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Repeated exposure to stress stimuli during ethanol consumption prolongs withdrawal-induced emotional abnormality in mice

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ABSTRACT

The present study was designed to ascertain the influence of repeated exposure to stress on the development of ethanol dependence in mice. Mice were chronically treated with 3% ethanol for 7 days, with or without exposure to restraint stress for 1 h/day. A significant loss of body weight was observed in the ethanol plus stress group compared with the other groups. In the ethanol plus stress group, mice exhibited persistent piloerection, which is considered to be a withdrawal sign that reflects emotion, after the discontinuation of ethanol treatment. The ethanol plus stress group also showed a marked increase in the intake of liquid diet, which is considered to be trying to avoid an unpleasant ethanol-withdrawal symptom, during ethanol withdrawal. In the hole-board test, a significant decrease in head-dipping behavior was observed in both the ethanol alone and ethanol plus stress groups at 6 h after the discontinuation of ethanol treatment. This abnormal emotion was recovered in the ethanol alone group, but not in the ethanol plus stress group, at 48 h after the discontinuation of ethanol treatment. These results suggest that repeated exposure to stress may affect the development of ethanol dependence and prolong the expression of withdrawal-induced emotional abnormality.

1. Introduction

Many studies have reported that stressful conditions are a significant risk factor for future excessive alcohol (ethanol) consumption, and thereby increase the risk for dependence and alcoholism (Enoch, 2006; Uhart and Wand, 2009). Several animal models have been used to better understand the relationship between stress and ethanol intake (Sillaber and Henniger, 2004). In addition, rodents have been widely used to evaluate developmental changes in stress-related behaviors, and repeated stress has been shown to induce many changes in behavior including an altered anxiety response, changes in locomotor activity, and aggressive behavior (Hefner and Holmes, 2007; McCormick et al., 2007; Spear, 2000, 2004).

Alcohol is one of the most commonly abused substances, and its excessive chronic intake leads to the development of ethanol dependence in both humans and laboratory animals. The development of ethanol dependence is associated with a withdrawal syndrome when ethanol consumption is discontinued or substantially reduced. A growing body of clinical evidence supports the major characteristics observed in alcoholics during an initial period of alcohol abstinence (Hershon, 1977). The most common signs include tremor, anxiety, insomnia, agitation, hypervigilance, irritability, piloerection and sometimes seizures (Hunter et al., 1974; Saitz, 1998). Similarly, animal models of ethanol dependence and withdrawal have been developed (Lester and Freed, 1973; McBride and Li, 1998). In particular, piloerection is known as emotional behavior, which relates to strong emotions such as fear or awe. Masuda et al. (1999) demonstrated emotional piloerection in mice given conditioned fear stress by means of a pass-through apparatus. In addition, we reported that the mouse exposure to repeated stress for 7 days produced a significant change in the head-dipping behavior using the hole-board test (Tsuji et al., 2000). Bilkei-Gorzó and Gyertyán (1996) reported that brighter lighting, which was highly aversive environment, increased the head-dipping behavior of rats. Indeed, it has been recognized that the change of piloerection and either increased or decreased head-dipping behavior, which were measured in the present study, are able to estimate objectively various emotional states of the animals. Several previous sociocultural studies have shown that stress may increase the risk of alcoholism. Exposure to stress is an environmental factor that has long been thought to increase alcohol consumption and predispose people to the development of alcoholism (Conger, 1956; Horton, 1943; Pohorecky, 1981, 1991; Volpicelli et al., 1999). However, the influence of repeated stress on emotional behaviors after ethanol withdrawal is not sufficiently clear.

The primary aim of the present study was to establish an animal model of ethanol-dependence using the liquid diet method (Iso, 1984,1990; Narita et al., 2000,2002) in ICR mice. Next, we

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investigated the influence of repeated exposure to stress stimuli during ethanol consumption on the changes in emotional behaviors after withdrawal.

2. Materials and methods

2.1. Animals

Male ICR mice (25–30 g) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Each mouse was kept individually in a polycarbonate cage (W22 cm × L32 cm × H13.5 cm) with sterilized PaperClean Bedding (Japan SLC Inc., Shizuoka, Japan) at a room temperature of 23 ± 1 °C and humidity of 50 ± 5% with a 12 h light-dark cycle (light on 7:00 a.m. to 7:00 p.m.). Food and water were available ad libitum until the experiment. All experiments were performed in accordance with the guidelines of the Care and Use of Laboratory Animals of International University of Health and Welfare and the Japanese Pharmacological Society.

2.2. Repeated restraint stress procedure

Mice were individually placed in a 50 ml plastic syringe (Terumo Co., Tokyo, Japan) once a day for 1 h from day 0 to day 6. Restraint stress was applied at the same time each day (between 9:00 a.m. and 10:00 a.m.) for 7 consecutive days.

2.3. Liquid diet method

To prepare the liquid diet, 99.5% ethanol (Wako Pure Chemical, Osaka, Japan) and sucrose (Wako Pure Chemical, Osaka, Japan) were mixed with skimmed milk according to the method described by Iso (1984, 1990) and Narita et al. (2000, 2002) with minor modifications. For chronic ethanol treatment, mice were individually housed and given access to a measured amount of liquid diet containing 3% (w/w) ethanol as their sole nutrient source for 7 days. Control mice were fed a liquid diet in which sucrose was substituted for ethanol in isocaloric quantities. Every 24 h, body weight and liquid diet consumption were measured and the liquid diet was replaced by fresh ethanol-containing liquid diet.

2.4. Ethanol withdrawal

Withdrawal was induced by replacing ethanol-containing liquid diet with normal liquid diet on day 7 of ethanol treatment. To quantify the intensity of the physical dependence on ethanol, withdrawal signs were rated on a scale of 0–5, as modified from a scoring system described previously (Ritzmann and Tabakoff, 1976). The ratings were as follows: 0, little or no reaction; 1, piloerection or jerking; 2, weak tremor; 3, severe tremor; 4, handling-elicited or spontaneous clonic-tonic seizure; 5, death while in a seizure. Withdrawal signs were observed at 0, 3, 6, 9, 12, 15, 18, 24, 36 and 48 h after the discontinuation of ethanol treatment. The emotional behavior after the discontinuation of ethanol treatment was evaluated using the hole-board test at 6 and 48 h after the discontinuation of ethanol treatment.

2.5. Apparatus

The exploratory behavior of mice in a novel environment was measured as previously described using an automatic hole-board apparatus (model ST-1, Muromachi Kikai Co., Ltd., Tokyo, Japan) (Takeda et al., 1998; Tsuji et al., 2000). The apparatus consisted of a gray wooden box (W50 cm × L50 cm × H50 cm, 170 lx) with four equidistant holes 3 cm in diameter in the floor. An infrared beam sensor was installed on the wall to detect the number and duration of rearing and head-dipping behaviors, and the latency to the first head-dipping. The distance of movement in the hole-board was recorded by an overhead digital video camera; the heads of the mice were painted yellow and the digital video camera followed their center of gravity. Data from the infrared beam sensor and the digital video camera were collected through a custom-designed interface (DVTrack, Model DVT-11; Muromachi Kikai) as a reflection signal. Head-dipping behaviors were double-checked via an infrared beam sensor and the overhead digital video camera. Thus, only when both the head intercepted the infrared beam and the head was detected at the hole by the digital video camera was head-dipping behavior counted. Data were analyzed and stored in a personal computer using analytical software (Comp ACT HBS, Muromachi Kikai).

2.6. Statistical analysis

Data are presented as the mean ± standard error of the mean (S.E.M.) or percentage of positive animals/total animals. The Bonferroni multiple comparison test and Chi-square test were used for the statistical evaluation of behavioral data ($P < 0.05$ and 0.01). The time course of withdrawal scores was analyzed using a two-way analysis of variance (ANOVA).

3. Results

3.1. Changes in daily body weight and intake of liquid diet in mice

Daily changes in body weight (g) are shown in Fig. 1A. On day 7, which was the last day of treatment with ethanol, a significant loss of body weight was observed in the "3% ethanol plus no stress" and "3% ethanol plus stress" groups compared with the "control milk (0% ethanol) plus no stress" group ($P < 0.05$, $P < 0.01$; Fig. 1B) and "control milk (0% ethanol) plus stress" group ($P < 0.05$; Fig. 1B). The body weight on day 7 in each of the groups was in the order "control milk (0% ethanol) plus no stress" group (33.9 ± 0.5 g) > "control milk (0% ethanol) plus stress" group (31.8 ± 0.5 g) > "3% ethanol plus no stress" group (29.5 ± 0.7 g) > "3% ethanol plus stress" group (27.4 ± 0.5 g).

Daily changes in the intake (g/10 g body weight) of liquid diet are shown in Fig. 2. On days 1–9, the control milk (0% ethanol)-treated groups consumed almost the same amount of liquid diet regardless of stress. Similarly, on days 1–7, the 3% ethanol-treated groups consumed almost the same amounts of liquid diet regardless of stress. On day 8, which was 24 h after the discontinuation of ethanol treatment, a marked increase in the intake of liquid diet was observed in the "3% ethanol plus stress" group (17.0 ± 0.6 g/10 g body weight) compared with that in the "3% ethanol plus no stress" group (13.3 ± 0.8 g/10 g body weight) ($P < 0.01$).

3.2. Expression of ethanol withdrawal in mice

Time-course changes in withdrawal scores after the discontinuation of chronic ethanol treatment are shown in Fig. 3. None of the control milk (0% ethanol)-treated mice exhibited withdrawal signs throughout the observation period, regardless of stress. On the other hand, all of the 3% ethanol-treated mice in both the stressed and non-stressed groups showed the withdrawal signs, such as piloerection, tremor and so on. The withdrawal scores in the "3% ethanol plus no stress" and "3% ethanol plus stress" groups peaked at 9 and 12 h after the discontinuation of chronic ethanol treatment, respectively. There was no significant difference between the "3% ethanol plus no stress" and "3% ethanol plus stress" groups during ethanol withdrawal (two-way ANOVA; 3% ethanol with or without stress × time interaction, $F(1,252) = 0.445$, $P = 0.510$). All of the mice in the "3% ethanol plus no stress" and "3% ethanol plus stress" groups exhibited