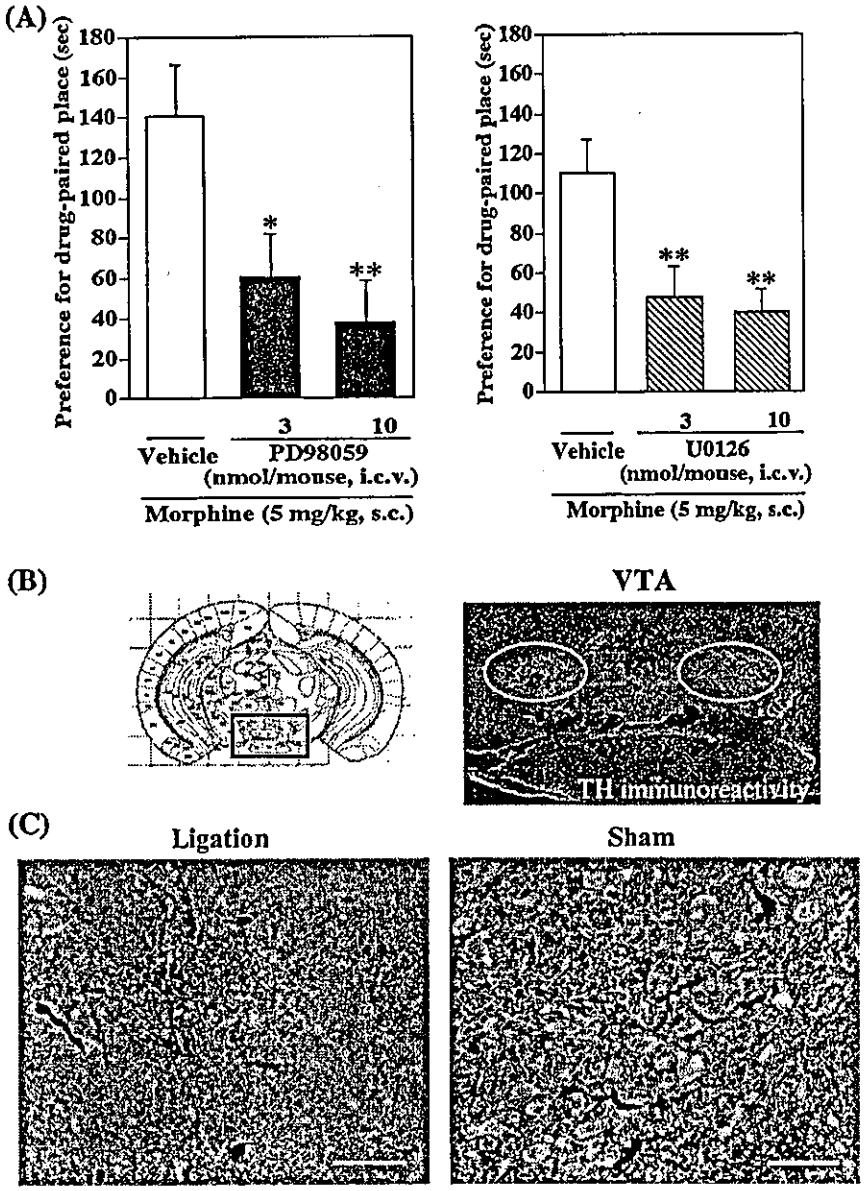

Fig. 9. (A) Effects of MEK inhibitors PD98059 (left) and U0126 (right) (3 or 10 nmol/mouse, i.c.v.) on the place conditioning produced by morphine (5 mg/kg, s.c.) in normal mice. Ordinate: mean difference (s) between time spent during the post-conditioning test and pre-conditioning test. Groups of mice were given i.c.v. with vehicle, PD98059 or U0126 at 30 min before s.c. injection of morphine or saline. Each value represents the mean ± S.E.M. of 8–16 mice. *$p < 0.05$, **$p < 0.01$: vs. vehicle-morphine group. (B) Atlas of the VTA at approximately bregma − 3.08 (according to the book by Paxinos and Frandlin, 2001). The box indicates the positions of the VTA imaged in the present study. The immunoreactivity of tyrosine hydroxylase (TH) is detected in the VTA. (C) Representative photomicrograph of phosphorylated-ERK (p-ERK) labeling in the VTA at 4 days after sciatic nerve ligation. The white-colored dot or circle represents p-ERK-positive cells. Scale bars, 100 μm.

by nerve denervation and intraplantar infiltration of carragennan (Morgan and Franklin, 1990; Saade et al., 1997). These findings indicate that the VTA may directly or indirectly receive nociceptive input with pain-related inhibitory action. If that is the case, neuropathic pain-like stimulation may activate the ascending inhibitory pathway from the spinal cord projecting indirectly to the VTA, resulting in the intracellular changes including the decrease in ERK activity within VTA DAergic neurons (Fig. 10).

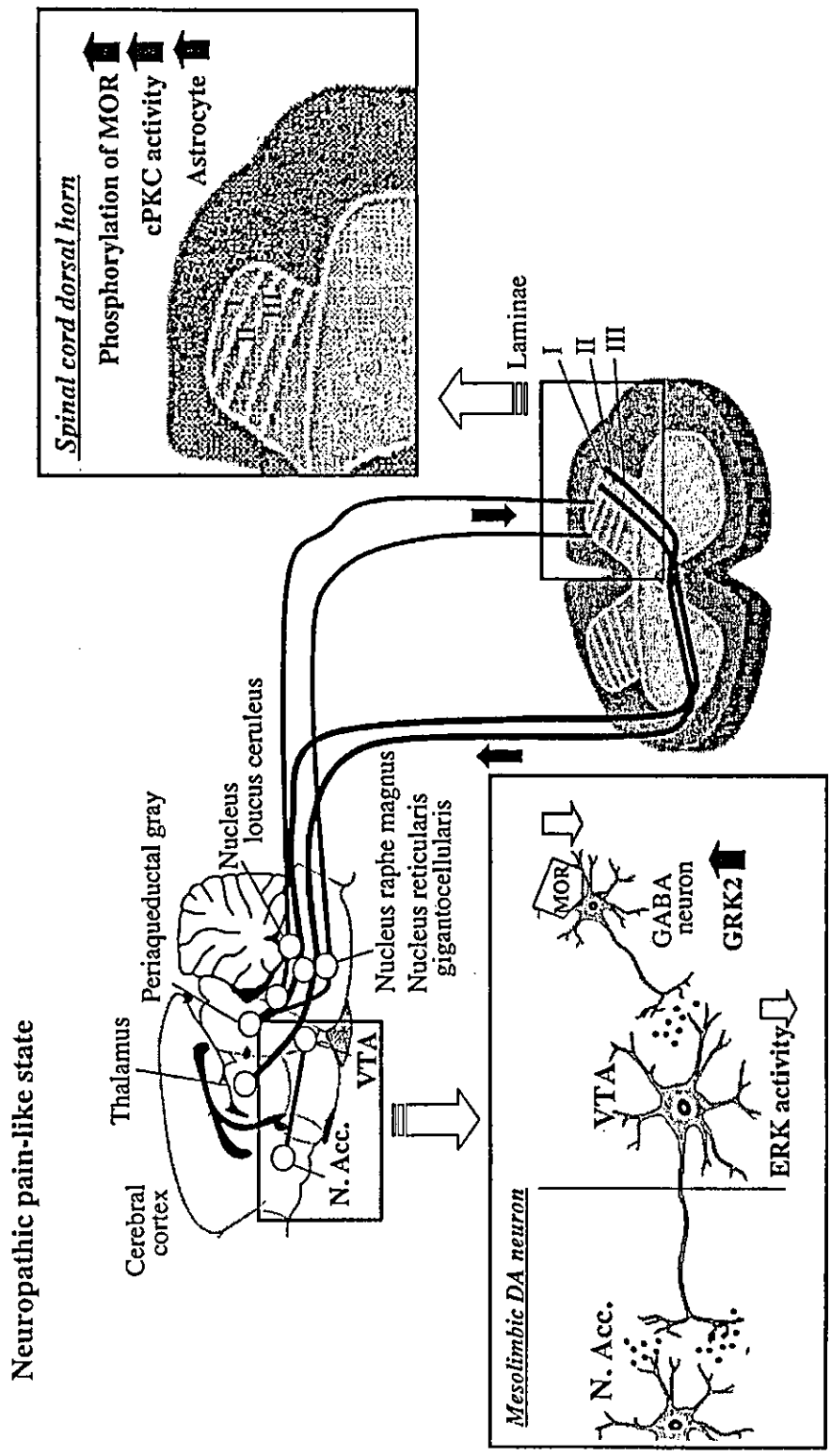

Fig. 10. Molecular mechanism of the suppression of morphine's effect under a neuropathic pain-like state described on the simplified schema of afferent sensory pathways and descending modulatory pathway. Rodents with sciatic nerve injury exhibit the increase in spinal cPKC activity, spinal astrocytic response, phosphorylation of spinal MOR and VTA-located GRK2, and the decrease in ERK activity and MOR function in the VTA. These changes in the spinal cord may cause the inhibition of morphine-induced antinociception. In contrast, the change in VTA dynamics may lead to the suppression of rewarding effect of morphine.

Attenuation of the morphine-induced rewarding effect under chronic inflammation

Several animal models of continuous inflammatory nociception resembling human clinical conditions have been developed and extensively studied in recent years. One such model involves the administration of complete Freund's adjuvant (CFA), carrageenan or formalin to the paw, joint or the tail to produce chronic inflammation. Under inflammatory nociception, the suppression of the development of tolerance to and physical dependence on morphine, changes in the endogenous opioid system, central sensitization of dorsal horn neurons and enhancement of extracellular excitatory amino acids have been observed (Iadarola et al., 1988; Skilling et al., 1988; Vaccarino and Couet, 1993; Vaccarino et al., 1993). We found that morphine-induced place preferences was significantly attenuated in formalin-or carrageenan-treated groups (Suzuki et al., 1996).

Many researchers have reported that κ-opioid systems negatively affect various functions of μ-opioid systems. For instance, the development of tolerance to morphine-induced antinociception is completely blocked by the co-administration of the κ-opioid receptor (KOR) agonist, U-50,488H (trans-(1S,2S)-3,4-dichloro-N-methyl-N-(2-[1-pyrollidinyl]cyclohexyl)bezeneacetamide) (Yamamoto et al., 1988). In addition, intravenous (i.v.) injection with the endogenous KOR agonists dynorphin A(1-13) and dynorphinA (2-17) suppresses the expression of tolerance to antinociception of morphine and naloxone-precipitated withdrawal signs in morphine-dependent mice (Hooke et al., 1995). Our previous study showed that U-50,488H markedly inhibited the rewarding effect of MOR agonists (Suzuki et al., 1992). Therefore, to elucidate the mechanism underlying the suppression of rewarding effects of morphine under inflammatory nociception, the effects of pretreatment with κ-and δ-opioid receptor antagonists, nor-binaltorphimine (nor-BNI) and naltrindole (NTI), on the development of the morphine-induced place preference under inflammation were examined. It is of interest to note that a significant attenuation of the morphine-induced place preference in the formalin-treated animals was completely reversed by pretreatment with nor-BNI, but not NTI. Furthermore, the morphine-induced increase in DA release in the limbic forebrain was suppressed by inflammation, and this suppression was abolished by the pretreatment with nor-BNI. These results suggest that endogenous κ-opioid systems may be activated by chronic inflammatory nociception. The activation of κ-opioid system may, then, inhibit DA release in the N. Acc., resulting in the suppression of the development of rewarding effects produced by morphine.

Conclusion

Our recent research suggests that activated spinal PKC plays a substantial role in the expression of a neuropathic pain-like state. Activated spinal PKC causes the phosphorylation of spinal MOR, indicating that this phenomenon is the one of key factor to suppress morphine analgesia by nerve injury. This activation of spinal PKC leads to the stimulation of the ascending nociceptive pathway, resulting in the changes in neurotransmission at supraspinal levels. In the behavioral study, we comfirmed that animals with chronic pain failed to exhibit the morphine-induced rewarding effect. This phenomenon strongly supports the clinical situation that psychological dependence on morphine is not a major concern of patients with chronic pain. It should be pointed out that sciatic nerve injury causes specific uncoupling between MOR and G-proteins in the VTA and produces a sustained and reduction in ERK activities of DAergic neurons in this area. In contrast, the enhanced κ-opioidergic system is

directly involved in the suppression of the morphine-induced rewarding effect under inflammation. These findings strongly indicate that treatment of morphine could be highly recommended for the relief of severe chronic pain.

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Direct Evidence for the Involvement of the Mesolimbic κ-Opioid System in the Morphine-Induced Rewarding Effect Under an Inflammatory Pain-Like State

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Recent clinical studies have demonstrated that when morphine is used to control pain in cancer patients, psychological dependence is not a major concern. The present study was undertaken to ascertain the modulation of psychological dependence on morphine under a chronic pain-like state in rats. The prototypical μ-opioid receptor agonist morphine (8 mg/kg, i.p.) induced a dose-dependent place preference. In the present study, we found that an inflammatory pain-like state following formalin injection significantly suppressed the morphine-induced rewarding effect. This effect was almost reversed by s.c. pretreatment with the κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI, 5 mg/kg). Furthermore, the morphine-induced increase in dopamine (DA) turnover in the limbic forebrain was significantly inhibited by treatment with formalin. This inhibition was also suppressed by pretreatment with nor-BNI. In addition, *in vivo* microdialysis studies clearly showed that the morphine-induced increase in the extracellular levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, in the nucleus accumbens (N.Acc.) was significantly decreased in rats that had been pretreated with formalin. This effect was in turn reversed by the microinjection of a specific dynorphin A antibody into the N.Acc. These findings suggest that the inflammatory pain-like state induced by formalin injection may have caused a sustained activation of the κ-opioidergic system within the N.Acc., resulting in suppression of the morphine-induced rewarding effect in rats. The present study provides further evidence of the clinical usefulness of morphine in patients suffering from severe pain.
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Keywords: opioid; inflammatory pain-like state; morphine dependence; nucleus accumbens; rat

INTRODUCTION

The World Health Organization publication Cancer Pain Relief (WHO, 1996) proposed a method for the relief of cancer pain based on a small number of relatively inexpensive drugs, including morphine. This approach is now used worldwide.

Recent clinical experience has shown that when morphine is used to control pain in cancer patients, psychological dependence is not a major concern (WHO, 1996). However, undue anxiety about psychological dependence on morphine in cancer patients has caused physicians and patients to use inadequate doses of opioids. Nociceptive stimuli produced physiological changes in the levels of some proteins and neuropeptides in dorsal horn neurons (Dubner and Ruda, 1992; Narita *et al*, 2000). We reported previously that morphine failed to induce rewarding effects under a neuropathic pain-like state induced by sciatic nerve ligation in the rat and mouse (Ozaki *et al*, 2002, 2003). Furthermore, this pain-like state leads to a reduction in μ-receptor function in the ventral tegmental area (VTA; Ozaki *et al*, 2002, 2003), which is the origin of the mesolimbic dopaminergic system and the major neural substrate of the rewarding effect produced by opioids (Koob, 1992; Nestler, 1996; Narita *et al*, 2001). These findings suggest that a state of pain could lead to physiological changes in neurotransmission at supraspinal levels, which could be responsible for the decrease in psychological dependence on opioids.

We also reported that morphine failed to induce rewarding effects under an inflammatory pain-like state produced by formalin or carrageenan (Suzuki *et al*, 1996,

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1999, 2001). However, there is, if any, direct evidence regarding possible changes at the supraspinal level after inflammation. Therefore, the aim of the present study was to investigate the modification of the rewarding effect induced by morphine under an inflammatory pain-like state produced by formalin injection.

EXPERIMENTAL PROCEDURES

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Every effort was made to minimize the numbers and suffering of animals used in the following experiments.

Animals

Male Sprague-Dawley rats (200–300 g) were obtained from Tokyo Laboratory Animals Science, Co., Ltd (Tokyo, Japan). The rats were housed at a room temperature of 23±1°C with a 12 h light–dark cycle (light on 0830–2030 h), and were allowed to adapt to this environment for 1 week before the experiments. Food and water were available *ad libitum*.

Induction of Inflammation

Formalin (2.5%, 50 μl) or vehicle (saline, 100 μl) was injected into the plantar surface of the right hind paw. Rats treated with formalin were housed individually.

Place Conditioning

Place-conditioning studies were conducted using an apparatus consisting of a shuttle box (width 30 cm × length 60 cm × height 30 cm) that was made of an acrylic resin board and divided into two colored compartments of equal size. One compartment was white with a textured floor and the other was black with a smooth floor. Conditioning sessions (two for morphine : two for saline) were started on the first day after formalin and vehicle injection into the rat paw, and conditioning was conducted once daily for 4 days (Suzuki *et al*, 1996, 1999, 2001). Immediately after i.p. injection of morphine (2–8 mg/kg), rats were placed in one compartment for 50 min. On alternate days, rats were treated with saline and placed in the other compartment for 50 min. The order of injection (morphine or saline) and compartment was counter-balanced (unbiased) across the subjects. The κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI, 5 mg/kg) was administered s.c. 6 h before i.p. treatment with morphine. On day 5, tests of conditioning were performed as follows: the partition separating the two compartments was raised to 12 cm above the floor, a neutral platform was inserted along the seam separating the compartments, and rats that had not been treated with morphine or saline were placed on the platform. The time spent in each compartment during a 900 s session was then recorded automatically with an infrared beam sensor (KN-80, Natsume Seisakusyo Co., Ltd, Tokyo, Japan). CPP scores represent the time spent in the drug (morphine)-paired place minus the time spent in the saline-paired place. All sessions were conducted under conditions of dim illumination (28 lux lamp) and white masking noise. Each group consisted of 8–10 rats.

Quantification of Dopamine Turnover

Using high-performance liquid chromatography with electrochemical detection (HPLC-ECD), the dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) levels were determined as previously described (Narita *et al*, 1993). Rats that had been pretreated with s.c. injection of nor-BNI (5 mg/kg) or saline were killed 30 min after s.c. injection of saline (1 ml/kg) or morphine (5 mg/kg). The brain was removed quickly, and the limbic forebrain (containing nucleus accumbens (N.Acc.) and olfactory tubercles) and the striatum were dissected on an ice-cold metal plate. The tissues were homogenized in 500 μl of 0.2 M perchloric acid containing 100 μM EDTA (2 Na) and 100 ng isoproterenol as an internal standard. The homogenates were then centrifuged at 20 000*g* for 30 min at 4°C, and the supernatants were maintained at pH 3.0 using 1 M sodium acetate. Samples were analyzed by HPLC-ECD. The HPLC system consisted of a delivery system (EP-10, Eicom Co., Kyoto, Japan), an analytical column (Eicompac, MA-5ODS, Eicom Co.), and a guard column (Eicom Co.). DA and its metabolites were separated by a column with a mobile phase containing sodium acetate (0.1 M), citric acid monohydrate (0.1 M), sodium 1-octane sulfonate (170 mg/l), EDTA (2 Na) (10 mg/l), and 15% methanol. The mobile phase was delivered at a flow rate of 1.0 ml/min. DA and its metabolites were identified according to the retention times of these standards, and the amounts were quantified by calculating the peak areas. The DA turnover, 'DA ratio', was calculated as (DOPAC + HVA)/DA.

Surgery

Stereotaxic surgery was performed under sodium pentobarbital (50 mg/kg, s.c.) anesthesia. Rats were placed in a stereotaxic apparatus and the skull was exposed. A small hole was then made using a dental drill. A guide cannula (AG-8, Eicom Co.) was implanted into the N.Acc. (AP: +1.5 mm and L: −1.5 mm from the bregma; V: −7.0 mm from the surface of the skull) according to the atlas of Paxinos and Watson (1998). The guide cannula was fixed to the skull with cranioplastic cement.

Microinjection and *In Vivo* Microdialysis

At 2–3 days after surgery, the animals were anesthetized with diethyl ether and injected with dynorphin A antibody (1 : 100) diluted in saline or saline alone in a volume of 2.0 μl/rat into the N.Acc. via a guide cannula using a Hamilton syringe at an infusion rate of 1.0 μl/min. These infusions were delivered over 2 min and the rats were returned to their home cages after microinjection. At 1 h after microinjection, microdialysis probes (AI-8-2; 2 mm membrane length, Eicom Co.) were slowly inserted into the N.Acc. through the guide cannulas under anesthesia with diethyl ether, and rats were awakened and placed in the

experimental cages (width 30 cm × depth 30 cm × height 30 cm). The probes were perfused continuously at a flow rate of 2.0 µl/min with artificial cerebrospinal fluid (aCSF) containing 147.0 mM NaCl, 4.0 mM KCl, and 2.3 mM $CaCl_2$ (pH 5.4). Outflow fractions were taken every 20 min. After three baseline fractions were collected, rats were treated with i.p. injection of morphine (8 mg/kg) or saline (1 ml/kg). For these experiments, dialysis samples were collected for 200 min after treatment with morphine or saline. Dialysis fractions were then analyzed using HPLC (Eicom Co.) with an ECD (Eicom Co.) system. The animals were killed by decapitation under sodium pentobarbital anesthesia at the end of the experiments. The brains were removed and the localization of the probes was confirmed by staining with Cresyl Violet.

Quantification of Dopamine and Its Major Metabolites

DA was separated by a column with a mobile phase containing sodium acetate (3.95 g/l), citric acid monohydrate (7.18 g/l), sodium 1-octane sulfonate (140 mg/l), EDTA (2 Na; 5 mg/l), and 17% methanol. The mobile phase was delivered at a flow rate of 0.21 ml/min. DA, DOPAC, and HVA were identified according to the retention times of the standard, and amounts were quantified by calculating peak heights.

Drugs

The drugs used in the present study were formaldehyde solution (Wako Pure Chemical Ind., Ltd, Osaka, Japan), morphine hydrochloride (Sankyo Co., Tokyo, Japan), and dynorphin A antibody (1-17) (Phoenix Pharmaceuticals, Inc., Mountain View, CA, USA). The specificity of this antisera has been determined by radioimmunoassay or ELISA. Briefly, this antibody did not show any cross-reactivities with endomorphin-1, endomorphin-2, Met-enkephalin, Leu-enkephalin-Lys, β-endorphin or β-neoendorphin (Tseng *et al*, 2000). Nor-BNI was synthesized by Dr Nagase (Toray Ind., Ltd, Kanagawa, Japan). All drugs were dissolved in sterile saline.

Statistical Analysis

Baseline microdialysis data were calculated as concentrations in the dialysates. Other microdialysis data were expressed as percentages of the corresponding baseline level. The data are expressed as the mean with SEM. The statistical analyses were performed using one-way and two-way analyses of variance with the Bonferroni/Dunn test.

RESULTS

The formalin-treated paws remained significantly swollen for 11 days ($F(1,10) = 511.54$, $p < 0.001$, Figure 1a). Furthermore, mechanical hyperalgesia in the formalin-treated paw persisted for 9 days after formalin injection ($F(1,16) = 14.94$, $p < 0.01$, Figure 1b). The time course of swelling paralleled that of the paw withdrawal threshold.

Under these conditions, we investigated whether the inflammatory pain-like state following formalin injection could affect the rewarding effect produced by morphine.

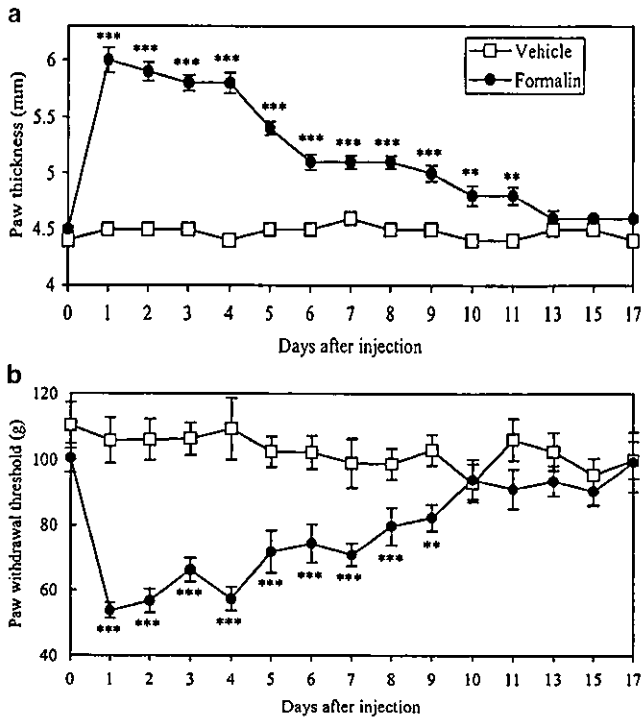

Figure 1 Time-course change in paw thickness (a) and withdrawal threshold (b) after the injection of formalin. Formalin-treated paws remained significantly swollen for 11 days. Furthermore, the pressure threshold in formalin-treated paws was markedly attenuated for 9 days. Each point represents the mean with SEM for 6 to 12 rats. $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* vehicle-treated group.

The i.p. injection of morphine produced a dose-related place preference in rats that had been treated with vehicle into the hind paw ($F(3,30) = 5.89$, $p < 0.01$, Figure 2a). This effect was significantly suppressed under the inflammatory pain-like state following formalin injection ($F(1,15) = 5.11$, $p < 0.05$, Figure 2a). In contrast, s.c. pretreatment with nor-BNI (5 mg/kg) dramatically reversed the suppression of the morphine (8 mg/kg)-induced place preference in rats that had been treated with formalin ($F(1,14) = 4.89$, $p < 0.05$, Figure 2b).

Since it is generally accepted that activation of the mesolimbic DAergic system is critically linked to the expression of the rewarding effect of morphine, we next investigated whether the suppression of the rewarding effect induced by morphine in rats treated with formalin could result from the changes in DA turnover in the limbic forebrain region including the N.Acc. The contents of DA and its metabolites in the rat limbic forebrain and striatum are shown in Table 1. None of the vehicle- or formalin-treated rat brain tissues exhibited a significant difference in the contents of DA and its metabolites. The DA turnover in the limbic forebrain and striatum was significantly increased by i.p. injection of morphine (limbic forebrain; $F(1,10) = 132.77$, $p < 0.001$, striatum; $F(1,10) = 158.45$, $p < 0.001$, Figure 3a, b). The morphine-induced increase in DA turnover in the limbic forebrain region, but not the striatum, was significantly suppressed in formalin-treated rats (limbic forebrain; $F(1,10) = 5.50$, $p < 0.05$, striatum; $F(1,10) = 0.09$, NS, Figure 3a, b). Interestingly, this

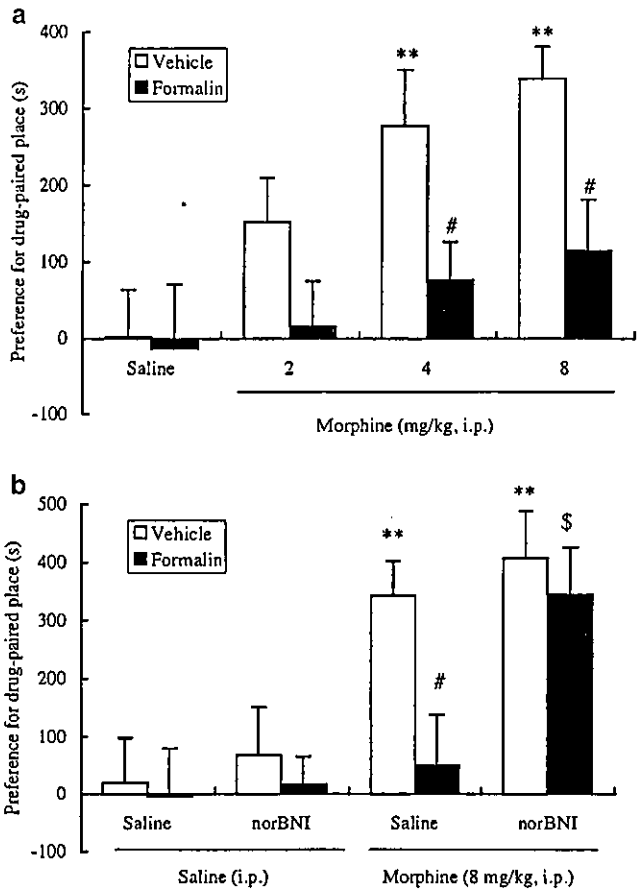

Figure 2 Effect of pretreatment with nor-BNI on the suppression of the place preference induced by morphine (2–8 mg/kg, i.p.) under an inflammatory pain-like state. (a) The morphine-induced rewarding effect was suppressed in rats treated with formalin. (b) The suppression of the morphine-induced rewarding effect by formalin injection was reversed by s.c. pretreatment with nor-BNI (5 mg/kg). The ordinate represents the preference for the drug-paired place. Each column represents the mean with SEM for 8 to 10 rats. $^{**}p<0.01$ *vs* Vehicle/Saline/Saline group, $^{\#}p<0.05$ *vs* Vehicle/Saline/Morphine group. $^{\$}p<0.05$ *vs* Formalin/Saline/Morphine group.

Table 1 Levels of Dopamine (DA) and Its Major Metabolites (DOPAC and HVA) in the Limbic Forebrain and Striatum of Rats Treated with Morphine or nor-BNI (5 mg/kg) Following the Injection of Vehicle or Formalin

	Contents (ng/g of wet tissue)		
	DA	DOPAC	HVA
Limbicfore brain			
Saline (i.p.)			
Vehicle+Saline	9192.5±771.6	1030.3±73.4	694.0±99.3
Vehicle+nor-BNI	6561.3±412.9	846.9±50.1	533.3±43.9
Formalin+Saline	8683.7±52.1	998.2±29.2	678.7±31.3
Formalin+nor-BNI	5763.9±367.9	635.9±53.0	445.5±27.2
Morphine (8 mg/kg i.p.)			
Vehicle+Saline	9649.9±419.8	2011.0±157.0	1486.7±82.3
Vehicle+nor-BNI	10791.7±370.0	2068.1±99.4	1566.9±77.1
Formalin+Saline	8635.1±684.6	1358.8±54.6	1030.3±38.6
Formalin+nor-BNI	10591.2±283.2	2037.3±89.3	1484.9±48.2
Striatum			
Saline (i.p.)			
Vehicle+Saline	25694.7±962.0	2158.4±117.5	1553.9±84.5
Vehicle+nor-BNI	19070.5±328.9	1657.5±22.5	1395.0±78.9
Formalin+Saline	26596.1±998.0	2158.8±102.1	1570.6±69.6
Formalin+nor-BNI	19514.1±1501.6	1537.2±163.1	1371.9±139.6
Morphine (8 mg/kg i.p.)			
Vehicle+Saline	28827.1±1162.3	3537.9±176.1	3174.9±102.3
Vehicle+nor-BNI	29385.3±686.0	3532.3±139.0	3089.8±73.6
Formalin+Saline	28537.6±1243.5	3496.5±208.0	3408.9±300.3
Formalin+nor-BNI	29499.6±1527.5	3628.6±182.1	3238.3±138.7

Each value represents the mean with SEM of 6 to 8 rats.

suppression of morphine-induced DA turnover in formalin-treated rats was significantly reversed by pretreatment with 5 mg/kg of nor-BNI ($F(1,10)=14.98$, $p<0.01$, Figure 3a).

We next demonstrated using *in vivo* microdialysis studies whether the κ-opioidergic system was responsible for suppression of the morphine-induced activation of mesolimbic DA neurons under an inflammatory pain-like state. Figure 4 shows the placement of microdialysis probes within the N.Acc. Regions that contained probes were localized in the N.Acc. Only data from rats in which probes had been accurately inserted in the N.Acc. were used for subsequent statistical analysis. There were no differences in levels of DA, DOPAC, and HVA among the groups (Table 2). The extracellular level of DA in the N.Acc. was markedly increased by i.p. injection of morphine (8 mg/kg) in the vehicle-treated control group (Vehicle/Saline/Saline group *vs* Vehicle/Saline/Morphine group, $F(1,84)=15.61$, $p<0.001$, Figure 5), whereas it was significantly decreased by pretreatment with formalin in the hind paw (Vehicle/Saline/Morphine group *vs* Formalin/Saline/Morphine group, $F(1,96)=25.53$, $p<0.001$, Figure 5). Microinjection of the specific antibody to dynorphin A into the N.Acc. did not affect the morphine-induced increase in the extracellular level of DA in the vehicle-treated control group. Under these conditions, the suppression of the morphine-induced increase in the extracellular level of DA in the N.Acc. produced by intraplantar injection of formalin was almost reversed by the microinjection of dynorphin A antibody into the N.Acc. (Formalin/Saline/Morphine group *vs* Formalin/Dynorphin A antibody/Morphine group, $F(1,96)=19.61$, $p<0.01$, Figure 5).

The i.p. injection of morphine also produced a significant increase in the major DA metabolites DOPAC (Vehicle/Saline/Saline group *vs* Vehicle/Saline/Morphine group, $F(1,84)=41.69$, $p<0.001$, Figure 6a) and HVA (Vehicle/Saline/Saline group *vs* Vehicle/Saline/Morphine group, $F(1,94)=93.79$, $p<0.001$, Figure 6b) in the vehicle-treated group. In contrast, these effects were significantly attenuated in rats that had been treated with formalin (DOPAC; Vehicle/Saline/Morphine group *vs* Formalin/Saline/

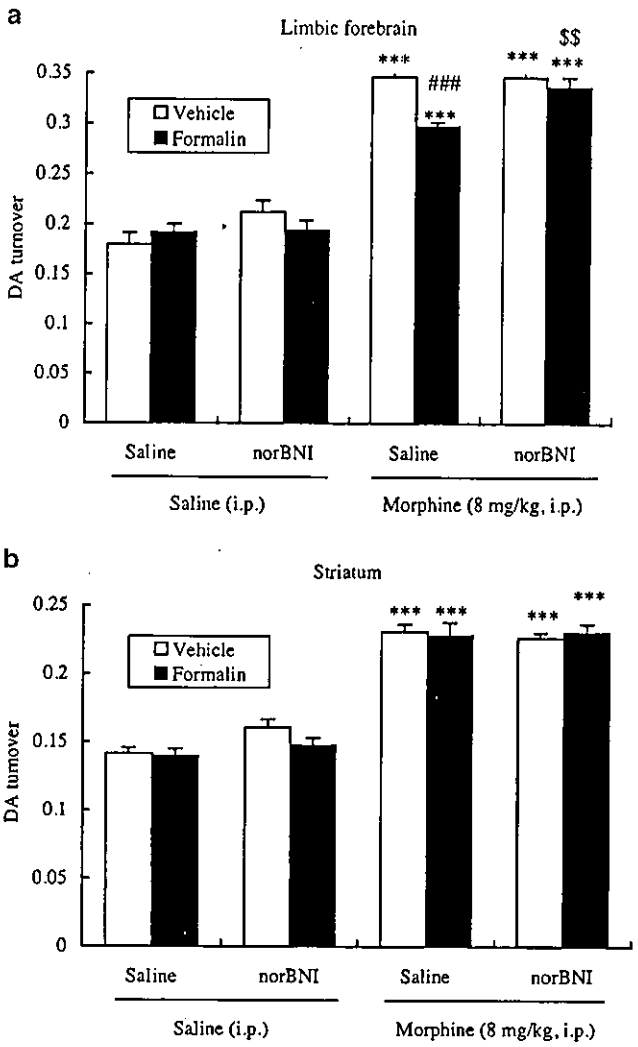

Figure 3 Effect of pretreatment with nor-BNI (5 mg/kg, s.c.) on the suppression of DA turnover induced by morphine (8 mg/kg, i.p.) under an inflammatory pain-like state. The morphine-induced increase in DA turnover in the limbic forebrain (a), but not the striatum (b), was suppressed in rats that had been treated with formalin. The suppression of morphine-induced DA turnover by formalin injection was reversed by s.c. pretreatment with nor-BNI. Each column represents the mean with SEM of 6–8 rats. The DA turnover (DA ratio) was calculated as (DOPAC + HVA)/DA. $^{***}p<0.001$ *vs* Vehicle/Saline or nor-BNI/Saline group, $^{###}p<0.001$ Vehicle/Saline/Morphine group *vs* Formalin/Saline/Morphine group, $^{\$\$}p<0.01$ Formalin/Saline/Morphine group *vs* Formalin/nor-BNI/Morphine group.

Morphine group, $F(1,84)=41.69$, $p<0.001$, Figure 6a; HVA; Vehicle/Saline/Morphine group *vs* Formalin/Saline/Morphine group, $F(1,96)=54.20$, $p<0.001$, Figure 6b). Furthermore, the suppression of the morphine-induced increase in the extracellular level of DA metabolites in the N.Acc. in rats that had been treated with formalin was almost reversed by the intra-N.Acc. injection of dynorphin A antibody (DOPAC; Formalin/Saline/Morphine group *vs* Formalin/Dynorphin A antibody/Morphine group, $F(1,84)=8.69$, $p<0.05$, Figure 6a; HVA; Formalin/Saline/Morphine group *vs* Formalin/Dynorphin A antibody/Morphine group, $F(1,108)=25.53$, $p<0.001$, Figure 6b).

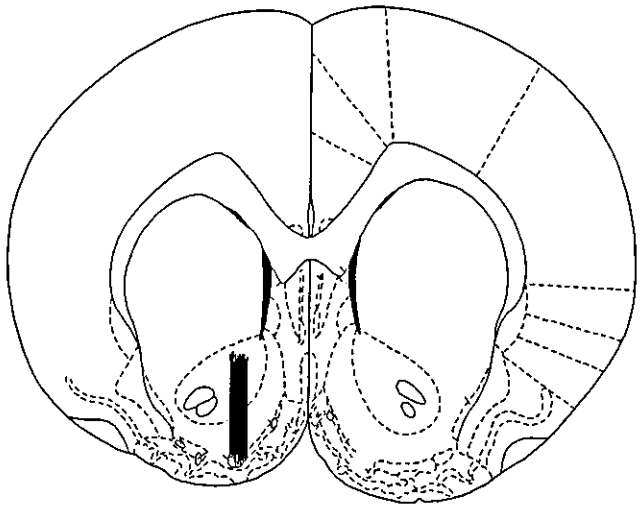

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Figure 4 Localization of microdialysis probes in the N.Acc. in rats. Stippled lines represent regions in the rat brain that contained probes. The schematic brain sections are from the atlas of Paxinos and Watson (1998).

Table 2 Basal Dialysate Levels of Dopamine (DA) and Its Major Metabolites (DOPAC and HVA) in the N.Acc. of Vehicle- or Formalin-Treated Rats

Group	DA (nM)	DOPAC (nM)	HVA (nM)
Vehicle/Saline/Saline	1.14±0.22	258.33±51.96	230.09±34.73
Vehicle/Saline/Morphine	1.13±0.11	289.22±39.77	210.04±26.16
Formalin/Saline/Saline	1.42±0.39	236.08±70.58	191.97±51.56
Formalin/Saline/Morphine	1.22±0.28	297.74±57.86	196.50±30.62
Vehicle/Anti-Dyn A/Morphine	1.07±0.15	278.84±49.56	248.03±33.42
Formalin/Anti-Dyn A/Morphine	1.14±0.34	310.07±71.68	237.95±47.85

Each value represents the mean with SEM of 6 to 8 rats.

DISCUSSION

Many studies have pointed to the mesolimbic DAergic system, projecting from the VTA of the midbrain to the N.Acc., as the critical substrate of the rewarding effect of morphine (Koob, 1992; Nestler, 1996; Narita *et al*, 2001). μ-Opioid receptor agonists have been shown to increase DAergic signals in the N.Acc. via the activation of DA cells in the VTA, an area that possesses high densities of μ-opioid receptors (Garzon and Pickel, 2001). We reported previously that the enhancement of DA turnover in the mesolimbic forebrain area containing the N.Acc. induced by morphine was associated with the expression of a rewarding effect by morphine (Funada *et al*, 1993; Narita *et al*, 2001). In the present study, we found that the injection of formalin into the plantar surface of the hind paw suppressed both the rewarding effect and activation of the mesolimbic DA system induced by morphine in rats. These results suggest that the mesolimbic DA system may not be facilitated by μ-opioid receptor agonists under an inflammatory pain-like state.

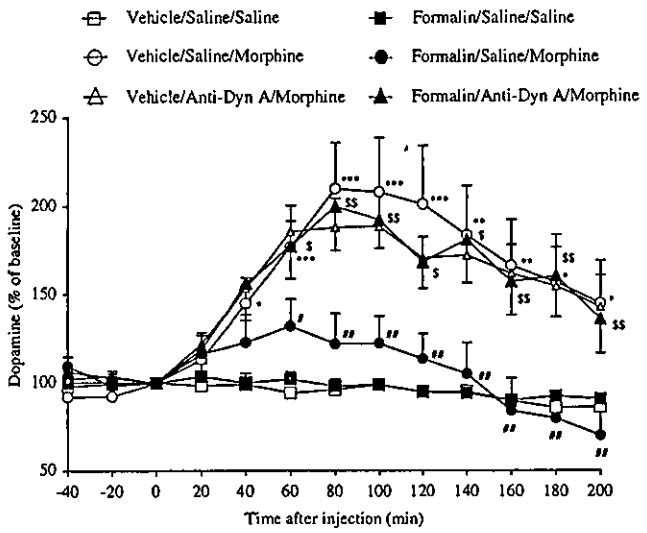

Figure 5 Effect of pretreatment with an antibody specific to dynorphin A (Anti-Dyn A) into the N.Acc. on the extracellular level of dopamine in the N.Acc. induced by morphine (8 mg/kg, i.p.) under an inflammatory pain-like state. The morphine-induced increase in dopamine release in the N.Acc. was suppressed in rats that had been treated with formalin. The suppression of the morphine-induced dopamine release by formalin injection was reversed by the intra-N.Acc. injection of Anti-Dyn A. Data are expressed as a percentage of the corresponding baseline levels with SEM of 4 to 5 rats. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ Vehicle/Saline/Saline group *vs* Vehicle/Saline/Morphine group. $^{\#}p<0.05$, $^{\#\#}p<0.01$ Vehicle/Saline/Morphine group *vs* Formalin/Saline/Morphine group. $^{\$}p<0.05$, $^{\$\$}p<0.01$ Formalin/Saline/Morphine group *vs* Formalin/Anti-Dyn A/Morphine group.

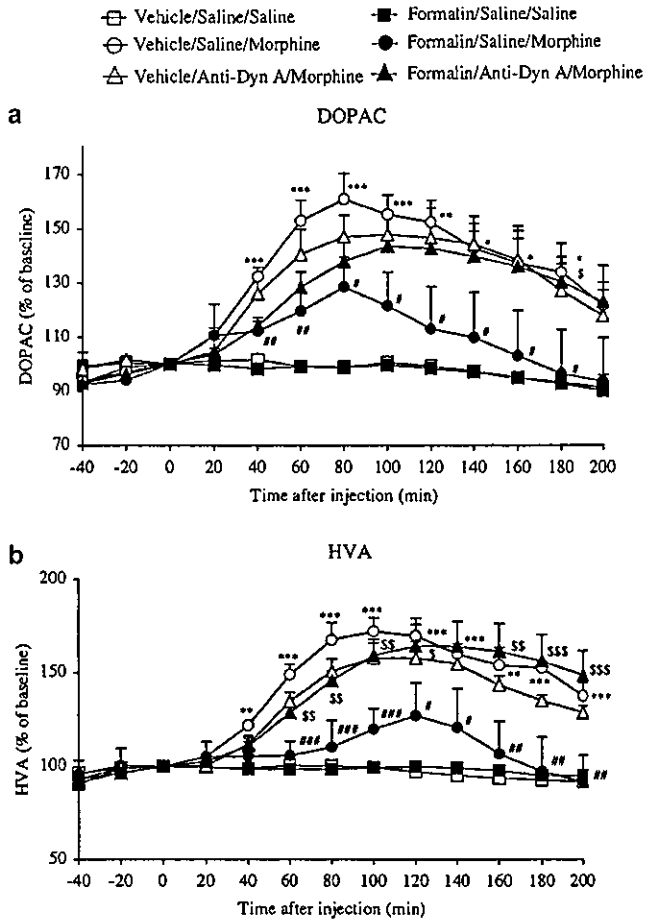

Figure 6 Effect of pretreatment with an antibody specific to dynorphin A (Anti-Dyn A) into the N.Acc. on the extracellular levels of DOPAC (a) and HVA (b) in the N.Acc. induced by morphine (8 mg/kg, i.p.) under an inflammatory pain-like state. The morphine-induced increase in DOPAC and HVA in the N.Acc. was suppressed in rats that had been treated with formalin. The suppression of the morphine-induced increase in the extracellular levels of DOPAC and HVA was reversed by the intra-N.Acc. injection of Anti-Dyn A in rats that had been treated with formalin. Data are expressed as a percentage of the corresponding baseline levels with SEM of 4 to 5 rats. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ Vehicle/Saline/Saline group *vs* Vehicle/Saline/Morphine group. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, Vehicle/Saline/Morphine group *vs* Formalin/Saline/Morphine group. $^{\$}p<0.05$, $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$, Formalin/Saline/Morphine group *vs* Formalin/Anti-Dyn A/Morphine group.

In a previous study, we observed that intra-VTA microinjection of the selective μ-opioid receptor agonist [D-Ala^2,NMPhe^4,Gly(ol)^5]enkephalin (DAMGO) produced a significant decrease in the extracellular γ-amino butyric acid (GABA) level in the VTA, indicating that disinhibition may be the key role of μ-opioid receptor-mediated function in the VTA. This contention can be supported by the finding that morphine and DAMGO each inhibits the firing frequency of non-DA cells in the VTA (Johnson and North, 1992; Bonci and Williams, 1997). These findings strongly suggest that the activation of μ-opioid receptor in the VTA may facilitate the mesolimbic DA system through the inhibition of GABAergic neurotransmission in the VTA, resulting in the induction of a rewarding effect induced by μ-opioid receptor agonists. Considering this background, we first hypothesized that either GABAergic or μ-opioidergic systems in the VTA could be altered by formalin injection. However, this is not a major effect, since the baseline levels of DA and its metabolites in the N.Acc. were not affected at all under an inflammatory pain-like state. These findings support the idea that other modulatory systems linked to the mesolimbic DA system might be altered under an inflammatory pain-like state.

Several lines of evidence indicate that the κ-opioidergic system negatively modulates the DA-related actions mediated by μ-opioid receptors (Funada *et al*, 1993; Narita *et al*, 1993, 2001). We found previously that pretreatment with the κ-opioid receptor agonist U50,488H at a dose that alone did not produce place aversion suppressed both the place preference and the elevation of DA metabolites in the rat limbic forebrain produced by morphine (Funada *et al*, 1993; Narita *et al*, 2001). In addition, the increase in the extracellular level of DA produced by morphine was attenuated by the microinjection of κ-opioid receptor agonist into the N.Acc., but not into the VTA (Spanagel *et al*, 1992), implying that the κ-opioidergic system within the N.Acc. may play an important role in the negative modulation of the mesolimbic DA-dependent motivational effect produced by μ-opioid receptor agonists.

Previous studies have demonstrated that the levels of dynorphin and pre-prodynorphin mRNA in some brain regions are increased under inflammatory pain-like states induced by the injection of carrageenan and complete Freund's adjuvant (Millan *et al*, 1987; Dubner and Ruda, 1992). These findings suggest that a state of chronic pain could lead to physiological changes in κ-opioidergic neurotransmission in the brain. In the present study, we

found that the elevation of DA turnover in the limbic forebrain induced by morphine was significantly suppressed by formalin injection and this effect was reversed by the selective κ-opioid receptor antagonist nor-BNI. In addition, the suppression of the morphine-induced increase in the extracellular levels of DA, DOPAC, and HVA in the N.Acc. following formalin injection was also reversed by the microinjection of an antibody specific to dynorphin A into the N.Acc. of rats that had been treated with formalin. Dynorphin A antibody failed to affect the basal level of DA in the N.Acc. following formalin injection, implying that this antibody has a specific effect on the morphine-induced increase in the extracellular levels of DA in the N.Acc. The injection of formalin did not directly affect the basal levels of DA, DOPAC, and HVA. These results support the fascinating possibility that the mild or moderate increase in the release of dynorphin A from κ-opioidergic terminals within the N.Acc. under an inflammatory pain-like state may not affect the basal state of the mesolimbic DA system. However, it may inhibit the overshooting of the mesolimbic DA system facilitated by morphine, resulting in suppression of the morphine-induced rewarding effect (Figure 7).

Endogenous μ- and κ-opioidergic systems can be physiologically balanced under a normal state. After morphine treatment, μ-opioidergic systems are superfluously excited, which may in turn upset the balance of the endogenous μ- and κ-opioidergic systems. This disruption may lead to the development of psychological dependence on morphine under a normal state. On the other hand, inflammatory pain may facilitate the endogenous κ-opioidergic system within the N.Acc., leading to negative modulation of the mesolimbic DA system. Under this condition, the biased balance of endogenous μ- and κ-opioidergic systems due to inflammation may actually be improved by morphine treatment. Therefore, psychological dependence on morphine may not be developed under an inflammatory pain-like state (Figure 8).

In conclusion, we demonstrated here that both the rewarding effect and facilitation of the mesolimbic DA system produced by morphine were significantly suppressed in rats that had been treated with formalin. These effects were reversed by blockade of the endogenous κ-opioidergic system in the N.Acc. These findings raise the possibility that an inflammatory pain-like state may cause a

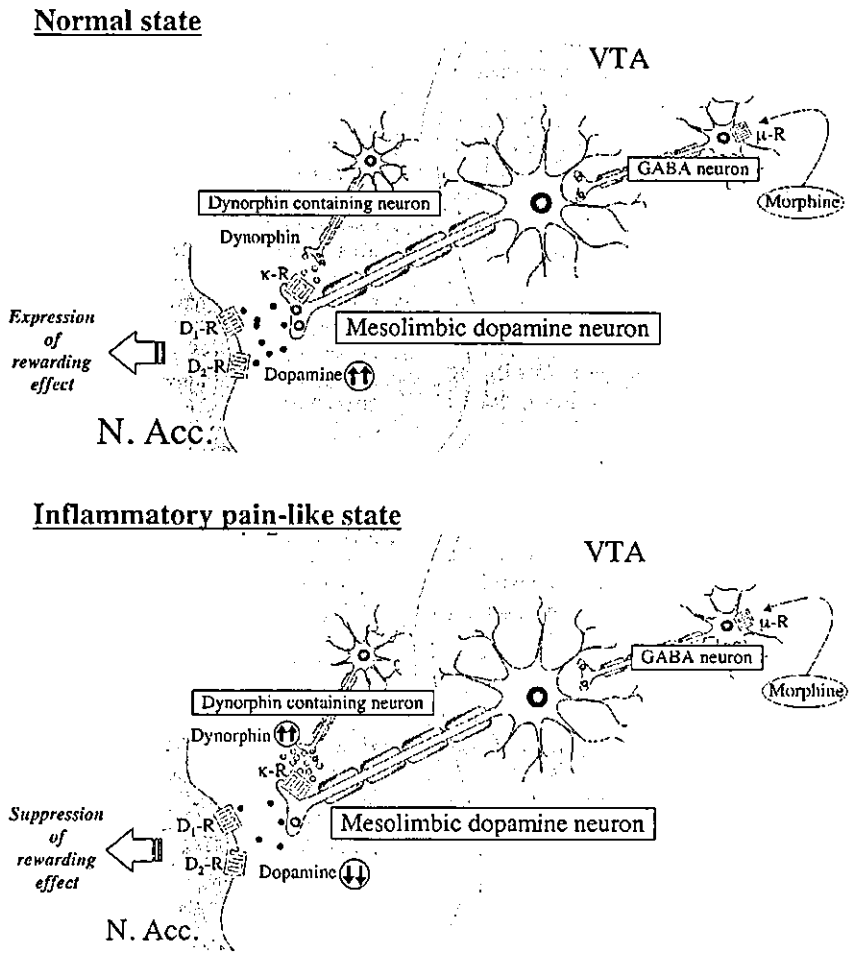

Figure 7 A schematic drawing of the suppression of the rewarding effect produced by morphine under an inflammatory pain-like state. In a normal state, morphine produces an increase in dopamine release in the N.Acc. through disinhibition of the GABAergic system in the VTA, resulting in the expression of a rewarding effect. In contrast, the morphine-induced rewarding effect is suppressed under an inflammatory pain-like state, due to the inhibition of dopamine release at dopaminergic terminals through facilitation of the endogenous κ-opioidergic system within the N.Acc.

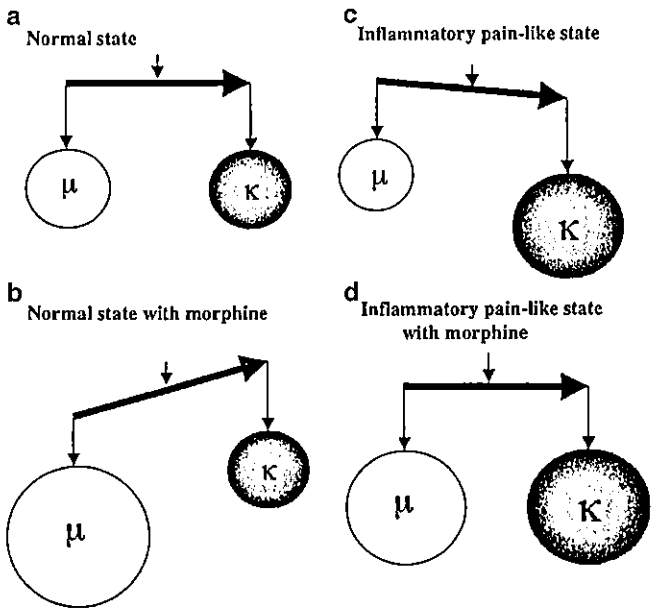

Figure 8 A schematic drawing of the presumed physiological balance between the endogenous μ- and κ-opioidergic systems within the mesolimbic dopaminergic system under normal and inflammatory pain-like states. (a) Endogenous μ- and κ-opioidergic systems may be balanced under a normal state. (b) Since μ-opioidergic systems are superfluously facilitated by morphine, psychological dependence on morphine develops. (c) On the other hand, inflammatory pain may cause a marked activation of the endogenous κ-opioidergic system. (d) Several lines of evidence have demonstrated that activation of the κ-opioidergic system suppresses the morphine-induced rewarding effect in rodents. Thus, we propose that psychological dependence on morphine may not develop under a state of inflammatory pain due to the activation of endogenous κ-opioidergic systems. Therefore, adequate treatment of patients with morphine is highly recommended for the relief of severe chronic pain.

sustained facilitation of the κ-opioidergic system within the N.Acc., resulting in suppression of the morphine-induced rewarding effect in rats.

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特集

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オピオイドローテーション

—その定義と考え方—

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はじめに

がん患者の80％以上は，オピオイドによる鎮痛を要する疼痛を経験する．海外では，表1に示すように多数のオピオイド製剤が発売され，がんの疼痛治療に使用されている．

これに対して，わが国でがん疼痛治療に対してこれまで使用されてきたオピオイドは，主としてモルヒネ製剤であり，その他の選択肢はコデイン，ブプレノルフィン（レペタン®），ペチジン（オピスタン®）に限られてきた．コデインは弱オピオイドであり，その鎮痛効果はモルヒネの1/5～1/10とされ，有効限界がある．また，強オピオイドであるブプレノルフィンには坐剤と注射剤しかなく，有効限界もあるためモルヒネの代替薬とすることは困難であった．そのため，日本のがん疼痛治療はモルヒネ偏重とならざるをえない状況であった．しかし，最近，経皮吸収型フェンタニルパッチ（デュロテップ®）が発売され，さらにオキシコドンも導入の可能性があることから，これまで

表1 がん疼痛治療に用いられる強オピオイド

薬剤		日本	イギリス	アメリカ	カナダ
morphine		○	○	○	○
hydromorphone	半合成オピオイド．モルヒネ不応性や耐性を生じた患者のモルヒネ代替薬			○	○
diamorphine			○		
oxycodone	半合成オピオイド		○	○	○
levolphanol				○	
methadone	phenylheptylamine 系合成オピオイド NMDA 受容体アンタゴニスト		○	○	○
fentanyl	phenylpiperidine 系合成オピオイド	○	○	○	○

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表2　オピオイドの反応に乏しい患者へのアプローチ[2]

1．副作用に対して積極的に治療する→鎮静に対して精神刺激薬など
2．より副作用と鎮痛のバランスのとれたオピオイドを探す→オピオイドの変更
3．薬理学的手法でオピオイドの全身投与の必要量を減らす→鎮痛補助薬，神経ブロックなど
4．非薬理学的手法でオピオイドの必要量を減らす→経皮的神経刺激など

のモルヒネのみに頼らざるをえなかったオピオイド使用法が変わる可能性が出てきた．

近年，海外の緩和ケア専門医の間では，がん疼痛治療の方法として「オピオイドローテーション(opioid rotation)」という概念が出てきている．そこで，オピオイドローテーションとはどのような概念なのか，海外における現状と，今後の日本での応用に関して考察を行いたい．

オピオイドの反応性

「オピオイドへの反応性（opioid responsiveness)」とは，鎮痛と副作用の好ましいバランスがとれるようにオピオイド量の調整ができるか否かということである．10〜30％のがん疼痛を持つ患者ではオピオイドへの反応が悪いとの報告もみられる[1]．モルヒネの副作用が強く現れるモルヒネ不耐性，神経因性疼痛など鎮痛剤に反応しにくい痛み，腎障害，脱水など活性代謝産物の蓄積が起きやすいなどがその要因となる．オピオイドへの反応は，個々の患者において差があり，一つのオピオイドに反応が悪い患者が，他のオピオイド治療すべてに反応が悪いというわけではない[2]．

疼痛治療では，オピオイドへの反応が悪い症例にどう対応するかは大きな問題となる．Portenoyら[1,2]は，表2に示すようなアプローチを提示している．

オピオイドローテーション

Mercadante[3]はオピオイドローテーションについて，「一つのオピオイドをより好ましい反応を得るために他のオピオイドに置換すること」と定義している．文献上では，rotationという単語が広く受け入れられているが，opioid changeという言葉が使われたり[4]，substitutionを好むとする著者もあり[5]，いまだ統一はされていない．

海外では，表1に示すように多数のオピオイド製剤が発売されている．これらの強オピオイドの中でも，後に述べるように，メサドン（methadone）がオピオイドローテーションに重要な役割を持っている．

オピオイドローテーションの適応

オピオイドローテーションの適応には，疼痛コントロールが不良であり，①オピオイドの毒性による強い副作用がある，②急速な耐性の出現がみられる，③難治な疼痛がある，などが挙げられる[3]．オピオイドローテーションの必要は40％前後の患者に生じ，その効果は約70％で得られるとされ，オピオイドローテーションを行った理由の内訳は，認知障害39％，幻覚症24％，疼痛コントロール不良16％，ミオクローヌス11％，嘔気9％との報告がある[6]．

オピオイド変更の薬理学的背景

オピオイドの中でも，強オピオイドはその作用する受容体，および生物活性により，得られる鎮痛効果，副作用には差があり，一つのオピオイドで耐性が出たからといって，すべてのオピオイドで耐性がみられるわけではない．

オピオイド受容体（特にμ受容体）への親和性，薬効を示すのに要する受容体占拠率などが，強オピオイド間に不完全交差耐性が存在する要因ともされている．また，オピオイドへの反応性には，オピオイド受容体の遺伝的要因も含め，患者個々の要素が強く反映しており，それぞれの症例に応じたアセスメントが必要である[3]．

表3　モルヒネ代謝産物の毒性による症状[3]

morphine-6-glucuronide (M6G)	morphine-3-glucuronide (M3G)
傾眠	認知障害
嘔気，嘔吐	ミオクローヌス，けいれん
昏睡	痛覚過敏
呼吸抑制	

1 モルヒネの代謝産物とその毒性

モルヒネは，オピオイド受容体（主としてμ受容体）に結合し，鎮痛作用など多彩な作用を発現する．経口モルヒネ製剤の生体内利用率は，約25％である．吸収されたモルヒネは主として肝臓と小腸粘膜で代謝され，多くはグルクロン酸抱合体になる．

鎮痛効果は，モルヒネと代謝産物の比が関与する．代謝産物としてはmorphine-3-glucuronide（M3G）とmorphine-6-glucuronide（M6G）が知られている．吸収されたモルヒネの約45％がM3Gに，10％がM6Gになるとされる．M6Gはモルヒネの活性代謝産物で，鎮痛作用をもつ．しかし，M3Gはモルヒネの主たる代謝産物ではあるが，オピオイド受容体に対する親和性は低く，鎮痛効果もないと考えられている．M6Gの腎からの排泄はモルヒネより遅く，腎機能障害患者においてはM6Gの蓄積が起こり，その毒性が遷延するとされている．また，M3Gが認知障害など中枢神経作用を起こす可能性が示唆されている（表3）．

2 フェンタニル

フェンタニルはphenylpiperidine系の合成オピオイドである．主としてμアゴニストであり，鎮痛作用はモルヒネの50～200倍の強さを持つといわれる．薬理作用はモルヒネとほぼ同等だが，鎮静効果は少ない．吸収されたフェンタニルの大部分は肝臓で代謝され，不活性な形で尿中に排泄される．フェンタニルの薬物動態は，肝硬変の患者でも健常者と差がない．

3 hydromorphone

半合成オピオイド鎮痛薬である．μアゴニストであり，特に侵害受容性疼痛に対する鎮痛作用をもつ．経口投与による生体内利用率は37～62％で，モルヒネより高い．力価がモルヒネの5～6倍と高いので，大量のオピオイドを必要とする患者の持続皮下投与に適している．また，モルヒネ不応性や，耐性を生じた患者のモルヒネ代替薬としても利用される．

4 オキシコドン

半合成オピオイド鎮痛薬である．μアゴニストとして鎮痛作用をもたらす．中等度以上の疼痛に用いられ，副作用はモルヒネに類似する．アセトアミノフェンやアスピリンとの合剤では，含まれる非オピオイド鎮痛薬の量が服用の極量を決定したが，単剤の徐放製剤が開発され，モルヒネと同等の効果が証明されている．

5 メサドン

メサドンはphenylheptylamine系薬剤である．依存を起こすが，作用持続時間が長いので，禁断症状は軽く，これを利用してヘロインなどの依存症の治療に使われてきた．メサドンは，ほぼ完全に腸管から吸収され，個人差が大きいモルヒネと比べ，生体内利用率が約80％と高い．活性代謝産物がなく，主として腸管から排泄されるため，肝疾患や腎疾患によるクリアランスの影響を受けない．タンパク結合率が高く，組織に蓄積しやすいため，特に高齢者に置いて累積毒性が問題となる．

μ受容体に対するアゴニストであるだけではなく，中枢神経系に広く存在し，興奮性伝達物質であるグルタミン酸の受容体のひとつ，*N*-methyl-D-aspartate（NMDA）受容体アンタゴニストでもある．NMDA受容体アンタゴニストとしては，このほかにケタミンが知られており，神経因性疼痛に対する鎮痛補助薬として使われる．メサドンは，NMDA受容体アンタゴニストとして，他のオピオイドに反応しにくい神経因性疼痛にも鎮痛効

果が得られる[5,7,8].

また，メサドンは非常にコストが安く(表4)[9]，活性代謝産物がなく，他のオピオイドと比べて耐性が生じにくいことから，将来first lineのオピオイドとして使われる可能性も出てきている[7]．緩和ケアで使用するオピオイドとしてのメサドンの特性を表5に示す[10].

オピオイドローテーションの実際

オピオイドローテーションの際は，オピオイドの力価の換算表をもとに計算して行う（表6～8）[1,11]．先に述べたように，オピオイドの耐性には個人差があり，さらにオピオイド間の不完全交差耐性も存在するため，換算量そのままではなく，減量して開始することが必要である．

表4　オピオイドの値段[9]

	カナダドル($)	日本円換算(¥)
diamorphine	0.50/8 mg	40
morphine	0.17/10 mg	13.6
hydromorphone	0.64/2 mg	51.2
methadone	0.04/10 mg	3.2

オピオイドローテーションの文献的考察

疼痛治療の手段としてのオピオイドローテーションは，欧米から多く報告がみられる．Bruera ら[12]は，強オピオイドを使用する際の考え方として以下の3点を挙げている．

①痛みのコントロールがうまくいかなかったり，副作用が現れた場合，オピオイドの変更を考える．

②鎮痛補助薬を使用する前にオピオイドの至適量を決めるべきである．

③可能なら多剤投与を避けるべきである．

オピオイドローテーションの考えが広まった背景には，Bruera のような考え方，すなわち，「鎮痛補助薬を使用する前に，オピオイドを増量，変更していく」という考え方があるかと思われる．実際，オピオイドローテーションを多数症例で検

表5　メサドンの特徴[10]

利　点	欠　点
・経口，経直腸，経静脈ルートがつかえ，経口で良好な生体内利用率が得られる ・知られている活性代謝産物がない ・安価である ・NMDA受容体のアンタゴニストであり，オピオイド抵抗性の疼痛や神経因性疼痛に有用である ・投薬間隔が長い ・便秘はゆっくりと出現する	・皮下ルートでは局所反応が強い ・半減期が長く，個人差が大きい ・それまでに使用したオピオイドによってメサドンと他のオピオイドの換算比が変わるため，変更が難しい ・元来薬物依存の治療に使われていたため，患者が不名誉に感じる ・第一選択のオピオイドとしては十分な研究がなされていない ・メサドンから他のオピオイドへの換算比は体系づけられていない

表6　10 mgのメサドンに対するオピオイド換算表[9]

	American Psychological Society	Agency for Health Care Policy and Research	最近の報告
methadone	10 mg	10 mg	10 mg
morphine	33 mg	20 mg	77.5 mg
hydromorphone	4.5 mg	3 mg	50 mg
fentanyl	200～400 μg		2000 μg