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Different roles of μ-, δ- and κ-opioid receptors in ethanol-associated place preference in rats exposed to conditioned fear stress

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Abstract

The present study was designed to investigate the role of the endogenous opioid system in the development of ethanol-induced place preference in rats exposed to conditioned fear stress (exposure to an environment paired previously with electric foot shock), using the conditioned place preference paradigm. The administration of ethanol (300 mg/kg, i.p.) with conditioned fear stress induced significant place preference. Naloxone (1 and 3 mg/kg, s.c.), a non-selective opioid receptor antagonist, significantly attenuated this ethanol-induced place preference. Moreover, the selective μ-opioid receptor antagonist β-funaltrexamine (3 and 10 mg/kg, i.p.) and the selective δ-opioid receptor antagonist naltrindole (1 and 3 mg/kg, s.c.) significantly attenuated ethanol-induced place preference. In contrast, the selective κ-opioid receptor antagonist nor-binaltorphimine (3 mg/kg, i.p.) significantly enhanced ethanol-induced place preference. Furthermore, 75 mg/kg ethanol (which tended to produce place preference) combined with the μ-opioid receptor agonist morphine (0.1 mg/kg, s.c.) or the selective δ-opioid receptor agonist 2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydroquinolino [2,3,3,-*g*] isoquinoline (TAN-67; 20 mg/kg, s.c.), at doses which alone did not produce place preference, produced significant place preference. However, co-administration of the selective κ-opioid receptor agonist *trans*-3,4-dichloro-*N*-(2-(1-pyrrolidinyl)cyclohexyl)benzenacetamide methanesulfonate (U50,488H; 0.3 and 1 mg/kg, s.c.) with ethanol (300 mg/kg, i.p.) dose dependently attenuated ethanol-induced place preference. Moreover, conditioned fear stress shifted the response curve for the aversive effect of U50,488H to the left. These results suggest that μ- and δ-opioid receptors may play critical roles in the rewarding mechanism of ethanol, and that κ-opioid receptors may modulate the development of the rewarding effect of ethanol under psychological stress.

Keywords: Ethanol; Conditioned place preference paradigm; Conditioned fear stress; Rewarding effect; Opioid system, endogenous; (Rat)

1. Introduction

It has been postulated that the interaction between stress and ethanol intake may play an important role in the etiology of alcoholism (Pohorecky, 1981). In fact, ethanol intake by humans increases in psychologically stressful situations. It has also been demonstrated that rats exposed to various types of stress, such as electric foot shock stress (Mills et al., 1977; Volpicelli et al., 1982; Caplan and Puglisi, 1986; Volpicelli et al., 1986), immobilization stress (Nash and Maickel, 1985; Rockman et al., 1987) and isolation stress (regarded as psychological stress) (Parker and Radow, 1974; Nash and Maickel, 1985; Wolffgramm, 1990), show increased ethanol intake. Recently, we reported that conditioned fear stress, a psychological stress (Fanselow, 1980; Conti et al., 1990; Inoue et al., 1993), potentiated ethanol-induced place preference in rats in the conditioned place preference paradigm, and speculated that psychological stress may play an important role in the development of the rewarding effect of ethanol (Matsuzawa et al., 1998).

There is evidence that the mesolimbic dopamine system, which is known to be under the influence of the endogenous opioid system (Gianoulakis, 1996; Herz, 1997), may play a role in the rewarding mechanism of ethanol (Koob, 1992). More importantly, the endogenous opioid system has been shown to play a critical role in the

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rewarding effect of ethanol. It has been suggested that ethanol stimulates the release of opioid peptides in the mesolimbic dopamine system (Gianoulakis, 1989). Small doses of an opioid agonist such as morphine have been shown to increase ethanol intake (Reid and Hunter, 1984; Hubbell et al., 1986, 1987), but the non-selective opioid receptor antagonist naloxone has been shown to decrease ethanol intake (Reid and Hunter, 1984; Samson and Doyle, 1985; Hubbell et al., 1986; Froehlich et al., 1990). Therefore, it appears that the mechanism of the rewarding effect of ethanol may be closely linked to the activation of opioid receptors in the mesolimbic dopamine system.

Previous reports have revealed that there are three opioid receptor types, μ-, δ- and κ-opioid receptors, in the central nervous system (Lord et al., 1977; Chang and Cuatrecasas, 1979), and that these opioid receptors play different roles in the motivational effects and reward mechanisms of drugs of abuse (Mucha et al., 1982; Mucha and Herz, 1985; Shippenberg et al., 1987; Bals-Kubik et al., 1990; Suzuki et al., 1991; Funada et al., 1993; Suzuki et al., 1993, 1994, 1996, 1997). In general, the activation of μ- and/or δ-opioid receptors results in a rewarding effect, while the activation of κ-opioid receptors results in an aversive effect (Mucha and Herz, 1985; Shippenberg et al., 1987; Bals-Kubik et al., 1990; Suzuki et al., 1991; Funada et al., 1993; Suzuki et al., 1993, 1996, 1997). Recently, we found that morphine-induced place preference is suppressed by the selective μ-opioid receptor antagonist β-funaltrexamine, the selective δ-opioid receptor antagonist naltrindole, and the selective κ-opioid receptor agonist U50,488H (Funada et al., 1993; Suzuki et al., 1993, 1994). These findings may support the notion that the rewarding effect of ethanol is modulated differently by each type of opioid receptor.

In the present study, we examined the role of the endogenous opioid system in the development of ethanol-induced place preference in rats exposed to conditioned fear stress, using opioid receptor antagonists and agonists. Ethanol was used at doses of 75 and 300 mg/kg, i.p. According to our published data for the dose (75, 150, 300, 600 and 1200 mg/kg, i.p.)–response relationship for ethanol-induced place preference during conditioned fear stress (Matsuzawa et al., 1998), ethanol produced significant conditioned place preference at 150 and 300 mg/kg, i.p., the dose–response curve for ethanol-induced place preference being bell-shaped over the dose range used. In addition, 300 mg/kg was more efficacious than 150 mg/kg.

2. Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, adopted by the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture, Japan.

2.1. Animals

Male Sprague–Dawley rats (Tokyo Experimental Animals, Tokyo, Japan), weighing 170–220 g, were housed in groups of four in a temperature-controlled room (22 ± 1°C) with a 12 h light–dark cycle (light on 0800 to 2000). Food and water were available ad libitum.

2.2. Apparatus

The test box consisted of a shuttle box (30 × 60 × 30 cm: width × length × height) which 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. The test box was placed under conditions of dim illumination (40 lux) and masking white noise.

2.3. Procedure

2.3.1. Habituation to the test box

On days 1 and 2, the partition separating the two compartments was raised 12 cm above the floor, and a neutral platform was inserted along the seam separating the compartments. Non-treated rats were placed on the platform of the test box and allowed to move freely in the test box for 15 min.

2.3.2. Pre-conditioning test (measurement of pre-conditioning scores)

On day 3, as the habituation session, non-treated rats were placed on the platform of the test box and allowed to move freely in the test box for 15 min. The time spent in each compartment during the 15 min session was measured automatically in a blind fashion by an infrared beam sensor (KN-80; Natsume Seisakusho, Tokyo, Japan).

2.3.3. Place conditioning

On days 4, 6, 8 and 10, the rats were individually subjected to intermittent electric foot shocks (10 min, 0.6 mA, 1 s on, 4 s off) through stainless steel floor grids by a shock generator (IT-2; O'Hara, Tokyo, Japan) in a gray shock chamber (27 × 18 × 27 cm). Twenty-four hours after the foot shocks (on days 5, 7, 9 and 11), the rats were again individually placed in the same shock chamber without foot shocks for 10 min. All the rats were then immediately injected with ethanol or saline and confined for 30 min to the non-preferred side in the pre-conditioning test following ethanol injection and to the preferred side in the pre-conditioning test following saline injection on alternate days (two for ethanol: two for saline).

An assessment of the motivational effect of U50,488H itself was also performed. In this case, non-conditioned fear stress rats were also used. The rats were individually

placed in a gray shock chamber without exposure to intermittent electric foot shocks for 10 min on days 4, 6, 8 and 10. After 24 h (on days 5, 7, 9 and 11), the rats were again individually placed in the same shock chamber without foot shocks for 10 min. All the rats (i.e., conditioned fear stress and non-conditioned fear stress rats) were injected with U50,488H or saline and confined for 30 min to the preferred side in the pre-conditioning test following U50,488H injection and to the non-preferred side in the pre-conditioning test following saline injection on alternate days (two for U50,488H: two for saline).

2.3.4. Post-conditioning test (measurement of post-conditioning scores)

On day 12, as in the pre-conditioning test session, the rats were placed on the platform of the test box and allowed to move freely in the test box for 15 min. The time spent in each compartment during a 15 min session was measured.

2.3.5. Injection procedure

Control rats were injected with saline instead of ethanol in each conditioning session. Naloxone (1 and 3 mg/kg, s.c.) was injected concomitantly with ethanol. β-funaltrexamine (3 and 10 mg/kg, i.p.), naltrindole (1 and 3 mg/kg, s.c.) and nor-binaltorphimine (1 and 3 mg/kg, i.p.) were injected 24 h, 45 min, and 4 h before ethanol treatment, respectively. Morphine (0.03 and 0.1 mg/kg, s.c.), TAN-67 (10 and 20 mg/kg, s.c.) and U50,488H (0.3 and 1 mg/kg, s.c.) were injected concomitantly with ethanol. In addition, in the test for the motivational effect of U50,488H, U50,488H (0.3, 1 and 3 mg/kg, s.c.) was injected instead of ethanol in each conditioning session.

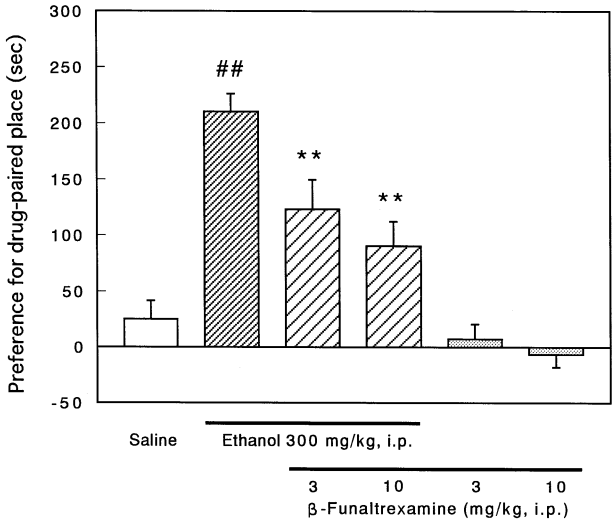

Fig. 2. Effect of β-funaltrexamine on ethanol-induced place preference during conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $^{\#\#}P < 0.01$ vs. saline-treated control group. $^{**}P < 0.01$ vs. ethanol alone-treated group.

2.4. Drugs

The drugs used in the present study were ethanol (Wako Pure Chemical, Osaka, Japan), naloxone hydrochloride (Research Biochemical, Wayland, MA, USA), β-funaltrexamine hydrochloride, naltrindole methanesulfonate, nor-binaltorphimine hydrochloride, morphine hydrochloride (Sankyo, Tokyo, Japan), TAN-67 (2-methyl-4aα-(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12aα-octahydroquinolino [2,3,3,-*g*] isoquinoline) and U50,488H (*trans*-

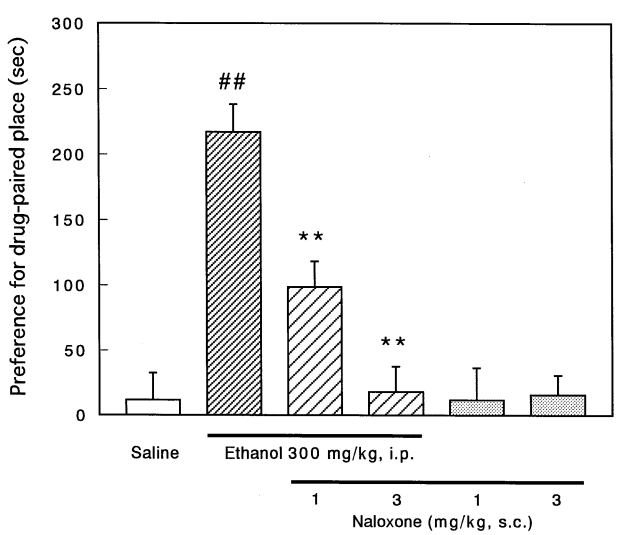

Fig. 1. Effect of naloxone on ethanol-induced place preference during conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $^{\#\#}P < 0.01$ vs. saline-treated control group. $^{**}P < 0.01$ vs. ethanol alone-treated group.

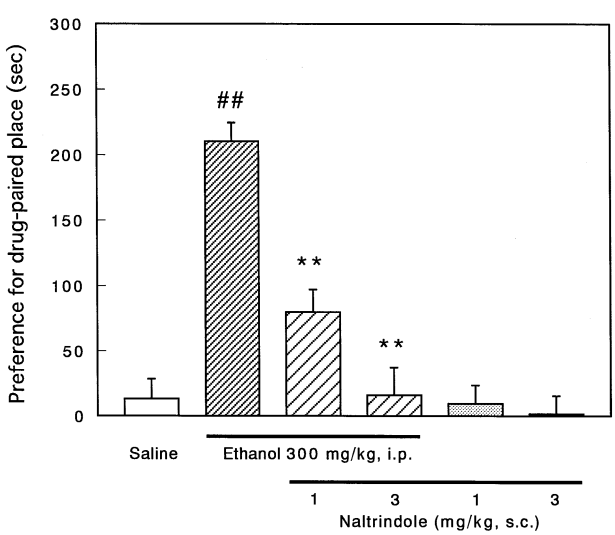

Fig. 3. Effect of naltrindole on ethanol-induced place preference during conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $^{\#\#}P < 0.01$ vs. saline-treated control group. $^{**}P < 0.01$ vs. ethanol alone-treated group.

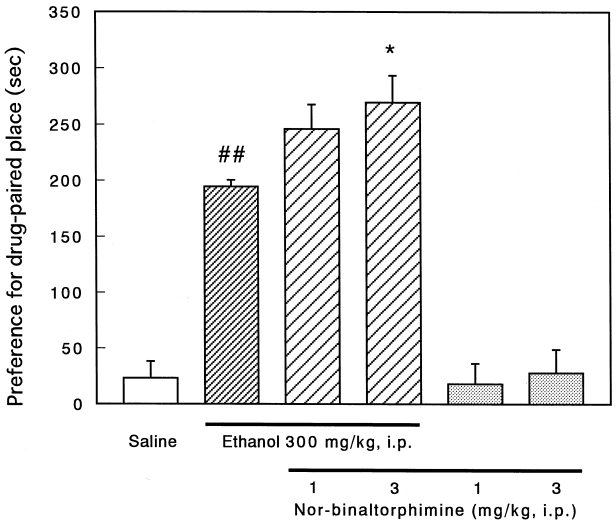

Fig. 4. Effect of nor-binaltorphimine on ethanol-induced place preference during conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $^{\#\#}P < 0.01$ vs. saline-treated control group. $^{*}P < 0.05$ vs. ethanol alone-treated group.

3,4-dichloro-*N*-(2-(1-pyrrolidinyl)cyclohexyl)benzeneacetamide methanesulfonate hydrochloride). β-Funaltrexamine, naltrindole, nor-binaltorphimine, TAN-67 and U50,488H were synthesized at Toray Industries. All the drugs were dissolved in saline. Ethanol was used at doses of 75 and 300 mg/kg. It was diluted to form a 20 (v/v) percent solution and injected intraperitoneally in different volumes.

2.5. Data analysis

Conditioning scores represent the difference in the time spent on the ethanol-paired side in the post-conditioning test minus the time spent on the non-preferred side in the pre-conditioning test, and are expressed as means ± S.E.M. The dose–response was analyzed using a one-way analysis of variance (ANOVA). Post-hoc analyses were carried out by Dunnett's test.

3. Results

3.1. Effect of naloxone on ethanol-induced place preference during conditioned fear stress

As shown in Fig. 1, ethanol at a dose of 300 mg/kg produced significant place preference ($P < 0.01$) in rats exposed to conditioned fear stress. Naloxone (1 and 3 mg/kg; $P < 0.01$) significantly attenuated ethanol-induced place preference in a dose-dependent manner ($F(5,42) = 16.48$; $P < 0.01$). Both 1 and 3 mg/kg of naloxone alone induced neither significant place preference nor place aversion.

3.2. Effects of β-funaltrexamine, naltrindole, and nor-binaltorphimine on ethanol-induced place preference during conditioned fear stress

The effects of pretreatment with β-funaltrexamine, naltrindole, and nor-binaltorphimine on ethanol-induced place preference are shown in Figs. 2–4, respectively. β-Funaltrexamine (3 and 10 mg/kg; $P < 0.01$) significantly attenuated ethanol-induced place preference in a dose-dependent manner ($F(5,42) = 20.74$; $P < 0.01$). Naltrindole (1 and 3 mg/kg; $P < 0.01$) also significantly attenuated ethanol-induced place preference in a dose-dependent manner ($F(5,42) = 25.18$; $P < 0.01$). In contrast, nor-binaltorphimine significantly enhanced ethanol-induced place preference in a dose-dependent manner ($F(5,42 = 41.47$; $P < 0.01$). Ethanol-induced place preference was significantly enhanced by nor-binaltorphimine at a dose of 3 mg/kg ($P < 0.05$). β-Funaltrexamine, naltrindole, and

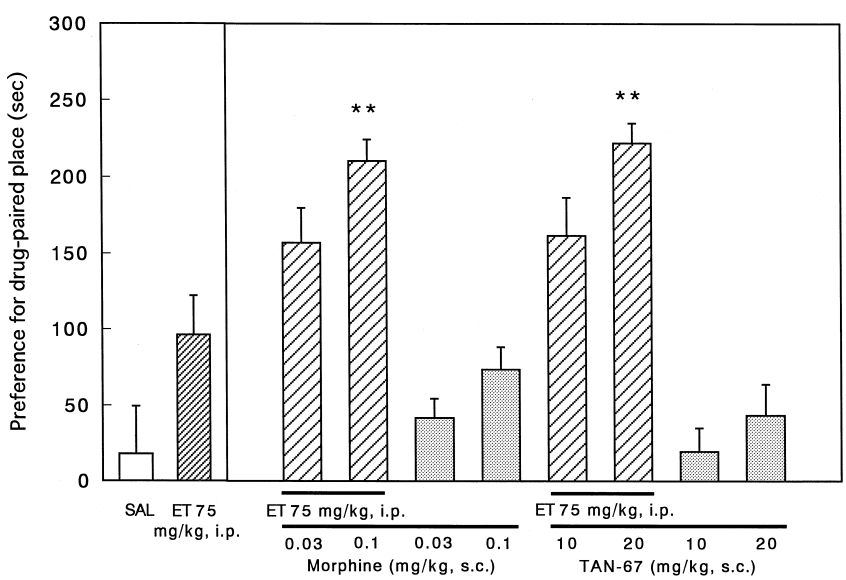

Fig. 5. Effects of morphine and TAN-67 on ethanol-induced place preference during conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $^{**}P < 0.01$ vs. ethanol alone-treated group. SAL: saline; ET: ethanol.

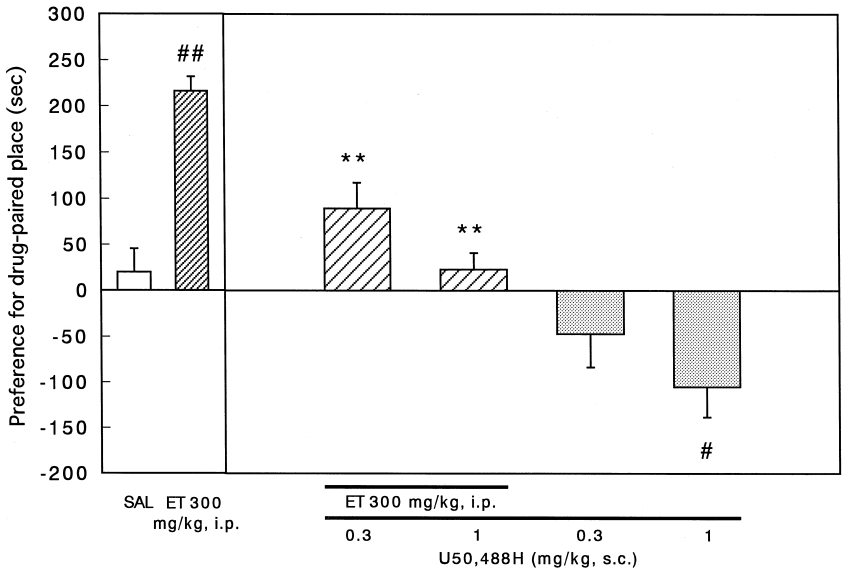

Fig. 6. Effect of U50,488H on ethanol-induced place preference during conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. saline-treated control group. $^{**}P < 0.01$ vs. ethanol alone-treated group. SAL: Saline; ET: Ethanol.

nor-binaltorphimine alone induced neither significant place preference nor place aversion.

3.3. Effects of morphine, TAN-67 and U50,488H on ethanol-induced place preference during conditioned fear stress

As shown in Fig. 5, although ethanol at a dose of 75 mg/kg tended to produce place preference in rats exposed to conditioned fear stress, the effect was not significant. Morphine dose dependently enhanced ethanol-induced place preference ($F(5,42) = 11.52$; $P < 0.01$). Morphine at a dose of 0.03 mg/kg tended to enhance ethanol-induced place preference, but this effect was not significant. At a dose of 0.1 mg/kg, morphine significantly potentiated ethanol-induced place preference ($P < 0.01$). TAN-67 also dose dependently enhanced ethanol-induced place preference ($F(5,42) = 13.49$; $P < 0.01$). Ethanol-induced place preference was significantly enhanced by TAN-67 at a dose of 20 mg/kg ($P < 0.01$). Morphine or TAN-67 alone induced neither significant place preference nor place aversion at the doses used in the present study. In contrast, as shown in Fig. 6, U50,488H (0.3 and 1 mg/kg) dose dependently attenuated ethanol-induced place preference ($F(5,42) = 16.81$; $P < 0.01$), and this attenuation was significant at doses of 0.3 and 1 mg/kg ($P < 0.01$). U50,488H alone induced significant place aversion at a dose of 1 mg/kg ($P < 0.05$), but not at 0.3 mg/kg.

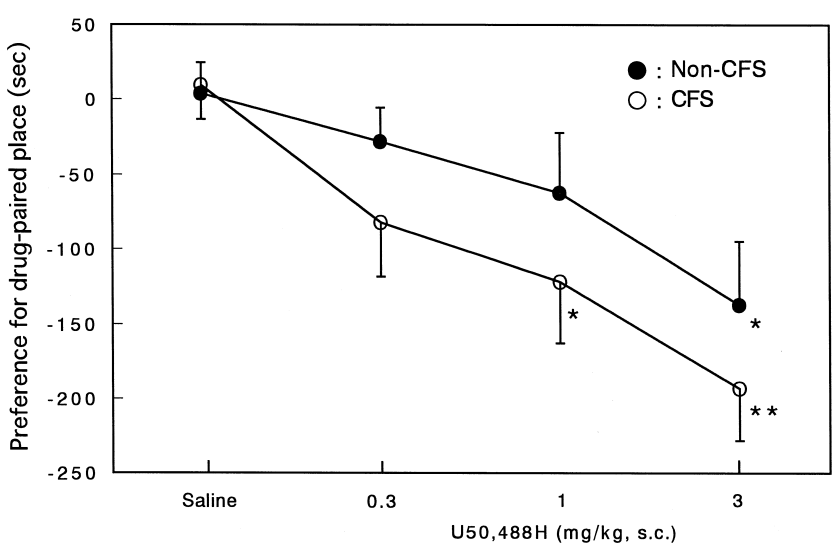

Fig. 7. Place-conditioning produced by U50,488H during non-conditioned fear stress (Non-CFS) or conditioned fear stress (CFS). The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for eight animals. $*P < 0.05$, $**P < 0.01$ vs. the respective saline-treated control group.

3.4. Place conditioning produced by U50,488H during non-conditioned fear stress or conditioned fear stress

As shown in Fig. 7, in rats that were not exposed to conditioned fear stress, U50,488H produced significant place aversion ($P < 0.05$) at a dose of 3 mg/kg, but not at 1 mg/kg. In contrast, U50,488H (1 and 3 mg/kg; $P < 0.05$ and $P < 0.01$, respectively) dose dependently produced significant place aversion ($F(3,28) = 6.01$; $P < 0.01$) in rats exposed to conditioned fear stress.

4. Discussion

In the present study, we found that ethanol produced significant place preference in rats exposed to conditioned fear stress. This result is consistent with our previous report showing that ethanol produces place preference in rats exposed to conditioned fear stress but not without the stress (Matsuzawa et al., 1998). It has been demonstrated that isolation stress (regarded as psychological stress) increases ethanol intake in rats (Parker and Radow, 1974; Nash and Maickel, 1985; Wolffgramm, 1990). These findings may support the hypothesis that the interaction between psychological stress and ethanol intake plays an important role in the etiology of alcoholism (Pohorecky, 1981). Psychological stress may act as an important motivating factor in the development of the rewarding effect of ethanol. Nevertheless, the mechanisms of the rewarding effect of ethanol under conditions of psychological stress are not yet clear.

There is evidence that the mesolimbic dopamine system, which contains key components of the reward pathway [ventral tegmental area (cell body) to nucleus accumbens (nerve terminal)], plays a critical role in the rewarding effect of drugs of abuse including ethanol (Koob, 1992). In addition, it is conceivable that the activation of the mesolimbic dopamine system mediated by stimulation of the endogenous opioid system is particularly important in the rewarding effect of ethanol (Gianoulakis, 1996; Herz, 1997). In the present study, we found that ethanol-induced place preference under conditioned fear stress was significantly attenuated by the non-selective opioid receptor antagonist naloxone (1 and 3 mg/kg, s.c.), which alone did not induce place aversion under the present experimental conditions. Naloxone has often been shown to induce place aversion (Mucha and Iversen, 1984). Although the detailed reason for this discrepancy is not understood, it may be due to the difference in experimental protocol, i.e., conditioned fear stress used as an additional procedure in the conditioned place preference paradigm in the present study. Since various types of stress including conditioned fear stress stimulate the release of endogenous opioid peptides in the brain (Nabeshima et al., 1987; Gianoulakis, 1989; Katoh et al., 1990; Nabeshima et al., 1992), it is possible that the response mediated by opioid receptors is changed (enhanced or diminished). If naloxone is used at higher doses (more than 3 mg/kg, s.c.), place aversion may be observed. It leads to the speculation that the motivational effect mediated by the endogenous opioid system is influenced in a complex way by conditioned fear stress in comparison with non-stressful situations.

Microdialysis studies have shown that ethanol increases extracellular dopamine concentrations in the rat nucleus accumbens (Imperato and Di Chiara, 1986; Di Chiara and Imperato, 1988b), indicating that ethanol activates the mesolimbic dopamine system. Furthermore, it is likely that ethanol stimulates the release of β-endorphin and enkephalins in the mesolimbic dopamine system (Gianoulakis, 1989). Conditioned fear stress increases dopamine release in the mesolimbic dopamine system, leading to activation of the dopamine pathway (Herman et al., 1982; Deutch et al., 1985; Inoue et al., 1994). As described above, various types of stress including conditioned fear stress have been shown to stimulate the release of endogenous opioid peptides (Nabeshima et al., 1987; Gianoulakis, 1989; Katoh et al., 1990; Nabeshima et al., 1992). These findings and the present results suggest that the development of ethanol-induced place preference may result from activation of the mesolimbic dopamine system by opioid receptors as a result of the interaction between ethanol and conditioned fear stress.

The existence of three opioid receptor types, μ-, δ- and κ-opioid receptors, in the central nervous system has been demonstrated (Lord et al., 1977; Chang and Cuatrecasas, 1979). There is evidence that the activation of these opioid receptors modifies the release of dopamine in the mesolimbic dopamine system and induces different motivational effects (rewarding or aversive effect). The activation of μ- and/or δ-opioid receptors produces a rewarding effect (place preference) through activation of the mesolimbic dopamine system, and the activation of κ-opioid receptors produces an aversive effect (place aversion) through inhibition of the mesolimbic dopamine system (Mucha and Herz, 1985; Shippenberg et al., 1987; Bals-Kubik et al., 1990; Suzuki et al., 1991; Funada et al., 1993; Suzuki et al., 1993, 1996, 1997). In the present study, with regard to μ- and δ-opioid receptors, β-funaltrexamine, a selective μ-opioid receptor antagonist, and naltrindole, a selective δ-opioid receptor antagonist, attenuated ethanol-induced place preference. In addition, morphine, a μ-opioid receptor agonist, and TAN-67, a selective δ-opioid receptor agonist, enhanced ethanol-induced place preference. Thus, the activation of μ- and/or δ-opioid receptors is involved in the development of ethanol-induced place preference. The evidence can be further supported by the observation that ethanol in combination with a small dose of morphine produces conditioned place preference in rats (Marglin et al., 1988). Moreover, small doses of the μ-opioid receptor agonist morphine increase ethanol intake (Reid and Hunter, 1984; Hubbell et al., 1986, 1987). Furthermore, the intracerebroventricular administration of β-endorphin, which

binds with higher affinity to μ- than to δ-opioid receptors, produces place preference by increasing dopamine release in the nucleus accumbens, and this effect of β-endorphin is attenuated by D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Pen-The-NH_2 (CTOP; a selective μ-opioid receptor antagonist) and *N*-*N*-diallyl-Tyr-Aib-Aib-Phe-Thr (ICI 174,864; a selective δ-opioid receptor antagonist) (Bals-Kubik et al., 1990; Spanagel et al., 1990). Tyr-D-Ala-Gly-[*N*MePhe]-$NH(CH_2)_2$-OH (DAMGO; a selective μ-opioid receptor agonist) and *cyclic*[D-Phe^2,D-Pen^5]enkephalin (DPDPE; a selective δ-opioid receptor agonist) also produce place preference by increasing dopamine release in the nucleus accumbens, and these effects of DAMGO and DPDPE are attenuated by treatment with CTOP and ICI 174,864, respectively (Bals-Kubik et al., 1990; Spanagel et al., 1990). We previously found that TAN-67 enhanced morphine-induced place preference through the activation of δ-opioid receptors (Suzuki et al., 1996). In addition, the increase in dopamine release induced by ethanol can be reduced by naloxonazine (an irreversible μ-opioid receptor antagonist), naltrindole and ICI 174,864 (Acquas et al., 1993; Di Chiara et al., 1996), suggesting that ethanol-induced dopamine release may be mediated by μ- and δ-opioid receptors. Considering these reports, our findings may support the hypothesis that the activation of β-endorphinergic and/or enkephalinergic systems in the mesolimbic dopamine system is critically linked to the development of ethanol-induced place preference during conditioned fear stress.

In contrast, we also found that nor-binaltorphimine, a selective κ-opioid receptor antagonist, significantly enhanced ethanol-induced place preference, while U50,488H, a selective κ-opioid receptor agonist, significantly attenuated ethanol-induced place preference. U50,488H has been reported to produce place aversion in animals (Mucha and Herz, 1985; Funada et al., 1993; Suzuki et al., 1993). This observation is supported by the fact that U50,488H depresses the firing rate of mesolimbic dopamine neurons and decreases dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988a). Previously, we demonstrated that U50,488H abolished morphine-induced place preference, and that this inhibitory effect of U50,488H was antagonized by nor-binaltorphimine (Funada et al., 1993). U50,488H reduced the morphine-induced elevation of dopamine metabolites in the mesolimbic dopamine system (Funada et al., 1993). Likewise, U50,488H may reduce the ethanol-induced and conditioned fear stress-induced elevation of dopamine release by activating κ-opioid receptors in the nucleus accumbens, resulting in attenuation of ethanol-induced place preference. An interesting finding of the present study is the effect of conditioned fear stress on the aversive effect of U50,488H. Conditioned fear stress caused a leftward shift of the dose–response curve for the aversive effect of U50,488H, indicating that conditioned fear stress increased sensitivity for the aversive effect of this drug. Conditioned fear stress has been shown to stimulate the release of dynorphin, which has high affinity for κ-opioid receptors in the brain (Katoh et al., 1990; Nabeshima et al., 1992), which suggests that the enhancement of dynorphin release induced by conditioned fear stress may potentiate the aversive effect of U50,488H. In addition, κ-opioid receptors may be involved in the response to psychological stress (Takahashi et al., 1990). Under conditions of psychological stress, κ-opioid receptors may affect the rewarding effect of ethanol. This possibility was supported by our finding that the development of conditioned fear stress-induced ethanol preference was markedly enhanced by nor-binaltorphimine and attenuated by U50,488H. Based on these findings, we suggest that κ-opioid receptors may negatively modulate the magnitude of the rewarding effect of ethanol during conditioned fear stress.

In conclusion, our findings suggest that μ- and δ-opioid receptors may play a key role in the rewarding mechanism of ethanol, and that κ-opioid receptors may negatively modulate the rewarding effect of ethanol during psychological stress.

Acknowledgements

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