to 180 min after the injection of morphine. Data are expressed as mean ± S.E. (n=5). (B) The occurrence of tolerance to morphine after 5 days treatment with morphine 10 mg/kg (i.p.) daily. Data are expressed as the area under the curve (AUC) and are mean ± S.E. (n=5). Significant differences (*p<0.05 vs. 1st day, +p<0.05, ++p<0.01 vs. no nalbuphine treatment group) between respective groups were determined with one-way analysis of variance followed by Dunnett's test.

Table I. Inhibitory effects of nalbuphine on morphine-induced withdrawal signs in morphine-dependent rats by repeated injections

Withdrawal signs[1]	Saline	Morphine	Morphine 10 + Nalbuphine 0.1	Morphine 10 + Nalbuphine 1.0
Teeth chattering	0/6	5/9*	6/11	6/11
Wet-dog shake	0/6	5/9*	4/11	3/11 #
Forepaw tremors	0/6	5/9*	1/11#	3/11
Diarrhea	0/6	0/9	0/11	0/11
Rearing	1/6	9/9*	3/11	2/11#
Ptosis	0/6	8/9*	4/11#	9/11
Penis-licking	0/6	7/9*	6/11	2/11#

Rats were received morphine (10 mg/kg, i.p.) and/or nalbuphine (0.1 or 1 mg/kg, i.p.) for 6 days, and were challenged with naloxone (10 mg/kg, i.p.) 24 hr after the final injection of morphine. [1]Numbers denote the number of rats showing positive signs over the total number of rats tested for 30 min after injection of naloxone. *p<0.05, compared with the saline group, #p<0.05, compared with the morphine group by Fischer-exact test.

Table II. Inhibitory effects of nalbuphine on morphine-induced withdrawal signs in morphine-dependent rats by by continuous infusion

Withdrawal signs[1]	Saline	Mor	Nalb	Mor:Nalb (10:1)
Teeth chattering	0/5	5/5*	0/5	1/5#
Wet-dog shake	0/5	5/5*	3/5	2/5
Diarrhea	0/5	0/5	3/5	0/5
Rearing	0/5	5/5*	1/5	1/5#
Ptosis	0/5	1/5	1/5	4/5
Penis-licking	0/5	3/5	3/5	1/5

Rats were infused with morphine (26 nmol/μL/h) and/or nalbuphine (2.6 nmol/μL/h) for 3 days, and were challenged with naloxone (10 mg/kg, i.p.) 7 h after the cessation of opioid infusion. [1]Numbers denote the number of rats showing positive signs over the total number of rats tested for 30 min after injection of naloxone. *P<0.05, compared with the saline group, #P<0.05, compared with the morphine group by Fischer-exact test.

induced withdrawal syndrome after repeated morphine injection was significant in the wet-dog shake, rearing, and penis licking, but does not inhibit the grooming, ptosis, and teeth chattering (Table I). To avoid the peripheral effect of opioid on withdrawal syndrome, opioid was infused into cerebroventricle *via* osmotic minipump for 3 day. The inhibitory action of nalbuphine coadministration on morphine withdrawal syndrome was significant in teeth chattering and rearing after 7 h abruption of opioid infusion (Table II).

Autoradiography

When the autoradiographs were quantitated, the binding of [^{3}H]MK-801 was found to be at the highest in the hippocampus and high in the cerebral cortical area (higher in the layers II-IV than the layers V and VI), caudate putamen, septum, and thalamus. However, the binding was low in the brainstem and cerebellum (Fig. 2, Table III). In case of morphine withdrawal, MK-801 binding was significantly increased in certain cortical area and hippocampal area and increased almost other brain regions during the time of withdrawal (7 h after stopping the infusion) from continuous morphine infusion. Interestingly, the elevation of [^{3}H]MK-801 binding was suppressed by the coadministration of nalbuphine in the temporal cortex and cingulated, dentate gyrus, and granule layer of cerebellum.

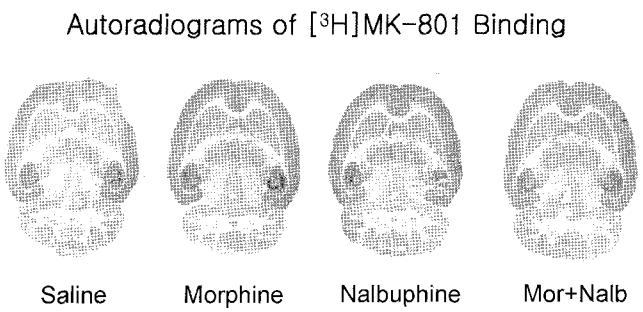

Fig. 2. Representative autoradiograms of [^{3}H]MK-801 in horizontal rat brain sections. Exposure time was 3 weeks. Representative autoradiograms of [^{3}H]MK-801 binding in horizontal rat brain sections. Morphine and/or nalbuphine were continuously infused (26 nmol/μL/h and/or 2.6 nmol/μL/h) into rat brain (i.c.v.) by osmotic minipump for 3 days and abruption of infusion for 7 hrs. Brain sections for MK-801 binding were incubated with 10 nM [^{3}H]MK-801 in 50 mM Tris-HCl buffer (pH 7.4) containing 30 mM glutamate and 10 mM glycine for 2 h at 25°C.

Table III. Changes in [^{3}H]MK-801 binding to brain regions of opioid-infused rats

Regions	[^{3}H]MK-801 binding (nCi/mg tissue)			
	Saline	Mor	Nalb	Mor+Nalb
Frontal Cortex				
Layers II, III, IV	4.59±0.31	5.04±0.34	4.75±0.12	3.40±0.19
Layers V, VI	3.58±0.26	3.95±0.29	4.02±0.11	3.87±0.16
Temporal	4.65±0.37	5.31±0.35*	4.95±0.13	4.61±0.19
Cingulate	4.95±0.33	5.41±0.36*	4.01±0.13	4.69±0.19
Caudate-putamen	2.78±0.22	3.07±0.30	2.58±0.11	2.60±0.15
Septum	3.09±0.38	3.42±0.40	2.84±0.21	2.80±0.24
Thalamus	3.24±0.24	3.60±0.25	3.02±0.25	3.05±0.12
Hippocampal formation				
CA1	6.21±0.48	6.75±0.43	5.93±0.15	6.52±0.09
CA3	5.26±0.34	5.75±0.38	5.25±0.13	4.95±0.21
Dentate gyrus	7.57±0.39	8.13±0.67*	7.21±0.16	8.04±0.24
Midbrain				
Inferior colliculus	0.78±0.09	0.98±0.09	0.68±0.09	0.72±0.11
Central gray	1.33±0.11	1.55±0.12	1.22±0.12	1.28±0.13
Cerebellum				
Granule layer	1.52±0.19	1.72±0.20*	1.38±0.18	1.40±0.15

Rats were infused intracerebroventricularly with morphine (26 nmol/1 μL/h), nalbuphine (2.6 nmol/1 μL/h), or combination (morphine 26 + nalbuphine 2.6 nmol/1 μL/h) for 3 days, and rendered withdrawal from opioid 7-h after cessation of infusion.
Values are expressed as mean ± SE from 5-6 rats. *p<0.05 for difference from respective control groups.

Immunoblot

The western blot was performed to examine the effect of nalbuphine coadministration on the modulation of the expression of NMDA subunits in the several brain regions

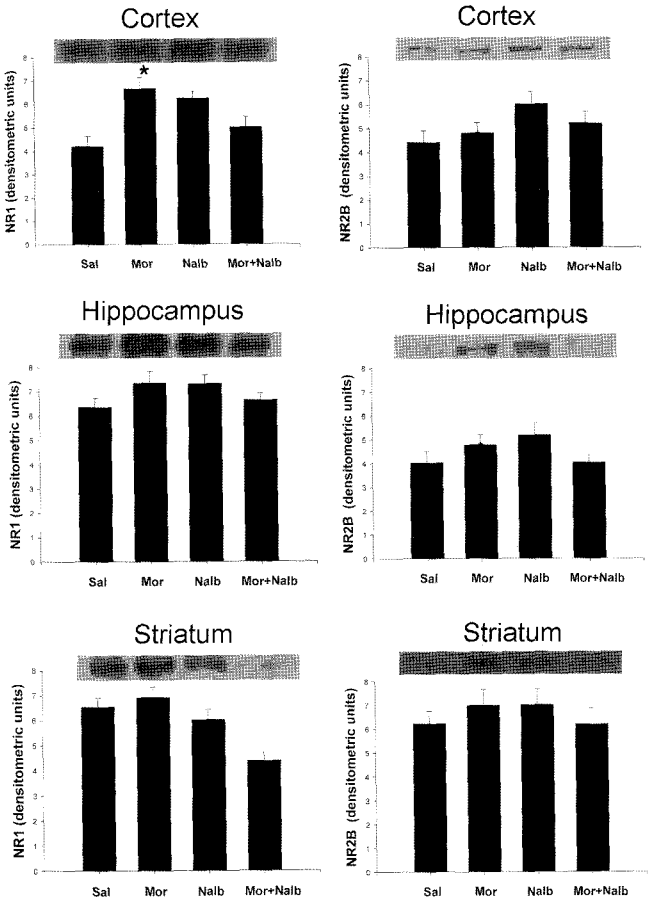

Fig. 3. The effects of nalbuphine on the expression of NMDA subunit (NR1, NR2B) detected by immunoblot in each brain areas of morphine infused rats. Morphine and/or nalbuphine were continuously infused (26 nmol/μL/h and/or 2.6 nmol/μL/h) into rat brain (i.c.v.) by osmotic minipump for 3 days and abruption of infusion for 7 h. Values are mean ± SE (n=5-6 rats per group). *significant from control (p<0.05).

after opioid infusion for 3 day. The level of NR1 was significantly elevated in cortex by the treatment with morphine, and the level of NR1 was significantly inhibited by the coinfusion with nalbuphine in the cortex. The inhibitory effect of nalbuphine on morphine-induced elevation of NR1 expression was shown in hippocampus and striatrum although it has not statistic significance (Fig. 3). Unexpectedly, the level of NR2B expression was increased by the infusion of nalbuphine but not by the morphine in the cortex and hippocampus.

DISCUSSION

In addition to the primary reinforcing effects in repeated opioid use, other factors come into play during long-term drug use that profoundly affect the pattern of use. Among these factors are the capacities of some substances to produce tolerance and/or physical dependence. These phenomena were often assumed inextricably linked to

each other and to the problem of compulsive drug use. Urinary retention is the most troublesome nonrespiratory side effect of epidural morphine, observed soon after injection and lasting for 14 h to 16 h regardless of the dose used (Rawal *et al.*, 1983).

Partial opiate agonists have become increasingly popular in clinical application, because of their reasonable analgesic effect and alleged less ability to induce dependence. Mice that received chronic treatment with nalbuphine and cyclorphan exhibited cross-tolerance to morphine, however, cross-tolerance to morphine was not observed after the chronic administration of buprenorphine or butorphanol (Gringauz *et al.*, 2001). Buprenorphine and cyclorphan are mixed agonist-antagonist with predominant μ receptor activity. Nalbuphine and butorphanol are more active on κ than on μ receptor (Pick *et al.*, 1992; Picker *et al.*, 1993; Bertalmio and Woods, 1992). The binding studies suggest that there may be interactions among the types of opioid receptors, including μ/δ receptors and μ/κ receptors interactions. Rothman *et al.* (1988) have proposed the existence of μ/δ opioid receptor complex using DADLE. Interestingly, U-50,488H, an exogenous κ agonist, could dose-dependently suppress the development of tolerance to morphine antinociception in mice (Narita, 1992). It has been also shown that antagonism at κ opioid receptor sites after morphine administration, positively modulates the development of μ opioid tolerance (Sofuoglu *et al.*, 1992). These results provide further evidence that the activation and blockade of κ receptors may suppress and potentiate the development of tolerance to morphine antinociception, respectively. More interestingly, it has been known that although both μ and κ agonists elicit the antinociceptive effects, there may be the opposing properties of μ and κ agonists on mesolimbic dopaminergic system underlying their different effects on motivation, motor behavior and biochemical analysis (Di Chiara and Imperato, 1988; Spanagel *et al.*, 1992). On the contrary, coadministration of either SCH23390 or haloperidol failed to suppress the development of antinociceptive tolerance to morphine (Narita, 1992). These findings indicate that the dopaminergic systems may not play a role in the development of antinociceptive tolerance to morphine.

Chronic administration of morphine in the rodent induces dependence, the physical component of which is exhibited in various specific behavioral and vegetative signs after withdrawal of morphine or administration of an opioid antagonist. The mechanisms responsible for opioid-induced physical dependence are among the most thoroughly studied. Aceto *et al.* (1986) have reported that a μ receptor antagonist, β-FNA, blocked the development of physical dependence in rats when infused simultaneously with morphine for 6 day. It has been reported that an endogenous κ agonist dynorphin may suppress morphine withdrawal signs (Aceto *et al.*, 1982). Furthermore, activation of κ receptors by morphine itself also plays an important role in the suppression of naloxone-precipitated body weight loss in morphine-dependent rats (Suzuki *et al.*, 1988). On the other hand, U-50,488H, an exogenous κ agonist, cannot suppress the development of physical dependence on morphine in rats (Fukagawa *et al.*, 1989). Thus, although there would be, at least in part, inhibitory κ opioid systems in the development of physical dependence on morphine and expression of withdrawal signs, it is still unclear what factors contribute to the differences in the effect of dynorphin and of U-50,488H on morphine physical dependence. It has been recognized that the μ2 and/or other receptors including peripheral opioid receptors are involved in withdrawal weight loss and diarrhea by using μ1 receptor-deficient CXBK mice (Suzuki and Misawa, 1990). However, administration of morphine with nalbuphine into cerebroventricle to avoid peripheral effect of opioid showed the less withdrawal signs in our experimental results. Whether these differences involve differences in receptor density, receptor sensitivity, or intracellular signaling mechanisms are not known.

Multiple cellular and receptor adaptations likely take place in opioid treatment. Interestingly, a signal transduction of κ opioids is quite different from those of μ and δ opioids. Instead of hyperpolarizing the neuronal membrane by opening K^+ channels, κ opioid inhibited transmitter release in these systems by attenuation of Ca^{2+} currents (Cherubini and North, 1985; Attali *et al.*, 1989). In addition, k opioid agonists do not affect the resting membrane potential or conductance of the myenteric neurons, but decrease the inward Ca^{2+} current underlying the action potentials of the neurons (Cherubini and North, 1985). However, the inhibition of Ca^{2+} current has been studied in cell somata and it is not known whether the findings apply to transmitter release sites. Thus such information for signal transduction of opioids will be essential for understanding the acute and chronic actions of opioids.

It has been known that the ligand binding affinities of recombinat NMDA receptors depend on subunit combination, and differences in regional subunit composition of NMDA receptors may underlie their functional and pharmacological heterogeneity (Laurie and Seeburg, 1994). When the heteromeric receptors were constructed from NR1 subunit and one of subunit of the NR2 subfamily, NR1-NR2B receptors display the highest affinity for [^{3}H]glutamte, whereas NR1-NR2A receptors show the highest affinity for competitive antagonists and bind [^{3}H]MK-801 with high affinity (Laurie and Seeburg, 1994). The present our data showed that the level of [^{3}H]MK-801 binding was increased in the cortical and hippocampal area by the morphine withdrawal, and the elevated MK-801 binding was suppressed by the coadministration of nalbuphine. Also the

elevated level of NR1 expression after morphine withdrawal was suppressed by the coadministration of nalbuphine in the cortex, hippocampus, and striatum. However, the level of NR2B expression was increased by the withdrawing from nalbuphine but not from morphine in the cortex and hippocampus. These results suggest the expressions of NR1 and NR2B are not changed identically in the opioid withdrawal. These discrepancies may denote the differential pharmacological action of mu-opioid receptor favoring agonist (morphine) and kappa-opioid receptor favoring agonist (nalbuphine). Based on the suggestion by Laurie and Seeburg (1994), our data can be interpreted as reflecting an increase in the number of the NMDA receptors after morphine withdrawal, associated with the [^{3}H]MK-801 binding and NR1 expression. And the NMDA receptor expression (activation) could be suppressed by the coadministration of nalbuphine.

In conclusion, our findings suggest that the coadministraion of morphine with nalbuphine may constitute a preferable superior approach to the treatment of pain, because nalbuphine may decrease some side-effects of morphine such as tolerance and physical dependence while keeping potent antinociceptive activity.

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