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Leuprolide – A luteinizing hormone releasing hormone agonist attenuates ethanol withdrawal syndrome and ethanol-induced locomotor sensitization in mice

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Abstract

Ethanol inhibits the synthesis, content and release of hypothalamic luteinizing hormone releasing hormone (LHRH), and LHRH modulates the activity of several neurotransmitters that experience adaptive changes on chronic exposure to ethanol, and also implicate in ethanol dependence. Hence, it was contemplated that LHRH agonist such as leuprolide may influence the behavioral consequences of withdrawing ethanol in dependent state. In the present study, ethanol dependence was produced in mice by providing ethanol liquid diet for 10 days; and its withdrawal on day 11 led to physical signs of hyperexcitability with its peak at 6th h. Acute treatment with leuprolide (20 ng/mouse, i.c.v.), 10 min prior to peak, significantly attenuated hyperexcitability. Such effect of leuprolide was evident even in castrated mice, and castration significantly increased the hyperexcitability in ethanol withdrawal state. Chronic treatment with leuprolide (10 ng/mouse, twice daily, i.c.v.) till day 10 significantly reduced the signs of hyperexcitability in ethanol withdrawal state. In another set of experiment, ethanol (2.4 g/kg, i.p.) was administered on day 1, 4, 7, 10 and 15, which caused gradual increase in locomotor activity indicating ethanol-induced sensitization. Leuprolide (20 ng/mouse, i.c.v.), 10 min prior to the challenge dose of ethanol (2.4 g/kg, i.p.) on day 15 significantly attenuated the expression of sensitization to hyperlocomotor effect of ethanol. Similarly, administration of leuprolide (20 ng/mouse, i.c.v.), 10 min prior to ethanol on day 1, 4, 7 and 10 not only reduced the gradual increase in locomotor activity but also attenuated the sensitized locomotor response on day 15, indicated attenuation of development of sensitization. Leuprolide *per se* did not affect physical signs and locomotor activity in control group. In conclusion, the present study demonstrated that leuprolide treatment attenuates expression and development of ethanol dependence and sensitization in mice.

Keywords: Gonadotropin-releasing hormone; Alcoholism; Substance dependence; Swiss mice; Castration; Neuromodulator; Neuropeptides; Locomotor activity

1. Introduction

Besides endocrine effect, LHRH analogues are demonstrated to exhibit variety of effects such as antidepressant, antianxiety, analgesic, anticonvulsant, catalepsy, drug discrimination learning, and inhibition of condition avoidance response, apomorphine/amphetamine-induced stereotypic behavior, and cage climbing behavior in rodents (Kádár et al., 1990, 1992; Jain and Subhedar, 1993; De Beun, 1999; Bobyntsev et al., 2006). These non-endocrine effects are probably because LHRH neurons are projected to some of the brain regions such as amygdala, ventral tegmental area (VTA), hippocampus, cerebral cortex, anterior

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cingulated cortex, frontal cortex, caudate, putamen, thalamus, and nucleus accumbens (Merchenthaler et al., 1984; Reubi and Maurer, 1984; Badr and Pelletier, 1987; Jennes et al., 1988; Leblanc et al., 1988; Rance et al., 1994; Izvolskaia et al., 2005; Quintanar et al., 2007), which are implicated in the regulation of behavior.

Ethanol administration to rats is reported to reduce hypothalamic content of LHRH (Morris et al., 1989; LaPaglia et al., 1997), suppress its secretion (Rettori and McCann, 1997; Lomniczi et al., 2000; Fernandez-Solari et al., 2004), reduce LHRH mRNA levels (Scott et al., 1992; Kim et al., 2003, 2005) and inhibit/perturb LHRH binding to its receptor (Bramley et al., 1999). Further, naltrexone, an approved drug in treatment of alcoholism is reported to attenuate ethanol-induced inhibition of LHRH secretion in rats (Masotto et al., 1989; Lomniczi et al., 2000). Whether these inhibitory effects of ethanol on LHRH are implicated in the characteristic ethanol withdrawal syndrome and sensitization to locomotor stimulant effect of ethanol is not known.

Dopamine and serotonin have received particular attention in alcohol addiction because of their putative role in the compulsive drug seeking behavior (McBride et al., 1991; Wallis et al., 1993), and also implicate in ethanol dependence and withdrawal syndrome (Rezvani et al., 1990; Sellers et al., 1992; LeMarquand et al., 1994; Uzbay et al., 1998). Incidentally, LHRH is reported to modulate the activity of serotonin, glutamate, dopamine and opioids (Pan et al., 1988; Kádár et al., 1992; Kinoshita et al., 2007). In addition, we have recently demonstrated that leuprolide (a LHRH agonist) exhibits anticompulsive-like effect, and also mediates the anticompulsive effect of fluoxetine (Uday et al., 2007). In view of these evidences, it was speculated that ethanol dependence and withdrawal syndrome may be related to perturbations of LHRH system. To substantiate such possibility, we investigated the influence of leuprolide on ethanol dependence by employing an animal model, wherein development of dependence is indicated by hyperexcitability signs in withdrawal state (Goldstein and Pal, 1971; Jung et al., 2005). Since, leuprolide modulates sex hormones levels which are reported to influence behavior, the studies were also carried out in castrated state.

Chronic use of ethanol not only produces tolerance to rewarding effects, but also simultaneously sensitizes drug seeking behavior. Sensitization plays a crucial role in drug dependence (Koob and Le Moal, 1997; Robinson and Berridge, 2001). Ethanol is reported to stimulate locomotor activity, which gradually increases with repeated dosing, and leads to a sensitized response on its administration after a brief abstinence. As this type of sensitization simulates well with drug seeking behavior in alcoholics (Lessov and Phillips, 1998; Robinson and Berridge, 2001), a method based on this principle described by Kotlinska et al. (2007) is employed in the present study to assess the effect of leuprolide on sensitization to locomotor stimulant effect of ethanol.

2. Materials and methods

2.1. Animals

Adult male albino Swiss mice (22–26 g) were randomized and group housed ($n = 6$–10) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25 ± 2 °C, $55 \pm 2\%$). They received standard rodent chow (Trimurti Feeds, Nagpur, India) and water *ad libitum*. Mice were acclimatized to laboratory conditions for 7 days before carrying out experiments. All the experiments were carried out in a noise-free room. Separate groups ($n = 6$ or 10) of mice were used for each set of experiments. The animal studies were approved by 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. Drug solutions and administration

Leuprolide acetate –a LHRH agonist (Sigma Aldrich, St. Louis, USA) was dissolved in artificial cerebrospinal fluid (aCSF) having composition 0.2 M NaCl, 0.02 M NaH_2CO_3, 2 mM KCl, 0.5 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.8 mM $MgCl_2$, 0.5 mM Na_2SO_4, and 5.8 mM D-glucose. All other chemicals were of analytical grade. The selected doses of leuprolide (5, 10 and 20 ng/mouse, i.c.v.) were based on our preliminary observations.

2.3. i.c.v. Cannulation

The i.c.v. cannulation was carried out as described earlier (Umathe et al., 2008). In brief, mice were anesthetized with ketamine (100 mg/kg, i.m.) and xylazine (5 mg/kg, i.m.). A guide cannula (Plastic One, VA, 24 gauge) was steriotaxically implanted with the stereotaxic coordinates from Paxinos and Franklin [AP −0.82 mm; ML +1.5 mm and DV +2.0 mm; related to bregma]. The guide cannula was secured to the skull using mounting screws (Plastic One) and dental cement (Dental Products of India, Mumbai). A stainless steel dummy cannula was used to occlude the guide cannula when not in use. The animals were then allowed to recover for a week under the cover of cefotaxim (50 mg/kg, s.c.), during which they were habituated to the experimental protocols to minimize non-specific stress. Injections were made using a Hamilton microliter syringe