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Ethanol, But Not the Anxiolytic Drugs Buspirone and Diazepam, Produces a Conditioned Place Preference in Rats Exposed to Conditioned Fear Stress

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MATSUZAWA, S. T. SUZUKI AND M. MISAWA. *Ethanol, but not the anxiolytic drugs buspirone and diazepam, produces a conditioned place preference in rats exposed to conditioned fear stress.* PHARMACOL BIOCHEM BEHAV **65**(2) 281–288, 2000.—The present study was designed to investigate the role of an anxiolytic effect in the development of a drug-associated place preference in rats exposed to conditioned fear stress, using the conditioned place-preference paradigm. The administration of a low dose of ethanol (300 mg/kg, IP) and the anxiolytic drugs, buspirone (1 and 2 mg/kg, IP) and diazepam (1.25 and 2.5 mg/kg, IP), did not produce a place preference in rats that were not exposed to conditioned fear stress. In rats that were exposed to conditioned fear stress, ethanol produced a significant place preference, while buspirone and diazepam failed to produce a place preference. In addition, ethanol, buspirone, and diazepam produced no place preference in rats treated with an anxiogenic dose of pentylenetetrazole (20 mg/kg, IP). A significant decrease in locomotor activity was observed in rats exposed to conditioned fear stress. Ethanol, but not buspirone and diazepam, significantly recovered or increased locomotor activity in rats exposed to conditioned fear stress. Further, the locomotor-stimulating effect of ethanol was markedly enhanced by repeated exposure to conditioned fear stress. These results suggest that the stimulating effect may be strongly related to the development of the rewarding effect of a low dose of ethanol under psychological stress, and that the conditioned place preference paradigm with conditioned fear stress may be useful for studying the rewarding mechanism of ethanol with regard to the interaction between ethanol and psychological stress. © 2000 Elsevier Science Inc.

Conditioned place preference Ethanol Buspirone Diazepam Locomotor activity Rats

PREVIOUS studies have indicated that stress is positively associated with the use and relapse of abused drugs such as psychostimulants, opioids, and ethanol. In particular, it has been postulated that the interaction between stress and ethanol intake may play an important role in the etiology of alcoholism (38). In fact, ethanol intake by humans increases under stressful situations. Likewise, rats exposed to various types of stress, such as foot shock stress (5,33,47,48), immobilization stress (34,40), and isolation stress (34,36,52) show an increase in ethanol intake. In our previous report (30), conditioned fear stress, as a form of psychological stress, markedly potentiated the ethanol-induced place preference in rats using the conditioned place preference paradigm, suggesting that psychological stress may play an important role in the development of the rewarding effect of ethanol.

It is well known that ethanol has two major emotional effects, i.e., euphoric and anxiolytic effects. Although the euphoric effect seems to be the main contributor to the rewarding effect of ethanol, the anxiolytic effect also seems to be an important motivating factor in the rewarding effect of ethanol, which is consistent with the "tension-reduction hypothesis" (6). In our previous study for assessing the rewarding ef-

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fect of ethanol (30), we used the conditioned place preference paradigm with conditioned fear stress, which has been proposed as a model of anxiety (10). Because the animals used in our previous study experienced anxiety during the conditioning period, ethanol's euphoric effect and its anxiolytic effect may have participated in the development of the ethanol-induced place preference. However, we speculated that the anxiolytic effect of ethanol may not be implicated in the development of the ethanol (300 mg/kg)-induced place preference because the dose of ethanol used in that study does not have a significant anxiolytic effect (31). If an anxiolytic effect is implicated in the development of the ethanol-induced place preference, it is possible that 1) the novel anxiolytic drug buspirone (a partial 5-HT_{1A} receptor agonist) and the potent anxiolytic drug diazepam (a prototypical benzodiazepine compound) could produce a place preference in the conditioned place preference paradigm with conditioned fear stress; and 2) ethanol could produce a place preference under anxiety induced by treatment with pentylenetetrazole as an anxiogenic stimulus (25).

The mesolimbic dopamine system plays an important role not only in the rewarding effect but also in the locomotor-stimulating effect of abused drugs. An increase in locomotor activity is one of the behavioral responses observed with several abused drugs, and results from pharmacological mechanisms related to the rewarding effect (51). Ethanol enhances locomotor activity in rodents at doses that increase dopamine levels in the nucleus accumbens (19). Hence, assessment of the locomotor-stimulating effect of ethanol is thought to be useful for studying the mechanism underlying its rewarding effect (27,49). Therefore, we also investigated the effect of ethanol on locomotor activity under conditioned fear stress to clarify the relationship between the locomotor-stimulating and rewarding effects of ethanol.

GENERAL METHODS

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

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 L:12 D cycle (lights on 0800 to 2000 h). Food and water were available ad lib. Eight rats were used for each treatment group of each experiment (Experiment 1: conditioned place preference, and Experiment 2: locomotor activity).

Drugs

The drugs used in the present study were ethanol (Wako Pure Chemical, Osaka, Japan), buspirone hydrochloride (Sigma Chemical, St. Louis, MO), diazepam (Profarma, Milan, Italy), and pentylenetetrazole (Sigma Chemical). All drugs were dissolved in saline, except diazepam, which was suspended in vehicle consisting of 9% Tween 80 (Kishida Chemical Co., Osaka, Japan) in saline. Ethanol was used at a dose of 300 mg/kg. It was diluted to form a 20 (v/v) % solution. Buspirone hydrochloride was used at doses of 1 and 2 mg/kg, whereas diazepam at doses of 1.25 and 2.5 mg/kg. All of the drugs were injected intraperitoneally.

EXPERIMENT 1: CONDITIONED PLACE PREFERENCE

Apparatus

The conditioned place preference test box consisted of a shuttlebox (30 × 60 × 30 cm: L W × H), 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 lx) and masking white noise.

Procedure

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. Nontreated rats were placed on the platform of the test box and allowed to move freely in the test box for 15 min.

Preconditioning test (measurement of preconditioning scores). On day 3, as the habituation session, nontreated 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). The compartment in which each nontreated rat spent for less time was regarded as the nonpreferred side for each animals. In the place-conditioning session, the drugs (ethanol, buspirone, and diazepam) were injected when the rats were confined to the nonpreferred side, whereas saline and vehicle were injected when the rats were confined to the preferred side; i.e., all of the rats were assigned to the nonpreferred side as the drug-paired side. The rats used in this experiment were assigned to the following treatment groups: (a) saline or vehicle-treated control group; saline or vehicle in the drug-paired side, saline or vehicle in the other side; (b) ethanol-treated group; ethanol in the drug-paired side, saline in the other side; (c) buspirone-treated group; buspirone in the drug-paired side, saline in the other side; (d) diazepam-treated group; diazepam in the drug-paired side, vehicle in the other side.

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: L × W × H). 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 of the rats were then immediately injected with drug (ethanol, buspirone, or diazepam) or saline (or vehicle) and confined for 30 min to the nonpreferred side in the preconditioning test following drug (ethanol, buspirone, or diazepam) injection and to the preferred side in the preconditioning test following saline or vehicle injection on alternate days (2 for drug: 2 for saline or vehicle). A pentylenetetrazole-treated group was prepared similarly to the other conditioning groups. However, instead of exposure to conditioned fear stress, rats were injected with pentylenetetrazole (20 mg/kg, IP) on days 5, 7, 9, and 11. All of the rats were then injected with drug (ethanol, buspirone, or diazepam) or saline (or vehicle) 10 min after pentylenetetrazole injection and confined for 30 min to the nonpreferred side in the preconditioning test following drug (ethanol, buspirone, or diazepam) injection, and to the preferred side in the preconditioning test following saline or vehicle injection on alternate days (2 for drug: 2 for saline or vehicle).

Postconditioning test (measurement of postconditioning scores). On day 12, as the preconditioning 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.

Data Analysis

Conditioning scores represent the difference in time spent on the drug-paired side in the postconditioning test minus the time spent on the nonpreferred side in the preconditioning test, and are expressed as mean ± SEM. The dose response was analyzed using a one-way analysis of variance (ANOVA). Post hoc analyses were carried out by Dunnett's test.

EXPERIMENT 2: LOCOMOTOR ACTIVITY

Apparatus

The locomotor activity test box consisted of a gray shuttle-box (30 × 60 × 30 cm: L × W × H). The floor of the test box was divided into 32 spaces of equal size. The test box was placed under conditions of dim illumination (40 lx).

Procedure

This experiment consisted of distinct sessions that were designed to be analogous to the conditioned place preference experiment.

Habituation to the test box. On days 1 and 2, non treated rats were allowed to move freely in the test box for 15 min.

Pretest (Test 1). On day 3, as the habituation session, non-treated rats were allowed to move freely in the test box for 30 min. During this time, locomotor activity was measured by an observer in terms of line crossing, and was counted when the animal moved either forward or backward over a line.

Test during exposure to conditioned fear stress (Tests 2 and 3). The rats used in this experiment were assigned to the following treatment groups: (a) saline or vehicle-treated control group; saline or vehicle on days 5, 7, 9, and 11: (b) ethanol-treated group; ethanol on days 5 and 9, saline on days 7 and 11; ethanol on days 7 and 11, saline on days 5 and 9; (c) buspirone-treated group; buspirone on days 5 and 9, saline on days 7 and 11; buspirone on days 7 and 11, saline on days 5 and 9; (d) diazepam-treated group; diazepam on days 5 and 9, vehicle on days 7 and 11; diazepam on days 7 and 11, vehicle on days 5 and 9.

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: L × W × H). 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 of the rats were then immediately injected with drug (ethanol, buspirone, or diazepam) or saline (or vehicle) and placed in the test box for 30 min on alternate days (2 for drug: 2 for saline or vehicle). As in the pretest session, line crosses were counted when each rat was paired with drugs according to the treatment groups (i.e., one group was measured on days 5 and 9; the other group was measured on days 7 and 11). Scores were derived from each of the two blocks of sessions [i.e., block 1 (= test 2) included drug injection trials 1 (on day 5) and 2 (on day 7), while block 2 (= test 3) included drug injection trials 3 (on day 9) and 4 (on day 11)].

Data Analysis

Line crosses were expressed as mean ± SEM and analyzed using a one-way analysis of variance (ANOVA). Post hoc analyses were carried out by Dunnett's test.

RESULTS

Experiment 1: Conditioned Place Preference

Motivational effects of ethanol, buspirone, and diazepam in rats that were not exposed to conditioned fear stress. As shown in Fig. 1, ethanol (300 mg/kg), buspirone (1 and 2 mg/kg), and diazepam (1.25 and 2.5 mg/kg) each failed to produce a significant place preference in rats that were not exposed to conditioned fear stress.

Motivational effects of ethanol, buspirone, and diazepam in rats exposed to conditioned fear stress. As shown in Fig. 2, ethanol (300 mg/kg) produced a significant place preference ($p < 0.01$), whereas buspirone (1 and 2 mg/kg) and diazepam (1.25 and 2.5 mg/kg) each failed to produce a significant place preference in rats exposed to conditioned fear stress.

Motivational effects of ethanol, buspirone and diazepam in rats treated with pentylenetetrazole. As shown in Fig. 3, ethanol (300 mg/kg), buspirone (1 and 2 mg/kg), and diazepam (1.25 and 2.5 mg/kg) each failed to produce a significant place preference in rats treated with pentylenetetrazole (20 mg/kg).

Experiment 2: Locomotor Activity

Effects of ethanol, buspirone, and diazepam on locomotor activity in rats exposed to conditioned fear stress. The locomotor activity of rats exposed to conditioned fear stress is shown in Fig. 4. Conditioned fear stress significantly ($p < 0.01$) decreased the locomotor activity of saline- or vehicle-treated rats in tests 2 and 3 compared to appropriate nontreated rats in test 1. Ethanol (300 mg/kg)-treated rats in tests 2 and 3 showed significantly ($p < 0.01$) greater locomotor activity than saline-treated rats in tests 2 and 3, respectively. Moreover, the locomotor activity of ethanol-treated rats in test 3 was significantly ($p < 0.01$) greater than that in test 2. Buspirone (2 mg/kg) and diazepam (2.5 mg/kg) had no effect on the locomotor activity of rats exposed to conditioned fear stress in tests 2 and 3.

DISCUSSION

In the present study, 300 mg/kg of ethanol produced a significant place preference in rats exposed to conditioned fear stress, but not without stress. This result is in agreement with our previous report (30) that psychological stress plays an important role in the development of the rewarding effect of ethanol (especially low doses of ethanol). However, the question of whether the anxiolytic effect of ethanol participates in the development of the ethanol-induced place preference is important, because we used conditioned fear stress, which has been proposed to be a model of anxiety (10), as an additional conditioning procedure in the conditioned place preference paradigm. Therefore, to clarify the participation of an anxiolytic effect in the development of a place preference, the motivational effects of anxiolytic drugs buspirone and diazepam were investigated using the conditioned place preference paradigm with and without conditioned fear stress.

The present study indicated that neither buspirone (1 and 2 mg/kg) nor diazepam (1.25 and 2.5 mg/kg) produced a place preference in rats that were not exposed to conditioned fear

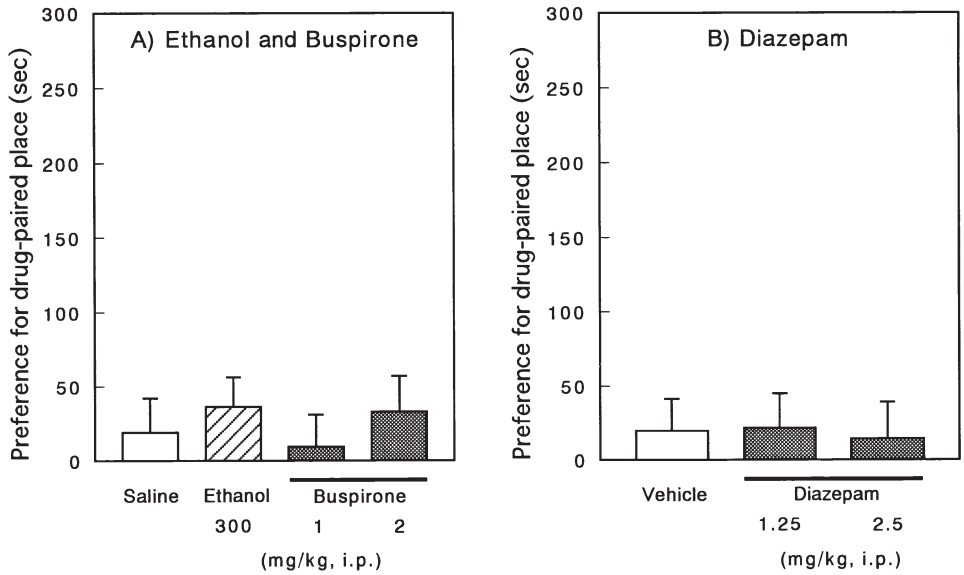

FIG. 1. Place conditioning produced by ethanol, buspirone, and diazepam in rats without conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with SEM of eight animals.

stress (i.e., under normal conditions). It has been shown that buspirone lacks a euphoric effect in humans (drug abusers and alcoholics) (9,15). Nevertheless, in several animal studies using the conditioned place preference paradigm, conflicting findings have been reported. On one hand, it has been reported that buspirone (0.25–1 mg/kg) does not produce a place preference in rats (13), indicating that buspirone has no reward potential. In contrast, Neisewander et al. (35) found that buspirone (1 and 3 mg/kg) produces a place preference in rats, indicating that buspirone does have some reward potential. On the other hand, the abuse liability of benzodiazepines has been documented among certain vulnerable humans such as alcoholics (4,8). In animal studies using the conditioned place preference paradigm, Di Scala et al. (11) and Meririnne et al. (32) have demonstrated that diazepam (1 and 2 mg/kg and 0.2–5 mg/kg, respectively) produces no place preference in rats, in agreement with our present results. In contrast, Spyraki et al. (43) have found that diazepam (1–5 mg/kg) produces a place preference in rats. Thus, there are contrastive findings about diazepam, as well as buspirone. One possible

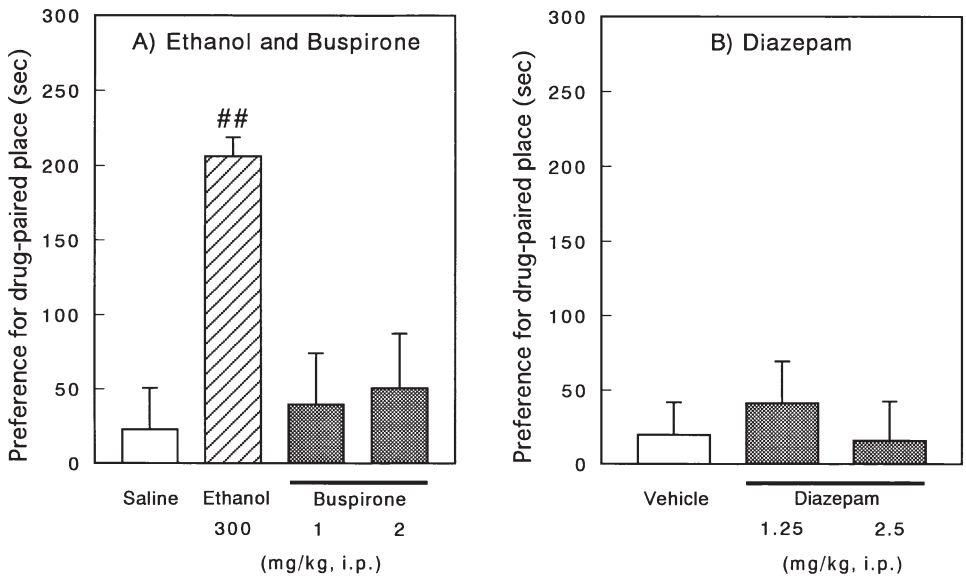

FIG. 2. Place conditioning produced by ethanol, buspirone, and diazepam in rats exposed to conditioned fear stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with SEM of eight animals. ## $p < 0.01$ vs. saline-treated control group (Dunnett's test).

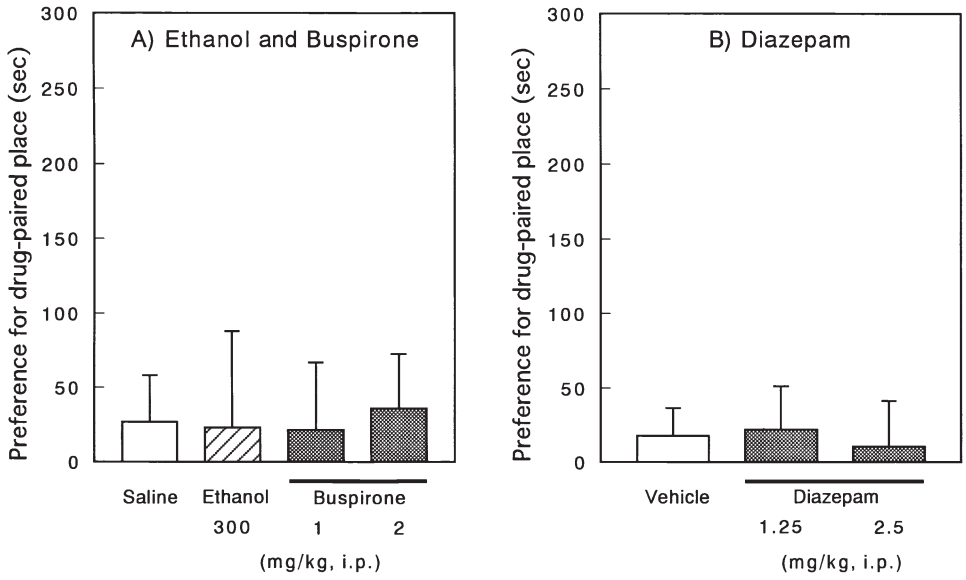

FIG. 3. Place conditioning produced by ethanol, buspirone, and diazepam in rats treated with pentylenetetrazole. The ordinate represents preference for the drug-paired place. Each column represents the mean with SEM of eight animals.

consideration of the discrepancy between these observations about buspirone and diazepam may be the procedural variations, such as the number of conditioning trials, the timing of animal confinement after the drug administration, and the dosage of these drugs. Although the reason for this discrepancy about buspirone and diazepam remains unclear, both buspirone and diazepam seemed to lack a rewarding effect at least in our experimental conditions.

On the other hand, we also found that buspirone and diazepam failed to produce a place preference in rats exposed to conditioned fear stress (i.e., under psychological stressful conditions). There is the evidence that both buspirone and diaz-

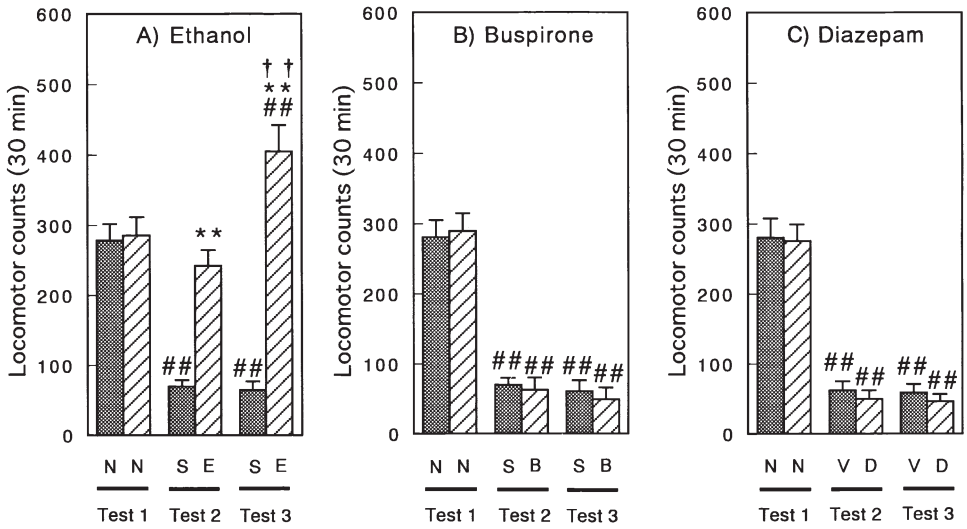

FIG. 4. Effects of ethanol, buspirone, and diazepam on locomotor activity in rats exposed to conditioned fear stress. The ordinate represents locomotor counts for 30 min in the drug-paired place. Each column represents the mean with SEM of eight animals. ##$p < 0.01$ vs. appropriate nontreated group in test 1 (Dunnett's test). **$p < 0.01$ vs. respective saline-treated control group in tests 2 and 3, respectively (Dunnett's test). ††$p < 0.01$ vs. ethanol-treated group in test 2 (Dunnett's test). N: Nontreated group; S: Saline-treated group; E: Ethanol (300 mg/kg, IP)-treated group; B: Buspirone (2 mg/kg, IP)-treated group; V: Vehicle-treated group; D: Diazepam (2.5 mg/kg, IP)-treated group.

epam generally exhibit anxiolytic effects in rats. For instance, according to the previous report of McCloskey et al. (31) using an animal conflict procedure, both buspirone and diazepam increase the number of shocks received in rats at the doses used in our present study. In contrast, ethanol, at a dose that has no significant anxiolytic effect (31), produced a significant place preference in rats exposed to conditioned fear stress. Furthermore, ethanol, buspirone, and diazepam produced no place preference in rats treated with a subconvulsive dose of pentylenetetrazole as an anxiogenic stimulus. Pentylenetetrazole is useful for inducing anxiety in animals, and for investigating the effect of anxiety on behavior, such as ethanol intake. Buczek et al. (3) reported that acute pentylenetetrazole (15 mg/kg) did not modify ethanol intake in rats. Moreover, McCloskey et al. (31) also demonstrated that the magnitude of the buspirone-induced anticonflict effect was considerably less than that observed with diazepam, indicating that buspirone has a weak anxiolytic effect compared with that of diazepam. In the present study, it is noteworthy that more potent anxiolytic drug diazepam (compared with buspirone) failed to produce a place preference in rats under psychological stressful conditions and anxiety-like conditions. The present results combined with these findings suggest that an anxiolytic effect by itself may not be involved in the development of a place preference using our method, and that the rewarding effect of ethanol, as assessed in the conditioned place-preference paradigm based on our methodology, may reflect its euphoric effect, but not its anxiolytic effect.

It is well known that the mesolimbic dopamine system is involved in the rewarding effect of ethanol (24). The increase in dopamine release in the nucleus accumbens is thought to be a key mechanism in the development of the rewarding effect of ethanol, as with other abused drugs. Based on these evidences, we discussed below about one possible speculation of the present result that ethanol, but not buspirone and diazepam, produced a place preference under conditioned fear stress in view of the effects of these drugs on dopamine release in the nucleus accumbens. A low dose of ethanol increased extracellular dopamine concentrations in the rat nucleus accumbens (19). With regard to buspirone, Neisewander et al. (35) demonstrated that buspirone (3 mg/kg) increases dopamine synthesis as measured by dopa accumulation in the nucleus accumbens in rats pretreated with a dopa decarboxylase inhibitor. However, dopa accumulation may not directly reflect extracellular dopamine concentrations (i.e., dopamine release) in the nucleus accumbens. In addition, the dose of buspirone used in that study was higher than that in our present study. Therefore, the effect of buspirone (1 and 2 mg/kg) on extracellular dopamine concentrations in the nucleus accumbens is not clear. Furthermore, 5-HT_{1A} receptor agonists such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) have no effect on extracellular dopamine concentrations in the nucleus accumbens (39,45), indicating that 5-HT_{1A} receptor agonists do not exert direct effects on dopaminergic neurotransmission in the nucleus accumbens. Ichikawa et al. (18) demonstrated that the activation of 5-HT_{1A} receptors inhibits the amphetamine-induced increase in extracellular dopamine concentrations in the nucleus accumbens. Hence, these results suggest that buspirone may have at most a weak effect on extracellular dopamine concentrations in the nucleus accumbens, and that 5-HT_{1A} receptor agonists may not be able to produce a rewarding effect, unlike ethanol, which increases extracellular dopamine concentrations in the nucleus accumbens. On the other hand, microdialysis studies have found that benzodiazepines including diazepam reduce the release of dopamine in the nucleus accumbens. For instance, Imperato et al. (20) demonstrated that diazepam at the anxiolytic dose of 2.5 mg/kg drastically decreased the basal release of dopamine in the nucleus accumbens in rats. Thus, it is likely that diazepam, in general, does not have the clear ability to increase dopamine release in the nucleus accumbens. Various types of stress including conditioned fear stress have been shown to increase dopamine levels in the nucleus accumbens. In addition, such stresses increase dopamine levels more selectively in the prefrontal cortex than in the nucleus accumbens. On the other hand, pentylenetetrazole (20 mg/kg) increases extracellular dopamine concentrations in the prefrontal cortex but not in the nucleus accumbens (1). Thus, differences exist in dopamine neural responses in the nucleus accumbens between conditioned fear stress- and pentylenetetrazole-induced anxiety. Moreover, buspirone (39,45,50), but not ethanol (1), clearly increases extracellular dopamine concentrations in the prefrontal cortex. Le Moal and Simon (26) demonstrated that the mesocortical and mesolimbic dopamine systems have different functions and roles in behavior. These findings support the hypothesis that an increase in dopamine levels in the nucleus accumbens, but not in the prefrontal cortex, may contribute to the potentiation of the rewarding effect of ethanol by psychological stress, and that the interaction between ethanol and psychological stress in the increase in dopamine release from the nucleus accumbens may be involved in the development of the rewarding effect of ethanol. This speculation may explain why buspirone failed to produce a place preference in rats that were exposed to conditioned fear stress and treated with pentylenetetrazole. More interestingly, with regard to diazepam, it has been demonstrated that diazepam decreases the release of dopamine not only in the nucleus accumbens but also in the prefrontal cortex in rats, and that diazepam does not affect the enhanced dopamine release in these brain areas induced by stress such as restraint stress (20).

Further, it has also been reported that diazepam rather decreases the enhanced dopamine release in the rat prefrontal cortex induced by stress such as conditioned fear stress (53), unlike a low dose of ethanol, which enhances the increase in dopamine release in the frontal cortex of the rat induced by stress such as immobilization stress (16). It, thus, appears that the effects of ethanol, especially a low dose, and diazepam on the basal dopamine release and the increase in dopamine release induced by stress in the nucleus accumbens and the frontal cortex are considerably different. Therefore, these different effects between these drugs on the release of dopamine in the nucleus accumbens (and in the prefrontal cortex) may possibly provide one support for the present result that ethanol, but not buspirone and diazepam, produced a place preference under conditioned fear stress.

The mesolimbic dopamine system plays an important role in mediating the locomotor-stimulating activity of abused drugs: i.e., an increase in locomotor activity is one of the behavioral responses observed with several abused drugs, and has been proposed to be a dopamine-related behavior that involves pharmacological mechanisms related to the rewarding effect (51). Several previous reports have demonstrated that ethanol enhances locomotor activity in rodents (14,23, 29,42,44). More importantly, in rodents including rats, ethanol-induced behavioral sensitization is thought to contribute to the development of the rewarding effect of ethanol (2,17), and the sensitization of mesolimbic dopaminergic neurons may be correlated with stress-induced behavioral sensitization between the response to stress and abused drugs (21,

22,28). For instance, exposure to restraint stress augments the subsequent locomotor-stimulating effect of morphine in rats (41). In the present study, ethanol increased locomotor activity in rats exposed to conditioned fear stress, and this effect was enhanced by repeated exposure to conditioned fear stress. In contrast, buspirone and diazepam had no effect on locomotor activity in rats exposed to conditioned fear stress. In several previous reports, buspirone either had no effect on or decreased locomotor activity (37,46), whereas diazepam depress locomotor activity (7,12), indicating a sedating effect. In the same dose range (i.e., the doses used in the present study), diazepam is known to have both anxiolytic and sedating effects. It, thus, may be that the sedating effect of diazepam is one possible factor of masking the rewarding effect of the drug in animal behavioral studies. However, a low dose of ethanol increases locomotor activity without any alteration of dopamine concentrations in the rat frontal cortex (1). Therefore, ethanol and conditioned fear stress-induced behavioral sensitization (enhancement of the increase in locomotor activity) through enhancement of the increase in dopamine concentrations in the nucleus accumbens may provide one possible mechanism underlying the development of the ethanol-induced place preference under conditioned fear stress.

In conclusion, ethanol, but not buspirone and diazepam, produced a place preference in rats exposed to conditioned fear stress. Ethanol, buspirone, and diazepam produced no place preference in rats treated with an anxiogenic dose of pentylenetetrazole. In addition, ethanol, but not buspirone and diazepam, increased locomotor activity in rats exposed to conditioned fear stress. The locomotor-stimulating effect of ethanol was significantly sensitized by repeated exposures to conditioned fear stress. These results suggest that some stimulating effect (probably an euphoric effect), but not an anxiolytic effect, may strongly participate in the development of the rewarding effect of a low dose of ethanol under psychological stress.

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