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ATTENUATION OF THE MORPHINE-INDUCED REWARDING EFFECT UNDER CHRONIC INFLAMMATORY PAIN-LIKE STATE IN RATS: IMPLICATION OF THE ACTIVATION OF ENDOGENOUS κ-OPIOID SYSTEM

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Recent clinical studies have demonstrated that when opioids are used to control pain, tolerance is not a major concern and patients rarely show any withdrawal sign. However, little information is available regarding changes in function at the supraspinal level under chronic pain state. Therefore, the present study was designed to investigate the opioid-induced place preference and the effect on the extracellular dopamine release by opioid in the nucleus accumbens (N.Acc), and its mechanism under inflammatory pain-like state in rats. In the present study, the paw pressure thresholds were significantly decreased maximally on day 1 after 2.5% formalin injection and gradually recovered over at least 11 days, using the Randall-Selitto test. The decrease in paw pressure threshold obviously indicated the development of mechanical hyperalgesia under inflammatory pain-like state. Under inflammatory pain state, we investigated the opioid-induced place preference in rats. Results of the study showed that the opioid-induced place preference was significantly attenuated by chronic inflammation. These findings suggest that pain can suppress the development of psychological dependence on opioids. In the present study, we also demonstrated that morphine-induced extracellular dopamine release in the N.Acc was significantly reduced under inflammatory pain-like state. In order to elucidate the mechanism of this attenuation, the effects of pretreatment with δ- and κ-opioid receptor antagonists, naltrindole (NTI) and nor-binaltorphimine (nor-BNI) on the development of the morphine-induced place preference under chronic inflammatory pain-like state were conducted in rats. We demonstrated that the attenuation of the morphine-induced place preference under chronic inflammatory pain-like state was effectively suppressed by pretreatment with κ-opioid receptor antagonist, nor-BNI but not the δ-opioid receptor antagonist, NTI. In conclusion, the present results suggest that the suppression of the opioid-induced place preference under inflammatory pain-like state may result from the activation of the endogenous κ-opioid systems and may implicate the reduction in extracelullar dopamine release in the N.Acc. These findings imply that psychological dependence is not a major concern for patients receiving opioids and opioid treatment is highly recommended for pain relief.

Keywords: *Opioid, rewarding effect, dopamine release, κ-opioid system, inflammatory pain*

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INTRODUCTION

Opioid receptors are classified into three types, μ-opioid receptor (MOP-R), δ-opioid receptor (DOP-R) and κ-opioid receptor (KOP-R). This classification is based upon pharmacological, behavioural and biochemical studies on opioid. Several investigators have demonstrated that either MOP-R or DOP-R agonists induce place preference, whilst KOP-R agonists induce place aversion (Suzuki *et al.* 1992; Narita *et al.* 1993; Pan 1998; Simonin *et al.* 1998). These opposing motivational effects are paralleled by the increase and decrease, respectively, of dopamine (DA) release in the nucleus accumbens (N.Acc) (Narita *et al.* 1993).

The place-conditioning procedure is used to evaluate motivational properties, such as rewarding or aversive effect of drugs. This was introduced in the early 1980s to compensate the methodological and interpretation difficulties associated with the self-administration technique and the conventional method for assessing reinforcing properties of drugs (Schuster and Johanson 1981). Conditioned place preference (CPP) paradigm has become the most frequently used method for evaluating motivational properties of drugs. Its use has been reported more frequently than the self-administration paradigm (Suzuki 1996).

Evidences suggest that the mesolimbic DA system, which originates in the ventral tegmental area (VTA) and projects to the N.Acc and other forebrain regions, have been identified as the critical part of the rewarding effect of opioids (Di Chiara and North 1992; Koob 1992). Opioid receptor agonists have been shown to increase dopaminergic 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). This activation could have resulted mainly from the inhibition of inhibitory GABAergic interneurons in the VTA. This is clearly supported by the finding that intra-VTA administration of [D-Ala^2, N-MePhe^4, Gly-ol^5]enkephaline (DAMGO) caused a dose-related preference for the drug-associate place. Furthermore, microinjection of DAMGO into the VTA also reduced the release of extracellular γ-aminobutyric acid (GABA) levels in the VTA (Narita *et al.* 2001). These findings strongly suggest that the activation of the μ-opioid receptor in the VTA may facilitate the mesolimbic DA system and increase the extracellular DA levels in the N.Acc through the inhibition of GABAergic neurotransmission in the VTA, resulting in the expression of a place preference.

Various studies provide arguments to support substantial roles for μ-, δ- and κ-opioid receptors, and their interactions in the development of physical and psychological dependence induced by morphine (Narita *et al.* 2001). Previous reports demonstrated that δ-opioid receptor agonists, [D-Pen^2, D-Pen^5]enkephalin and deltorphine II produced a significant place preference, and the morphine-induced place preference is completely blocked by δ-opioid receptor antagonist naltrindole (NTI) (Suzuki *et al.* 1994). In contrast, κ-opioid receptor agonists produce aversive effects, and pretreatment with κ-opioid receptor antagonists, U-50, 488H and E-2070 abolished the morphine-induced place preference (Funada *et al.* 1993). In 1992, we reported for the first time that pretreatment with κ-opioid agonists reduced the morphine-induced elevation of DA metabolites in the limbic forebrain (Suzuki *et al.* 1992).

Recent clinical studies have demonstrated that when opioids are used to control pain, tolerance is not a major concern and patients rarely show any withdrawal signs. It was previously reported that inflammation in rats induced by formalin or carrageenan in the hind paw showed suppression of morphine-induced place preference (Suzuki *et al.* 1996a). Furthermore, we also found that the morphine-induced increase of DA turnover in the limbic forebrain was significantly attenuated under inflammatory condition. These findings raised the possibility that being in a state of pain could lead to physiological changes in neurotransmission at supraspinal levels and that this could be responsible for the suppression of the opioid dependence. However, little information is available regarding changes in function at the supraspinal level under inflammatory pain state. Therefore, the present study was designed to investigate the morphine-induced place preference and the effect of morphine-induced extracellular DA release in the N.Acc under inflammatory pain-like state in rats. To clarify the mechanism of the attenuation of the morphine-induced rewarding effect under inflammatory pain state, we investigated the effects of pretreatment with δ- and κ-opioid receptor antagonists, NTI and nor-binaltorphimine (nor-BNI) respectively, on the morphine-induced place preference in rats.

METHODS

The present study was conducted in accordance with Guiding Principles for the Care and Use of Laboratory Animals Hoshi University, as adopted

by the Committee on Animal Research of Hoshi University. Every effort was made to minimise the number of animals used and their sufferings in the following experiments.

Animals

Male Sprague-Dawley rats (Tokyo Laboratory Animals Science Co. Ltd, Tokyo, Japan) weighing 250—300 g were housed in a room with temperature maintained at 23 ± 1°C under a 12 hr light/dark cycle (light on 08:00–20:00 hr). Food and water were available *ad libitum*.

Induction and measurement of inflammation

Formalin (2.5%, 50 μL) was injected into the plantar surface of the right rat paw. The paw pressure threshold was measured using Randall-Selitto analgesymeter (MODEL TK201, Muromachi Kikai Co. Ltd., Tokyo, Japan). Each rat was gently restrained and increased pressure was applied *via* a wedge-shaped, blunt piston onto the surface of the hind paw by an automated gauge. The hind paw withdrawal or vocalisation was considered to be a nociceptive response. The measurements were performed before induction of inflammation (day 0) and on 1, 3, 5, 7, 9, 11, 14 and 18 days after induction of inflammation.

Effect of inflammation on place preference induced by morphine

Place conditioning was performed according to our previous report (Suzuki *et al.* 1994). The apparatus consisted of a shuttle box (30 x 60 x 30 cm) that was divided into two compartments of equal size. One compartment was white with a textured floor and the other was black with a smooth floor. For conditioning, rats were confined to one compartment after drug injection and to the other compartment after saline injection. The orders of the injection (drug or saline) and the compartment (white or black) were counterbalanced across the subjects.

Conditioning sessions (3 days for drug: 3 days for saline) were started on the day 1 after the induction of inflammation. Immediately after subcutaneous (s.c.) injection of morphine (4 or 8 mg/kg), animals were placed in one compartment for 1 hr. On alternate days, animals receiving saline were placed in the other compartment for 1 hr. On day 7, tests of conditioning were performed as follows: the partition separating the two

compartments was raised to 12 cm above the floor, and the neutral platform was inserted along the seam separating the compartments. The rats were not treated with drugs or saline, and then placed on the platform. The time spent in each compartment during a 900 sec session was then recorded automatically in a blinded fashion using an infrared beam sensor (KN-80, Natsume Seisakusyo Co., Ltd, Tokyo, Japan). All sessions were conducted under the dim illumination (28 lux lamp) and white masking noise.

Effect of inflammation on dopamine release in the N.Acc induced by morphine using in vivo microdialysis study

Stereotaxic surgery was performed under sodium pentobarbital (50 mg/kg, s.c.) anesthesia. Rats were mounted in stereotaxic frame (Stoelting, IL, USA) and the skull was exposed. A small hole was made using a dental drill. Guide cannula (AG-8, Eicom CORP., Kyoto, Japan) was implanted into the N.Acc (A/P + 1.5 mm, L -1.5 mm, V -7.0 mm) according to the atlas of Paxinos and Watson (1998). The guide cannula was fixed to the skull with cranioplastic cement.

Three to five days after the surgery, the microdialysis probe (A-I-8-2; 2 mm membrane length, Eicom) was slowly inserted into the N.Acc through the guide cannula under anesthesia with diethyl ether, and the rats were settled in experimental cages (width 30 cm x ~depth 30 cm x ~ height 30 cm). The rats were habituated for 3–5 hr after the probe insertion. The probe was perfused continuously at a flow rate of 2 μL/min with artificial cerebrospinal fluid (aCSF) containing 147.0 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$ and 0.9 mM $MgCl_2$. The outflow fractions were collected every 20 min. Following the subsequent collection of three baseline fractions, rats were treated with s.c. injection of morphine (8 mg/kg) or saline (1 mL/kg) for these experiments. Dialysis samples were collected for 210 min after morphine or saline treatment. Dialysis fractions were then analysed using high performance liquid chromatography (HPLC, Eicom) with an electrochemical detection (ECD, Eicom) system. The animals were killed by decapitation under pentobarbital anesthesia at the end of the experiments. The brain was removed and the localisation of the probe was confirmed by staining with cresyl violet.

DA was separated by a column (Eicom-PAK CA-5ODS, 21 mm id x 150 mm, Eicom, Kyoto, Japan) with a mobile phase containing sodium

acetate (4.05 g/L), citric acid monohydrate (7.35 g/L), 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 2 μL/min. Identification of dopamine was determined according to the retention times of a dopamine standard, and the amount of dopamine was quantified by calculating peak area. The microdialysis data were expressed as percentage of the corresponding baseline level.

Effects of NTI and nor-BNI on the morphine-induced place preference under inflammatory pain-like state

Conditioning started on the day 1 after induction of inflammation. Control rats were injected with saline instead of morphine for each of the conditioning sessions. Rats were confined to one compartment on the day 1 and the other compartment on the next day after saline injection. This conditioning session was repeated three times. NTI or nor-BNI control rats were injected with saline and NTI (3 mg/kg, i.p.) or nor-BNI (5 mg/kg, i.p.), 45 min or 6 hr before treatment of saline, respectively. In combination study, rats were injected with NTI (3 mg/kg, i.p.) and nor-BNI (5 mg/kg, i.p.) 45 min and 6 hr before treatment with morphine, respectively. Morphine (8 mg/kg, i.p.) and saline (1 mL/kg, i.p.) were injected s.c. on alternate days. The rats were immediately confined to the respective compartment after the injection. After three conditioning sessions, tests were performed and time spent in each compartment was measured. Conditioning scores represent the difference between the time spent in the drug-paired place and the saline-paired place.

Drugs

The drugs used in the present study were morphine hydrochloride (Sankyo Co., Tokyo, Japan) and formaldehyde solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan). NTI and nor-BNI were synthesised by Dr Nagase (Basic Research Laboratories, Toray Industries, Inc., Kamakura 248, Japan). All drugs were dissolved in saline.

Statistical analysis

The data were presented as the mean ± S.E.M. The statistical significance of differences between the groups was assessed with a two-way ANOVA, followed by Bonferroni/Dunn or Student's t-test.

RESULTS AND DISCUSSION

Induction and measurement of inflammatory hyperalgesia

The development of the mechanical hyperalgesia after induction of inflammation is demonstrated in Figure 1. There was no difference in the paw pressure threshold between saline and formalin injection in the right hind paw (ipsilateral) before the induction of inflammation (day 0). The paw pressure threshold of the ipsilateral-paw was maximally decreased on day 1 after the induction of inflammation but gradually recovered over at least 11 days later. Inflammatory pain is characterised by hyperalgesia and edema. Hyperalgesia is a typical element of chronic pain state. The decrease in paw pressure threshold obviously indicates the development of hyperalgesia. Therefore, in the present study, we performed the CPP assay during the reduction in the paw pressure threshold produced by formalin.

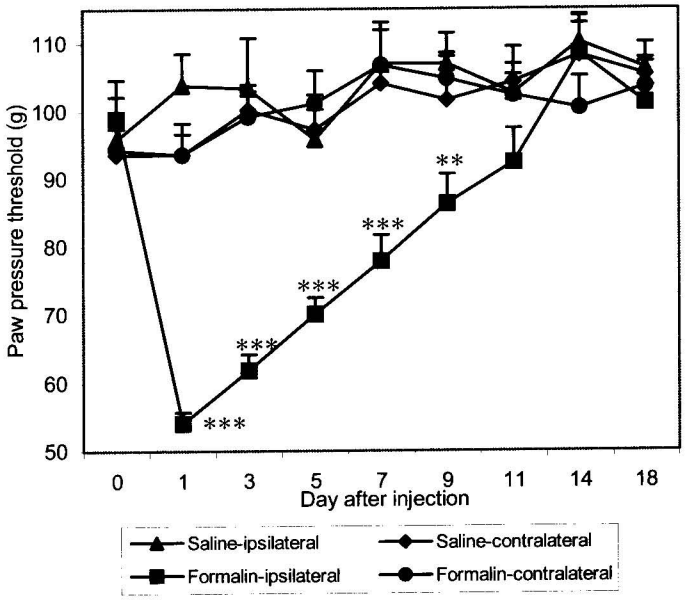

Fig. 1: Time-course of paw pressure threshold after injection of 2.5% formalin, saline-ipsilateral, saline-contralateral, 2.5% formalin-ipsilateral, 2.5% formalin-contralateral was injected into the plantar surface of the right paw. Each point expressed the mean ± S.E.M. of 6-12 rats. The asterisks denote significant difference (**$p < 0.01$, ***$p < 0.001$).

Effect of inflammation on place preference induced by morphine

Under inflammatory pain-like state, we investigated whether inflammation could affect the place conditioning induced by morphine. Injection of morphine (4 or 8 mg/kg, s.c.) produced a dose-related preference for the drug-associated place in saline-control rats. Under inflammatory pain, the morphine-induced place preference was significantly suppressed as compared to saline-control rats (Fig. 2A). These results suggested that morphine-induced place preference was significantly attenuated by inflammation. It was previously reported that the morphine-induced place preference was significantly attenuated under inflammatory pain-like state induced by formalin in rats (Suzuki *et al.* 1996a).

Effect of inflammation on dopamine release in the N.Acc produced by morphine using *in vivo* microdialysis

In the microdialysis study, basal levels of DA in the N.Acc were not different among all groups (saline-saline, saline-morphine, formalin-saline, formalin-morphine). The DA levels were markedly increased by s.c. injection of morphine 8 mg/kg as compared with saline treatment (control). However, the levels of extracellular DA in the N.Acc stimulated by morphine 8 mg/kg were significantly suppressed under inflammatory pain-like state (Fig. 2B).

The mesolimbic DA system, which originates in the VTA and projects to the N.Acc, has been identified as critical site for the initiation of opioid reinforcement (Funada *et al.* 1995; Koob *et al.* 1998; Narita *et al.* 2001). Electrophysiological studies have demonstrated that morphine and DAMGO inhibit the firing frequency of non-DA cells in the VTA (Matthews and German 1984; Johnson and North 1992). Furthermore, opioids have been shown to increase the DA release and DA metabolites in the mesolimbic DA terminal fields (Kalivas *et al.* 1983; Di Chiara and Imperato 1988; Funada *et al.* 1995). The morphine- or DAMGO-induced place preference could be blocked by either DA antagonists or neurochemical destruction of the N.Acc (Philips *et al.* 1983; Shippenberg *et al.* 1993). These findings indicate that the DA-containing neurons of the midbrain VTA, an area of high densities of μ-opioid receptors, play a major role in the rewarding effect of opioid. We postulated that the suppression on morphine-induced place preference under inflammatory pain-like state might result from the changes in the extracellular DA

release in the N.Acc. In the present study, we clearly demonstrated that extracellular DA release in the N.Acc induced by morphine was significantly reduced under inflammatory pain-like state.

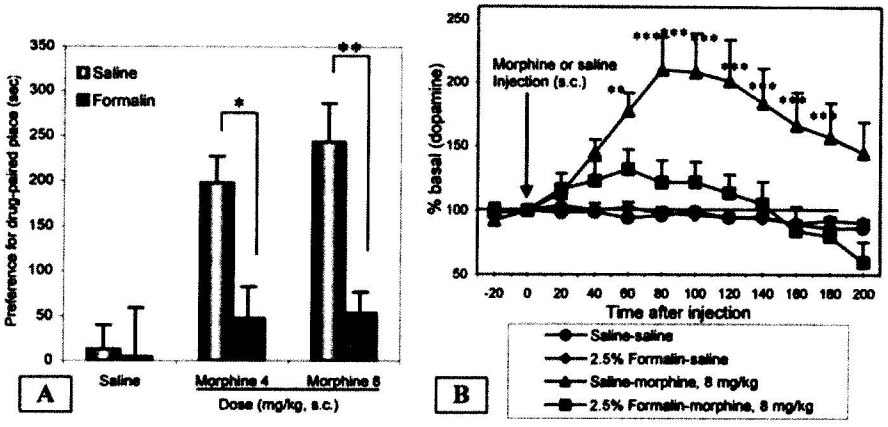

Fig. 2: Effect of inflammatory pain-like state on the (A) morphine-induced place preference and (B) extracelullar DA release in the N.Acc in rats. Each column and point expressed the mean ± S.E.M. of 6–12 rats and as a percentage of the corresponding baseline levels ± S.E.M. of 4–5 rats for place preference and microdialysis study, respectively. The asterisks denote significant difference versus saline (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$).

Effects of NTI and nor-BNI on the morphine-induced place preference under inflammation

Figure 3 shows that none of saline-control or formalin-treated rats that received saline in conditioning sessions produced a significant place preference. Pretreatment saline, NTI or nor-BNI on the saline-control or formalin-treated rats that receive saline did not produce a significant place preference. In the saline-control group, the significant place preference produced by morphine was significantly attenuated by pretreatment with NTI; whereas under inflammatory pain state, the significant attenuation of the morphine-produced place preference was not affected by pretreatment with NTI. In the saline-treated group, the significant place preference produced by morphine was not affected by pretreatment with nor-BNI. However, under inflammatory pain state, the significant suppression of the morphine-induced place preference was completely reversed by pretreatment with nor-BNI.

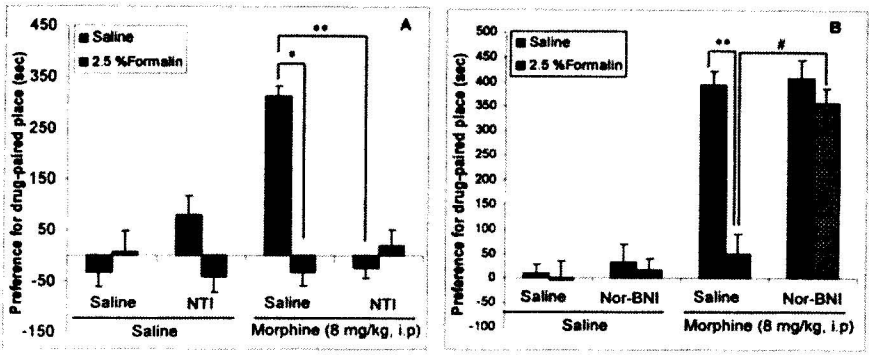

Fig. 3: Effect of pretreatment with (A) NTI and (B) nor-BNI on the morphine-induced place preference under chronic inflammatory pain. Each column represents the mean ± SEM. of 8–10 rats. The ordinate represents the preference for drug-paired place. *p < 0.05, **p < 0.01, significantly different versus morphine-induced place preference in saline treated group. #p < 0.05, significantly different versus morphine-induced place preference without nor-BNI.

Many studies suggested the interaction of the μ- and δ-opioid system. We reported that although it is important to block δ_2-opioid receptors rather than δ_1-opioid receptors to suppress the development of physical dependence on morphine, both δ_1- and δ_2-opioid receptors may play an important role in the development of dependence (Suzuki *et al.* 1997). On the other hand, it was reported that the morphine-induced place preference is completely prevented by both δ_1- and δ_2-opioid receptor antagonists (Suzuki *et al.* 1994), and the δ-opioid receptor agonist TAN-67 potentiates morphine-induced place preference (Suzuki *et al.* 1996b). In the present study, in the non-inflammed group, the non-selective δ-opioid receptor antagonist NTI significantly suppressed the morphine-induced place preference. This result is consistent with a previous report (Suzuki *et al.* 1994). However, under inflammatory pain state, NTI did not block the attenuation of morphine-induced place preference. This result suggests that δ-opioid systems play a small role in the attenuation of morphine-induced place preference under inflammatory pain state.

Evidences indicate that activation of the κ-opioid receptor opposes a variety of μ-opioid receptor-mediated actions throughout the brain and spinal cord (Pan 1998). The pretreatment with a κ-opioid receptor agonist U-50, 488H abolishes the morphine-induced place preference and increase in DA turnover in the limbic forebrain (Funada *et al.* 1993). It is well known that the development of tolerance to morphine antinociception is

completely blocked by the co-administration of U-50, 488H (Yamamoto *et al.* 1988). In addition, injection of the endogenous κ-opioid receptor agonist dynorphine A (1-13) and dynorphin A (2-17) suppressed the expression of tolerance to morphine antinociception and naloxone-precipitated withdrawal signs in morphine-dependent mice (Hooke *et al.* 1995). It was also reported that levels of dynorphin B were significantly increased under chronic arthritis in various brain regions, but not followed by the change in μ-opioid receptor expression (Spetea *et al.* 2002). The present study demonstrated that the attenuation of the morphine-induced place preference under formalin-induced inflammation was effectively suppressed by pretreatment with a selective κ-opioid receptor antagonist nor-BNI, but not with δ-opioid receptor antagonist NTI. Accumulating experimental evidence indicates that the selective activation of (μ and perhaps δ) or κ receptors produces opposite behavioural and physiological effects, especially DA-related responses. It is therefore likely that suppression of morphine-induce place preference and suppression of extracellular DA release in the N.Acc under inflammatory pain-like state may result from the enhancement of the endogenous κ-opioidergic system.

CONCLUSION

In conclusion, the present results suggest that the suppression of the morphine-induced place preference under inflammatory pain-like state may result from the reduction in the extracelullar DA release in the N.Acc. In addition, pretreatment with κ-opioid receptor antagonist nor-BNI but not the δ-opioid receptor antagonist NTI completely prevented the attenuation of the morphine-induced place preference under inflammatory pain state. These results suggest that the attenuation of morphine-induced place preference under inflammatory pain state may result from the activation of the endogenous κ-opioidergic system.

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