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European Journal of Pharmacology 341 (1998) 127–130

ejp

Short communication

Conditioned fear stress induces ethanol-associated place preference in rats

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Received 10 July 1997; revised 21 October 1997; accepted 24 October 1997

Abstract

The purpose of the present study was to establish ethanol-induced place preference under conditioned fear stress (exposure to an environment paired previously with electric foot shock) in rats by using the conditioned place preference paradigm. The administration of ethanol (300 mg/kg, i.p.) with conditioned fear stress, but not without conditioned fear stress, induced a marked and significant place preference. Furthermore, additional exposure to conditioned fear stress immediately before the post-conditioning test further enhanced the development of ethanol-induced place preference. These results suggest that psychological stress may play an important role in the development of ethanol-induced place preference, and the present method may be useful for studying the mechanism of the rewarding effect of ethanol. © 1998 Elsevier Science B.V.

Keywords: Ethanol; Conditioned place preference paradigm; Place preference; Conditioned fear stress; Rewarding effect; (Rat)

1. Introduction

Ethanol is a well-known drug of abuse in everyday human life. To clarify the multiple mechanisms of the rewarding effect of ethanol in light of the etiology of alcoholism, it is important to establish animal models for human alcohol abuse. The rewarding effects of abused drugs are currently assessed by using self-administration, intracranial self-stimulation and conditioned place preference paradigms in animals. The conditioned place preference paradigm has been widely used to study the mechanisms of the rewarding effects of abused drugs, since this paradigm can assess the rewarding effects of drugs in much less time than the self-administration paradigm. However, most previous attempts to establish ethanol-induced place preference have not been successful (Van der Kooy et al., 1983; Asin et al., 1985). Therefore, the mechanism of the rewarding effect of ethanol has hitherto been poorly understood.

It has been postulated that an interaction between psychological stress and ethanol intake may play an important role in the etiology of alcoholism (Pohorecky, 1981); i.e., psychological stress may be an important motivating factor in the initiation and maintenance of ethanol intake by humans. In rats, it has been demonstrated that isolation stress, used as a psychological stress, produces an increase in ethanol intake (Parker and Radow, 1974; Nash and Maickel, 1985; Wolffgramm, 1990). Therefore, it is hypothesized that psychological stress may potentiate the rewarding effect of ethanol.

The present study attempted to establish ethanol-induced place preference in rats with conditioned fear stress as a psychological stress, using the conditioned place preference paradigm, and examined the effect of conditioned fear stress on the development of ethanol-induced place preference.

2. Materials and 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.

2.1. Animals

Male Sprague–Dawley rats (Tokyo Experimental Animals, Tokyo), weighing 170–220 g, were housed in groups

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of 4 in a temperature-controlled room (22 ± 1°C) with a 12 h light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). Food and water were available ad libitum.

2.2. Apparatus

The test box consisted of a shuttlebox (30 × 60 × 30 cm) 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 join separating the compartments. Untreated 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 in the habituation session, untreated 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). The time spent in the ethanol-associated compartment was defined as the pre-conditioning score.

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) 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 (CFS group). A control (non-CFS) group was also prepared. The control rats were individually placed in the shock chamber without foot shocks, and 24 h later were again individually placed in the same shock chamber without foot shocks for 10 min. All of 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 (2 for ethanol: 2 for saline).

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

Before this session on day 12, the rats in the CFS group were divided into two groups: the rats in one group were again individually placed in the same shock chamber without foot shocks for 10 min before the post-conditioning test (CFS/CFS group); the rats in the other group were not placed in the shock chamber (CFS/non-CFS group). 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. The time spent in the ethanol-associated compartment was defined as the post-conditioning score.

2.4. Drugs

Ethanol (Wako Pure Chemical, Osaka) was used at doses of 75, 150, 300, 600 and 1200 mg/kg, i.p. It was diluted in saline to form a 20 (v/v) % solution and injected intraperitoneally in different volumes.

2.5. Data analysis

Conditioning scores represent the difference in time (s) spent on the ethanol-paired side in the post-conditioning test versus the pre-conditioning test and are expressed as means ± S.E.M. The Wilcoxon test was used to determine whether each ethanol-treated group exhibited significant conditioning.

3. Results

As shown in Fig. 1, ethanol-treated control (non-CFS) rats showed no preference for the ethanol-associated place compared to saline-treated control (non-CFS) rats.

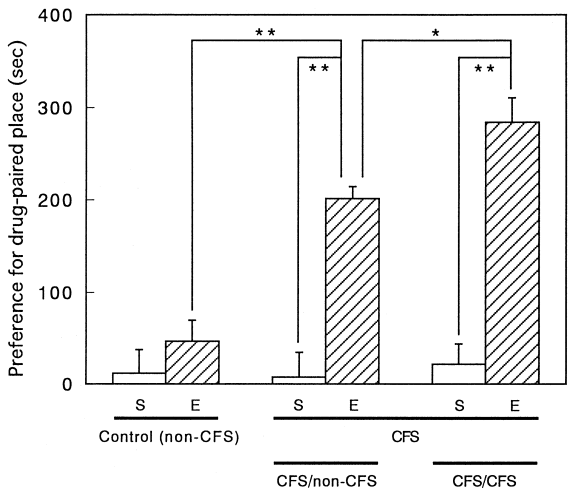

Fig. 1. Ethanol (300 mg/kg, i.p.) induced place preference under conditioned fear stress (CFS). Conditioned fear stress (exposure to an environment paired previously with electric foot shock) is regarded as a psychological stress. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for 8 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ versus respective control (Wilcoxon test). S: Saline; E: Ethanol.

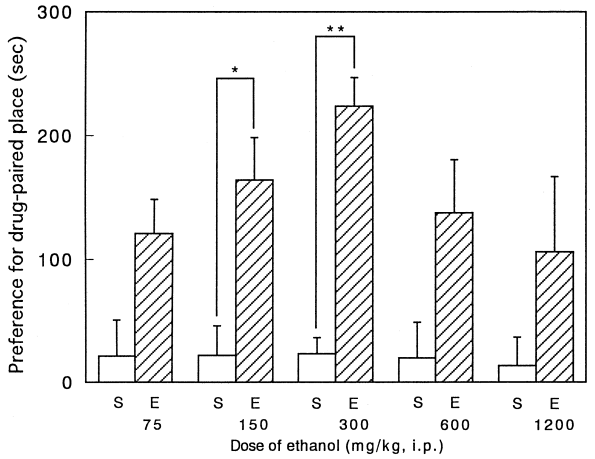

Fig. 2. Dose–response of ethanol-induced place preference under conditioned fear stress (CFS). Conditioned fear stress (exposure to an environment paired previously with electric foot shock) is regarded as a psychological stress. All animals had received CFS/non-CFS treatment. The ordinate represents preference for the drug-paired place. Each column represents the mean with S.E.M. for 8 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ versus respective control (Wilcoxon test). S: Saline; E: Ethanol.

Ethanol-treated CFS/non-CFS rats showed significant place preference (201.4 ± 12.8 s) compared to saline-treated CFS/non-CFS rats (7.6 ± 27.4 s) ($P < 0.01$) and to ethanol-treated control rats ($P < 0.01$). Moreover, the ethanol-induced place preference was significantly enhanced in ethanol-treated CFS/CFS rats (283.9 ± 26.5 s) compared to ethanol-treated CFS/non-CFS rats ($P < 0.05$) and to saline-treated CFS/CFS rats (21.8 ± 22.2 s) ($P < 0.01$).

As shown in Fig. 2, ethanol (150 and 300 mg/kg) treated CFS/non-CFS rats showed significant place preference (164.0 ± 34.3 s and 223.8 ± 23.0 s, respectively) compared to saline-treated CFS/non-CFS rats (21.9 ± 24.1 s and 23.1 ± 13.3 s) ($P < 0.05$ and $P < 0.01$). Ethanol (75, 600 and 1200 mg/kg) treated CFS/non-CFS rats showed no significant place preference compared to saline-treated CFS/non-CFS rats.

4. Discussion

Most reports have found that ethanol does not produce place preference or rather produces place aversion in rats (Asin et al., 1985; Van der Kooy et al., 1983). Generally, it has been shown that ethanol produces dose-dependent stimulation or depression (Pohorecky, 1977): low doses induce excitation, while high doses result in depression. In addition, ethanol produces a primary transient excitation followed by depression. Reid et al. (1985) hypothesized that the rewarding effect of ethanol might be due to the initial excitatory effect of the drug, resulting in ethanol-induced place preference. Furthermore, the primary excitatory effect of ethanol is thought to be reinforcing (Van der Kooy et al., 1983) and the secondary sedative effect of ethanol may mask its reinforcing effect (Asin et al., 1985). These findings suggest that the development of ethanol-induced place preference can be strongly influenced by the dosage of ethanol and the duration of conditioning after ethanol injection, and that the rewarding effect of ethanol can be clearly identified soon after drug injection.

It is commonly assumed that humans drink alcohol for its euphoric effect and to reduce anxiety induced by psychological stress. It has been shown that ethanol intake by humans increases in stressful situations (Pohorecky, 1981). It has also been demonstrated that psychological stress, in the form of isolation stress, increases ethanol intake in rats (Parker and Radow, 1974; Nash and Maickel, 1985; Wolffgramm, 1990).

Based on the above findings, we focused on the role of psychological stress in the rewarding effect of ethanol, and attempted to establish ethanol-induced place preference in rats that were exposed to conditioned fear stress as a psychological stress, using a low dose (300 mg/kg) of ethanol with a 30 min period of conditioning. The present study found that ethanol produced neither place preference nor place aversion in rats in the control (non-CFS) group, confirming the findings of previous reports (Asin et al., 1985; Suzuki et al., 1992). Most importantly, ethanol produced pronounced place preference in rats in the CFS group. Furthermore, this ethanol-induced place preference was markedly enhanced by the additional exposure to conditioned fear stress before the post-conditioning test. These data suggest that psychological stress may play an important role in the development and enhancement of the rewarding effect of ethanol.

It is known that the mesolimbic dopamine system is activated by ethanol (Gessa et al., 1985; Imperato and Di Chiara, 1986). Furthermore, this system may be involved in the rewarding effect of a low dose of ethanol (Koob, 1992). In fact, a low dose of ethanol markedly increased extracellular dopamine concentrations in the rat nucleus accumbens during the first 20 min after intraperitoneal injection (Imperato and Di Chiara, 1986). Moreover, the increase in locomotor activity produced by ethanol may be related to the rewarding effect of ethanol (Waller et al., 1986; Lewis and June, 1990), as is the case of other abused drugs (Wise and Bozarth, 1987). Lewis and June (1990) reported that open-field locomotor activity increased during the first 10 min after intraperitoneal injection of a low dose of ethanol, whereas this effect was not observed 30–40 min after the injection. Furthermore, they indicated that the threshold for brain stimulation reward decreased and the response rate increased during the first 20 min, but not 30–50 min after ethanol injection. These findings suggest that the stimulatory effect during the early period after the injection of a low dose of ethanol may be an important factor in the expression of the rewarding effect of ethanol. Conditioned fear stress is known to activate the mesolimbic dopamine system (Herman et al., 1982; Deutch et al., 1985; Inoue et al., 1994). Moreover,

Cabib and Puglisi-Allegra (1996) have demonstrated that short-term (approximately 10 min) exposure to stress enhances dopamine release from the nucleus accumbens in the mesolimbic system, promotes behavioral activation and facilitates reinforced responding. Our present results, combined with these findings, suggest that a neuronal interaction between the ethanol-enhanced mesolimbic dopamine system and psychological stress may be involved in the development and enhancement of ethanol-induced place preference. In addition, we speculate that the additional exposure to conditioned fear stress just before the post-conditioning test might provoke motivation for the central effect of ethanol (probably, euphoric effect), resulting in the enhancement of ethanol-induced place preference. Thus, it is also suggested that psychological stress may increase the motivation to resume ethanol intake.

It has been demonstrated that ethanol has not only a euphoric effect but also an anxiolytic effect, and that its anxiolytic effect appears to be a motivating factor in ethanol intake (Wilson, 1988). Conditioned fear stress-induced freezing behavior has been proposed as a model of anxiety. In place conditioning sessions in the present study, rats were immediately confined to each side of the test box after exposure to conditioned fear stress. It is likely that the rats experienced anxiety during the conditioning period. Therefore, it is possible that the anxiolytic effect of ethanol may also contribute to the development and enhancement of ethanol-induced place preference. Several behavioral studies have shown that a low dose of ethanol has little anxiolytic effect. For example, Pokk et al. (1996) reported that 500 mg/kg, but not 250 mg/kg, ethanol produced an anxiolytic effect in the plus-maze test. They also found that ethanol (250–1000 mg/kg) had little anxiolytic effect in mice that were exposed to stress. Because a low dose (e.g., 300 mg/kg) of ethanol does not have a significant anxiolytic effect, the anxiolytic effect of ethanol may not participate in the rewarding effect of ethanol in the present study.

In conclusion, we found that ethanol produces place preference under conditioned fear stress, and that additional exposure to conditioned fear stress immediately before the post-conditioning test further enhances this place preference. In addition, the ethanol-induced place preference under conditioned fear stress can be observed with a low dose of ethanol which probably has no significant anxiolytic effect. These findings suggest that psychological stress may play an important role in the rewarding effect of ethanol, and this animal model may be useful for studying the mechanism of the rewarding effect of ethanol.

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