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Original Research Article

Role of L-lysine in Ethanol Induced Behavioral Changes in Mice

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Abstract

Lysine, (S)-2,6,-diaminohexanoic acid, is a basic amino acid. Following ingestion, L-lysine is absorbed by the active transport process from the lumen of the small intestine into the enterocytes. L-lysine is a 5-HT_4 antagonist that can increase and decrease ethanol intake when they are given intraperitoneal administration. 5-HT_4 antagonist can block the rewarding and motivation effect as indicated by attenuation of sensitization to the locomotors stimulant effect of ethanol, decreased ethanol-induced conditioned placed preference, and reduced ethanol drinking. Young healthy mice (21–30 g) were group-housed (five per cage) in opaque polypropylene cages Animals were naive to drug treatment and experimentation at the beginning of all studies. Each experimental group was comprised of five mice. Testing was carried out in counterbalanced order concerning the treatment conditions in the noise-free room. Locomotor activity and conditioned place preference was assessed followed by acute and chronic exposure of ethanol to animals. The results revealed that acute as well as administration of L-lysine (20 and 40 mg/kg, i.p.) pre-treatment, 30 min before the test significantly reduced place preference in ethanol control-treated groups. In locomotor activity L-lysine (20 and 40 mg/kg, i.p.) pre-treatment, 30 min before the test significantly reduced locomotor count in ethanol control-treated groups in both acute and chronic groups. In conclusion, Results indicated that l-lysine exhibited an inhibitory influence against ethanol-induced behavioral changes in mice.

Keywords: L-lysine; 5-HT_4 antagonist, Ethanol dependence; Locomotor Activity; Conditioned Place Preference.

Introduction:

Ethanol is one of the most widely abused addictive drugs and has hazardous health consequences resulting from its chronic use[2]. Ethanol produces a striking array of behavioral effects in humans that are dependent on the dose of ethanol administered[3]. When used in low to moderate quantities, it relieves anxiety and fosters a feeling of well-being and euphoria. Alcohol abuse is a pattern of drinking that results in harm to one's health, interpersonal relationships, or ability to work[9]. Alcohol abuse can result in brain damage which causes impairments in executive functioning

such as impairments to working memory, visuospatial skills, and can cause an abnormal personality as well as affective disorders to develop[11]. Several neurotransmitter systems like glutamate, noradrenaline, dopamine, serotonin, opiates, gamma-aminobutyric acid (GABA), and certain plasma membrane ion channels like voltage-sensitive calcium channels have been implicated in the effects of ethanol[4,15].

Serotonin is produced in and released from neurons that originate within discrete regions, or in the nuclei[12]. The neurotransmitter serotonin has long been a target of interest for potential pharmacotherapies for alcoholism because of the well-established link between serotonin depletion, impulsivity, and alcohol drinking behavior in mice and humans[6].

Lysine, (S)-2,6, -diaminohexanoic acid, is a basic amino acid. Following ingestion, L-lysine is absorbed by the active transport process from the lumen of the small intestine into the enterocytes[13]. The 5-HT_4 antagonist increased and decreased ethanol intake when they are given intraperitoneal administration. 5-HT_4 antagonist can block the rewarding and motivation effect as indicated by attenuation of sensitization to the locomotors stimulant effect of ethanol, decreased ethanol-induced conditioned placed preference, and reduced ethanol drinking. Functional and binding studies have shown that the 5-HT4 receptor, is the G-protein coupled receptor which positively coupled to adenylate cyclase and increases the cAMP production or by generating IP_3 /DAG as second messengers. 5-HT receptor subtype 5-HT4 receptor may involve in the control of voluntary ethanol intake in mice. The high densities of this receptor in the nucleus accumbens and other regions suggest the influence of ethanol intake with its reinforcing properties. The present study is carried out to evaluate the efficacy of serotonin 4 receptor antagonist on ethanol-induced motivational and rewarding effect in mice[1].

Animals

Experimental Animals (Swiss Albino) Mice will be procured from Animal House of Rajarshi Shahu College of Pharmacy Buldana, Dist.-Buldana, and will be used in this study. The animal care and handling will be done according to the guidelines set by the CPCSEA. Young healthy mice (21–30 g) were group-housed (five per cage) in opaque polypropylene cages (28×21×14 cm) and maintained at the local conditions (23±2 ^{0}C and 40±2 % humidity) under 12:12 h light/dark cycle, with free access to rodent chow and tap water. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India. Animals were naive to drug treatment and experimentation at the beginning of all studies. Each experimental group was comprised of five mice. Testing was carried out in counterbalanced order concerning the treatment conditions in the noise-free room.

Drugs and solutions

L-LYSINE [Base] Monohydrate [$C_6H_{14}N_2O_2H_2O$] M.W.164.21 (Research-lab Fine Chem Industries Mumbai), ethanol (Changshu Yangyuan Chemical, China) used in the present study were dissolved in 0.9% saline. Drug solutions were prepared fresh, and doses of L-lysine are expressed in terms of its free base. The doses of l-lysine were selected based on previous reports[5].

Role of l-lysine in alcohol addiction

The 5-HT_4 antagonist increased and decreased ethanol intake when they are given intraperitoneal administration. 5-HT_4 antagonist can block the rewarding and motivation effect as indicated by attenuation of sensitization to the locomotors stimulant

effect of ethanol, decreased ethanol-induced conditioned placed preference, and reduced ethanol drinking. Functional and binding studies have shown that the 5-HT4 receptor, is the G-protein coupled receptor which positively coupled to adenylate cyclase and increases the cAMP production or by generating IP_3 /DAG as second messengers. 5-HT receptor subtype 5-HT4 receptor may involve in the control of voluntary ethanol intake in mice. The high densities of this receptor in the nucleus accumbens and other regions suggest the influence of ethanol intake with its reinforcing properties[8].

Methods

Locomotor Activity

Locomotor activity was assessed in an acto-photometer (VJ Instruments, Amaravati, India), having a diameter of 40 cm, equipped with three infrared beam cells pair (pair: one emitter and one receiver), 20 cm apart from each other and located on the walls of the circular arena and were connected to the digital counter. Locomotor activity was expressed in terms of the total number of counts of beams interruptions in 30 min.[7].

Conditioned place preferences

CPP experiments were conducted in a three-chambered place conditioning apparatus connected to a digital recorder and housed in a light- and sound-attenuated chamber. Two (6.6″×5″) conditioning chambers, one white with a rough floor and the other black with a smooth floor, were connected by a smaller (2.85″×5″) central access chamber with gray walls and a smooth polyvinyl chloride floor. In addition, each chamber was equipped with a house light, with the luminance (40 lx) adjusted such that the environmental (visual and tactile) cues should not produce a significant baseline preference for a specific chamber. All chambers were separated by manually operated guillotine doors. The individual chamber activity was determined by photo beam breaks recorded by a digital recorder. The CPP test was carried out using Swiss mice as per the method described by Font et al. (2008) with slight modifications. This experiment involved three phases such as habituation (one session), conditioning (eight sessions), and testing (one session)[3].

Administration of Drug

L-lysine was administered intraperitoneally

Statistical analysis

The data from the present investigations were analyzed by parametric tests[15], using either one-way ANOVA followed by Tukey's test or two-way repeat measure ANOVA followed by the Bonferroni test for multiple comparisons. All values are expressed as the mean _ SEM of 6–7mice per group. A value of *p* 0.05 was considered statistically significant in all the cases.

Result

Table 1A: Acute effect of L- Lysine on the ethanol-induced conditioned place preference

Groups	Conditioned Place Preference in (sec)
Vehicle + saline	10.33 ±51.10
L- Lysine (10mg/kg i.p.) + Saline	39.00±24.12
L- Lysine (20mg/kg i.p.) + Saline	29.67±32.91
L- Lysine (40mg/kg i.p.) + Saline	15.50±32.41
Vehicle + Ethanol	383.0±26.3$^{\#}$

L- Lysine (10mg/kg i.p.) + Ethanol	363.2±43.16
L- Lysine (20mg/kg i.p.) + Ethanol	108.8±50.69*
L- Lysine (40mg/kg i.p.) +Ethanol	88.67±40.83**

Each value represents the Mean ± S.E.M. (n=6) using one-way ANOVA following Tukey's multiple comparison test &P < 0.001 as compared to control, *P<0.05 and **P < 0.01 as compared to ethanol.

Table 1B: Chronic effect of L- Lysine on the ethanol-induced conditioned place preference.

Groups	Conditioned Place Preference in (sec)
Vehicle + Saline	36.50±62.69
L- Lysine (10mg/kg i.p.) + Saline	32.50±72.05
L- Lysine (20mg/kg i.p.) + Saline	47.33±58.08
L- Lysine (40mg/kg i.p.) + Saline	52.00±45.17
Vehicle + Ethanol	587.0±46.25#
L- Lysine (10mg/kg i.p.) + Ethanol	328..7±56.45*
L- Lysine (20mg/kg i.p.) + Ethanol	286.7±29.68**
L- Lysine (40mg/kg i.p.) +Ethanol	105.2±13.79***

Each value represents the Mean ± S.E.M. (n=6) using one-way ANOVA following Tukey's multiple comparison test #P < 0.001 as compared to control, *P<0.05, **P < 0.01 and ***P < 0.001 as compared to ethanol.

Table 2A: Effect of L- Lysine on the expression sensitization to the locomotor stimulant effect of ethanol.

Groups	Number of locomotor counts (in 30 min)
Saline(ch) + Vehicle(a) + Saline(a)	228.3±55.77
Saline(ch) + Vehicle(a)+ Ethanol(a)	496.5±31.81@
Ethanol(ch)+Vehicle(a)+Ethanol(a)	1163±58.08#
Ethanol(ch)+ L- Lysine (10mg/kg)(a) + Ethanol(a)	988.2±42.61
Ethanol(ch)+ L- Lysine (20mg/kg)(a) + Ethanol(a)	849.5±57.39*
Ethanol(ch)+ L- Lysine (40mg/kg)(a) + Ethanol(a)	819.8±62.82**

Each bar represents the mean ± S.E.M. of n=6 mice per group. @P < 0.05 vs. [saline (ch) + vehicle (a) + saline (a)]; #P < 0.001 vs. [saline (ch) + vehicle (a) + ethanol (a)], *P < 0.05, **P < 0.01 vs. [ethanol (ch) + vehicle (a) + ethanol (a)] treated group (One-way ANOVA followed by Tukey's multiple comparison test). (a): acute; (ch): chronic.

Table 2B: Effect of L- Lysine on the development of sensitization to locomotor stimulant effect of ethanol.

Groups	Number of locomotor counts (in 30min)
Saline(ch) + Saline(ch) + saline(a)	203.5±17.03
Saline(ch) + Saline(ch)+ Ethanol(a)	333.3±17.85
Saline(ch)+Ethanol(ch)+Ethanol(a)	825.3±46.37$
L- Lysine (10mg/kg)(ch)+ Ethanol(ch)+Ethanol(a)	702.2±37.85*
L- Lysine (20mg/kg)(ch)+ Ethanol(ch)+Ethanol(a)	613.9±31.08#
L- Lysine (40mg/kg)(ch)+ Ethanol(ch)+Ethanol(a)	519.1±40.10@

Each bar represents the mean ± S.E.M. of n=6 mice per group. $P < 0.001vs. [saline (ch) + saline (ch) + ethanol(a)]; *P<0.05 [saline (ch)+ ethanol(a) +ethanol (a), #P < 0.01, @P < 0.001 vs. [Saline (ch) + ethanol (ch) + ethanol (a)] treated group (One-way ANOVA followed by Tukey's multiple comparison test). (a): acute; (ch): chronic.

Conditioned Place Preference

Acute effect of L- Lysine on the ethanol-induced conditioned place preference.

L-lysine (20 and 40 mg/kg, i.p.) pretreatment, 30 min before the test significantly reduced place preference in ethanol control-treated groups. Values are mean _ SEM of six observations per group. * p< 0.001 vs. respective vehicle treatment in ethanol diet withdrawn group (one-way ANOVA followed by Tukey's posthoc test). [F (5,30) 294.33, p < 0.0001], whereas a lower dose (10 mg/kg, i.p.) did not influence reduced place preference (p > 0.05).

Chronic effect of L- Lysine on the ethanol-induced conditioned place preference.

Chronic treatment with L-lysine (20 and 40 mg/kg, i.p.), during ethanol conditioning sessions, significantly reduced place preference in ethanol control-treated groups. Values are mean _ SEM of six observations per group. * p< 0.001 vs. respective vehicle treatment in ethanol diet withdrawn group (one-way ANOVA followed by Tukey's posthoc test). [F (5,30) 482, p < 0.0001], whereas a lower dose (10 mg/kg, i.p.) shows less influence on place preference (p > 0.05).

Locomotor Activity:

Effect of L- Lysine on the expression sensitization to the locomotor stimulant effect of ethanol.

L-lysine (20 and 40 mg/kg, i.p.) pretreatment, 30 min before the test significantly reduced locomotor count in ethanol control-treated groups. Values are mean _ SEM of six observations per group. * p< 0.001 vs. respective vehicle treatment in ethanol diet withdrawn group (one-way ANOVA followed by Tukey's posthoc test). [F (5,30) 243.20, p < 0.0001], whereas a lower dose (10 mg/kg, i.p.) did not significantly lower the locomotor count (p > 0.05).

Effect of L- Lysine on the expression sensitization to the locomotor stimulant effect of ethanol.

Chronic treatment with L-lysine (20 and 40 mg/kg, i.p.) pretreatment, 30 min before the test significantly reduced locomotor count in ethanol control-treated groups. Values are mean _ SEM of six observations per group. * p< 0.001 vs. respective vehicle treatment in

ethanol diet withdrawn group (one-way ANOVA followed by Tukey's posthoc test). [F (5,30) 305.90, $p < 0.0001$], whereas a lower dose (10 mg/kg, i.p.) shows less significant decreases in the locomotor count ($p > 0.05$)

Discussion

Ethanol acts on many cellular targets of several neuromodulators within many neural networks in the brain. There is evidence that suggests that 5-HT_4 is an important target for ethanol in the brain. Ethanol actions at 5-HT_4 -R contribute to the behavioral changes in animals and humans. 5-HT_4 receptor-mediated neurotransmission is involved in the development of behavioral sensitization produced by the drug of abuse. It has been shown that the locomotor stimulatory and stereotype-inducing effects of these drugs can be attenuated by both competitive and uncompetitive 5-HT_4 receptor antagonists. 5-HT_4-R antagonist treatment has the potential to interfere with neuroadaptive changes in the brain that contribute to the maintenance of addictive behavior. It has been found that chronic consumption of ethanol is responsible for the upregulation of the 5-HT_4 receptor and 5-HT_4 -R appears to adapt to the inhibitory effects of alcohol by increasing their excitatory activity through glutamate. Therefore, the drugs which can antagonize the 5-HT_4 -R are useful in the reduction of ethanol dependence and tolerance by suppression of the hyper glutaminergic state. L-lysine is a non-selective 5-HT_4 -R antagonist found to inhibit the acquisition of morphine self-administration in rats.

It has been reported that 5-HT4 receptors are involved in the modulation of acetylcholine release. 5-HT_4 agonist such as cisapride is all able to increase Ach release from electrically or nicotine stimulated gastrointestinal tract since they also show an affinity for other subtypes of 5HT receptor as well as for dopaminergic and muscarinic ones[10]. The simultaneous administration of L-lysine and zinc reduced the lead and ethanol-induced biochemical alterations. Literature also suggests that lysine affects5-HT_4 receptor not only in the guts but also in the brain[16]. The mechanism by which L-lysine inhibited the rewarding effects of ethanol is not known. Recently Evidence was obtained suggesting that 6 g/day of adjunctive L-lysine treatment is a sufficient dose for increasing blood L-lysine levels above the nutritional, naturally occurring levels, without inducing adverse side-effects. In addition, there was a significant decrease in the positive PANSS scores and WCST over the whole study period[17]**.**

We observed that the acute effect of L-Lysine (20 and 40 mg/kg, i.p.) pretreatment, 30 min before the test significantly reduced place preference in ethanol control-treated groups. When chronic treatment was carried out with L-lysine (20 and 40 mg/kg, i.p.), during ethanol conditioning sessions significantly reduced place preference in ethanol control-treated groups. Further, the locomotor activity was observed where, acute treatment with L-lysine (20 and 40 mg/kg, i.p.) pretreatment, 30 min before the test significantly reduced locomotor count in ethanol control-treated groups. When chronic treatment was carried out with Chronic treatment with L-lysine (20 and 40 mg/kg, i.p.) pretreatment, 30 min before the test significantly reduced locomotor count in ethanol control-treated groups.

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