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Increased marble-burying behavior in ethanol-withdrawal state: Modulation by gonadotropin-releasing hormone agonist

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Abstract

A characteristic behavior in alcohol abstinence state indicates the possibility of obsessive–compulsive behavior in alcoholics. Ethanol is known to reduce hypothalamic synthesis, release, and mRNA expression of gonadotropin-releasing hormone (GnRH) that modulates serotonergic, dopaminergic, and glutamatergic systems, which experience adaptive changes on chronic exposure to ethanol. Such changes are also evident in obsessive–compulsive disorder. Therefore, it was proposed to investigate the effect of ethanol-withdrawal on marble-burying behavior in mice, particularly because it simulates some aspects of obsessive–compulsive behavior; further, the influence of GnRH agonist was studied on the same. Ethanol-withdrawal state was induced after its chronic administration, and marble-burying behavior was observed at 0, 6, 24, 48, and 96 h interval. Further, the influence of leuprolide—a GnRH agonist (50–600 μg/kg, s.c.) or fluoxetine (5–30 mg/kg, i.p.) was investigated on ethanol-withdrawal-induced changes in marble-burying behavior. The results indicated that ethanol-withdrawal led to a gradual increase in marble-burying behavior upto 48 h with peak at 24 h interval. Administration of leuprolide (100–600 μg/kg, s.c.), 30 min prior to 24 h interval, dose dependently reduced ethanol-withdrawal-induced increase in marble-burying behavior, and this effect was comparable to that of fluoxetine (15 and 30 mg/kg, i.p.). Further, twice daily administration of leuprolide (50 μg/kg, s.c), concomitant with ethanol, prevented the gradual increase in marble-burying behavior after ethanol-withdrawal and this effect was comparable to fluoxetine (5 mg/kg, i.p.). In conclusion, ethanol-withdrawal on chronic administration increases marble-burying behavior in mice; its development and expression is attenuated by leuprolide.

Keywords: Luteinizing hormone-releasing hormone; Obsessive–compulsive disorder; Leuprolide; Alcoholism; Fluoxetine

1. Introduction

Obsessive–compulsive disorder is an anxiety disorder, characterized by persistent thoughts (obsessions) that are ego-dystonic and associated with seemingly purposeful behaviors (compulsions). Obsessive–compulsive disorder is often evident in alcohol dependent patients (Lima et al., 2005), particularly in state of abstinence (Neziroglu et al., 1994). Suzuki et al. (2002) suggested that the characteristic behavior of chronic alcohol users may derive at least in part, from the co-morbidly existing obsessive–compulsive behavior. An urge to drink alcohol and relapse from abstinence is deeply associated with obsession (Nakazawa, 1999). In addition, it is reported that obsessive–compulsive disorder and alcohol dependence are associated with mutated epsilon sarcoglycan gene, which imply common genetic disposition (Hess et al., 2007). Moreover, both the conditions are associated with similar neurohumoral changes; e.g., serotonin dysfunction, hyperactivity of dopamine and glutamate (Badawy, 2003; Denys et al., 2004; El Mansari and Blier, 2006; Heinz et al., 1998, 2003; Koob, 2000; Patkar et al., 2003; Pierce and Kumaresan, 2006). This indicates that ethanol abuse may predispose obsessive–compulsive behavior.

Ethanol administration is reported to decrease GnRH secretion, content, mRNA levels in hypothalamus (Fernandez-Solari et al., 2004; Kim et al., 2003, 2005; LaPaglia et al., 1997; Morris et al., 1989; Rettori et al., 2002; Rettori and McCann, 1997); perturb GnRH/GnRH-receptor system (Bramley et al., 1999). Opioid antagonists are known to attenuate inhibitory influence of ethanol on GnRH secretion in rats (Lomniczi et al., 2000; Masotto et al., 1989), and in addition, reported to reduce

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compulsive alcohol drinking in chronic alcoholics (Pettinati et al., 2006). Recently, we have demonstrated that GnRH mediates the anti-compulsive effect of fluoxetine (Uday et al., 2007), which is used to attenuate ethanol-withdrawal syndrome (Uzbay et al., 2004; Williams, 2005). GnRH receptors have been identified in amygdala, hippocampus, anterior cingulated cortex, caudate, putamen, and thalamus (Badr and Pelletier, 1987; Jennes et al., 1988; Rance et al., 1994; Reubi and Maurer, 1984; Reubi et al., 1987). Incidentally, these regions are involved in variety of activities including the behavior seen in obsessive–compulsive disorder (Aouizerate et al., 2004) and alcoholism (Adinoff, 2004; Beresford et al., 2006; Deshmukh et al., 2005; Goldstein and Volkow, 2002). Thus, some of the behavioral changes in ethanol dependent state might be consequent to the inhibitory influence of ethanol on GnRH.

Marble-burying behavior of mice simulates some aspects of obsessive–compulsive behavior; therefore, it is often used to screen anti-compulsive drugs due to high predictive and good face validity (Joel, 2006). Hence in the present investigation, we first studied the influence of ethanol-withdrawal state on marble-burying behavior of mice, and then investigated the influence of leuprolide—a GnRH agonist on the same; particularly because it is reported to attenuate marble-burying behavior as well as ethanol-withdrawal syndrome in mice (Uday et al., 2007; Umathe et al., 2008).

2. Materials and methods

2.1. Animals

Adult male albino Swiss mice (22–25 g) were group housed (n=6–10) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*. Mice were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6 or 10) of mice was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

2.2. Drugs

Leuprolide acetate was purchased from Sigma-Aldrich Ltd., USA. Fluoxetine hydrochloride was a gift from Reliance Laboratories Ltd., India. Leuprolide acetate and fluoxetine hydrochloride were dissolved in 0.9% saline. Drug solutions were prepared fresh and doses are expressed in terms of their free bases.

2.3. Assessment of marble-burying behavior and locomotor activity

Marble-burying behavior of mice was studied as described previously (Njung'e and Handley, 1991; Uday et al., 2007). In brief, each mouse was individually placed in a plastic cage (21×38×14 cm), containing 5 cm thick sawdust bedding, and three photo cells connected to digital counter. Twenty small glass marbles (diameter 10–12 mm) were arranged on the bedding evenly spaced in four rows. After 30 min exposure, the number of unburied marbles was counted. A marble covered at least two-third of its size by saw dust was considered as 'buried'. Total number of light beam interruptions in 30 min was considered as an index of locomotor activity.

2.4. Ethanol-withdrawal state

Ethanol-withdrawal state was produced in experimental (ethanol diet) group as described previously (Hirani et al., 2002; Tabakoff et al., 1978). In brief, on day 1, all groups received nutritionally balanced liquid diet containing sucrose (9.68% w/v) and vitamin supplement (Novartis India Ltd., Mumbai) in 100 ml calibrated drinking bottle. From day 2, (8.00 h) till day 9 (8.00 h), the experimental group received liquid diet containing ethanol (5.96% w/v) instead of sucrose; while control group was pair-fed sucrose and vitamin containing liquid diet. On day 9 (8.00 h), ethanol was replaced with sucrose (ethanol-withdrawal).

2.5. Influence of ethanol-withdrawal on marble-burying behavior in mice

Marble-burying behavior was assessed at 0, 6, 24, 48, and 96 h time interval after ethanol-withdrawal. The time interval at which mice exhibited maximum marble-burying behavior was recorded in experimental (ethanol diet) group. The locomotor activity was recorded simultaneously.

2.6. Effect of acute treatment with leuprolide or fluoxetine on marble-burying behavior after ethanol-withdrawal

The earlier experiment revealed that experimental (ethanol diet) group exhibited maximum marble-burying at 24 h time interval after the withdrawal of ethanol. Therefore, experimental group was treated with leuprolide (50, 100, 300, and 600 μg/kg, s.c.) or fluoxetine (5, 10, 15, and 30 mg/kg, i.p.) or vehicle (10 ml/kg, s.c. or i.p.), 30 min prior to the recording of marble-burying behavior and locomotor activity at 24 h time interval after ethanol-withdrawal. For each of the above treatment and dose separate group (n=10) of mice was employed.

2.7. Effect of chronic treatment with leuprolide or fluoxetine on marble-burying behavior after ethanol-withdrawal

In another set of experiment, the experimental (ethanol diet) group was treated twice daily (8.00 h and 20.00 h) with leuprolide (25 and 50 μg/kg, s.c.) or fluoxetine (2.5 and 5 mg/kg, i.p.) or vehicle (10 ml/kg, s.c. or i.p.) whereas control (sucrose diet) group daily received saline (10 ml/kg, s.c. or i.p.). The above drug treatment was terminated on day 9 at 08.00 h i.e., a time at which ethanol was withdrawn. Marble-burying behavior and locomotor activity was recorded at 0, 6, 24, 48, and 96 h following ethanol-withdrawal.