

SHORT COMMUNICATION

NMDA ANTAGONISTS INHIBIT THE DEVELOPMENT OF ETHANOL DEPENDENCE IN RATS

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The influence of non-competitive NMDA receptor antagonist, 1-amino-3,5-dimethyl-adamantane (memantine), and glycine_B site antagonist, 7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2(1H)-quinolone (L-701,324), on the development of ethanol dependence was investigated in Wistar rats. The development of ethanol dependence was induced by intragastric administration of 20% w/v ethanol, three times a day at increasing doses. The results were quantified using withdrawal audiogenic seizures, 12 h after the last ethanol administration. Memantine (3.75 or 7.5 mg/kg) and L-701,324 (2.5 or 5 mg/kg), given before ethanol administration, prevented the development of ethanol dependence. Our results support the data that NMDA receptors are involved in the development of ethanol dependence.

Key words: *ethanol, dependence, memantine, L-701,324, rats*

INTRODUCTION

Accumulated evidence suggests that ethanol withdrawal seizures are blocked by the competitive and non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists (see [14] for review). However, the therapeutic usefulness of most of these substances is limited since they cause psychosis-like side effects in man.

Among the non-competitive NMDA antagonists, memantine is clinically used for the treatment of dementia and spasticity [7]. Hölter et al. [6] reported that memantine reduced ethanol deprivation symptoms without any sedative, disphoric, or stimulant side effects in rats. L-701,324 is a highly selective, orally active antagonist of NMDA/glycine (glycine_B) site [13]. Given acutely, it is a potent inhibitor of seizure activity, induced by the cessation of chronic ethanol administration [8]. Moreover, L-701,324 and memantine produced an almost complete generalization to the ethanol cue in rats trained to discriminate ethanol [2, 9].

In the present study, the effects of the clinically useful, non-competitive NMDA receptor antagonist, memantine, and glycine_B site antagonist, L-701,324, on the development of ethanol dependence was studied in Wistar rats.

MATERIALS and METHODS

Male Wistar rats (180–250 g) were subjected to adaptation for 7 days, prior to the experiment, and during this time they were kept at constant temperature, humidity, and controlled light-dark cycle.

Chronic administration of ethanol

The currently used ethanol regimen was based upon procedures first presented by Majchrowicz [11] with the modification described by Adams et al. [1]. Ethanol administration was initiated by giving orally 3 g/kg ethanol (20% w/v; 95% ethyl alcohol in tap water) at 8 a.m. Ethanol administration was repeated every 8 h during day 1. On the second day, the ethanol dose was increased to 3.5 g/kg. After this initial period, a dose of 4 g/kg of ethanol was given every 8 h for 5 days. Rats (8–10 per group) that had fully lost their righting reflex, were given only half of the ethanol dose. The last ethanol dose was given around 6 a.m. on day 8 of the treatment. Using this regimen, the animals received 10–12 g/kg ethanol per day. At

10–12 h after the last ethanol dose, rats were tested individually for ethanol withdrawal signs by bringing one animal at a time to the experimental room, where they were placed in a wide, glass cylinder. At this time the occurrence of audiogenic seizures was examined by ringing an electric buzzer (92 dB, signal lasting up to 90 s). The audiogenic stimulus was immediately discontinued upon the initiation of seizure activity.

Drugs

Glycine_B site antagonist, L-701,324 (7-chloro-4-hydroxy-3-(3-phenoxy)phenyl-2-(1H)-quinolone, Merck Sharp & Dohme, Natick, USA), was given intragastrically (*ig*), twice a day as a suspension in a 0.5 % solution of methyl cellulose, 30 min before ethanol or tap water administration for 7 days. The non-competitive NMDA receptor antagonist, memantine (1-amino-3,5-dimethyl-adamantane, Merz & Co., Frankfurt am Main, Germany), was dissolved in physiological saline and given intraperitoneally (*ip*) three times a day, 15 min before each ethanol or tap water administration during the development of ethanol dependence (7 days). Control animals received tap water instead of ethanol.

Data analysis

The audiogenic seizure activity was analyzed using the χ^2 test with Yates' correction. Values between $p < 0.05$ to $p < 0.001$ were considered as statistically significant.

RESULTS and DISCUSSION

The influence of glycine_B site antagonist, L-701,324, and non-competitive NMDA receptor antagonist, memantine, on the development of ethanol dependence was evaluated. The results were expressed as a percent of animals with audiogenic seizures during ethanol withdrawal.

As shown in Figure 1, a significant increase in the number of animals showing audiogenic seizures was observed in groups chronically treated with ethanol, that were withdrawn from the ethanol treatment for 12 h, as compared to the vehicle-treated control animals (showing no seizures). Earlier findings have shown that the intensity of withdrawal signs reaches maximum at 10–12 h after discontinuation of the applied ethanol regimen. The chronic administration of the glycine_B site antagonist, L-701,324 (2.5 or 5.0 mg/kg, *ig*,

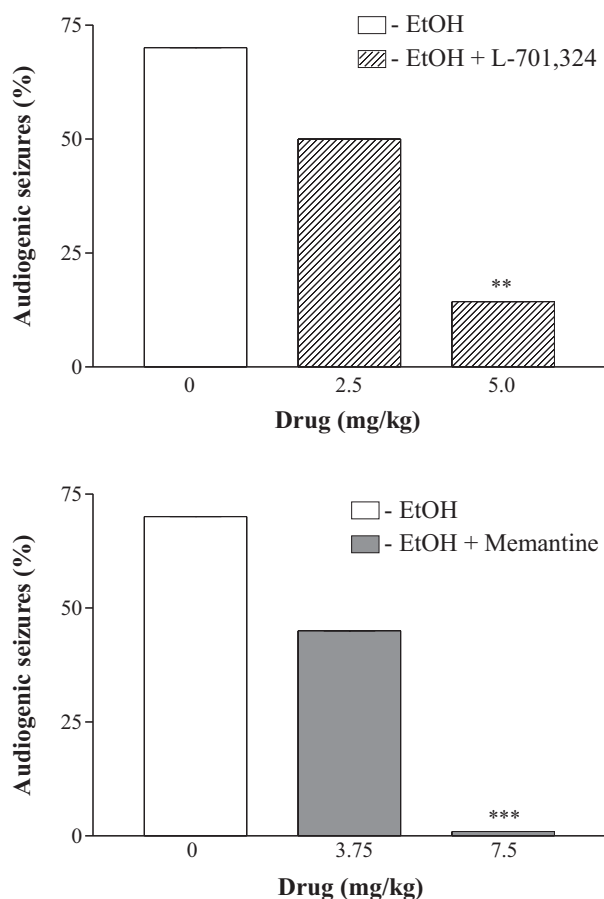


Fig. 1. Effect of chronic administration of the glycine_B antagonist, L-701,324 (2.5 or 5.0 mg/kg, *ig*, twice a day; upper panel) and non-competitive NMDA receptor antagonist, memantine (3.75 or 7.5 mg/kg, *ip*, three times a day; bottom panel), on the development of ethanol dependence measured as ethanol withdrawal audiogenic seizures in rats. Chronic administration of ethanol significantly increased the number of audiogenic seizures in rats that were withdrawn from the ethanol treatment for 12 h. Data represent percentage incidence of audiogenic seizures in the group of rats (8–10 per group). Control animals that received tap water instead of ethanol, showed no seizures (data not shown). ** $p < 0.01$; *** $p < 0.001$ as compared to the ethanol withdrawal rats

twice a day), and non-competitive NMDA receptor antagonist, memantine (3.75 or 7.5 mg/kg, *ip*, three times a day), prevented the development of ethanol dependence measured as an ethanol withdrawal seizures in rats. None of the rats that had received L-701,324 or memantine, and tap water (instead of chronic administration of ethanol) indicated audiogenic seizures in the final experimental test.

Abstinence, after chronic exposure to ethanol, often leads to a physical withdrawal syndrome including symptoms in humans such as tremor, agitation, delirium and in severe cases, convulsions and

brain damage [15]. The convulsive seizures, spontaneous or induced by sound (audiogenic seizures), are one of the severe withdrawal signs in rats [11]. NMDA receptors are a subclass of excitatory neurotransmitter receptors that play important physiological role in vertebrate nervous systems [12]. Since the studies have demonstrated that intoxicating concentrations of ethanol inhibit the NMDA receptors [10], it is conceivable that a substantial component of alcohol withdrawal hyperexcitability may be related to an adaptive upregulation of NMDA receptor function due to chronic exposure. Administration of NMDA receptor antagonists reduces the behavioral score of the withdrawal symptomatology [14] while NMDA receptor agonists worsen it [3]. On the basis of our study we can say that non-competitive NMDA antagonist, memantine, and glycine_B site antagonist, L-701,324, given chronically before every ethanol administration could block NMDA receptors more stably than ethanol, take control over these receptors, and in this way they could prevent the influence of ethanol on these receptors. Because these substances are probably devoid of abuse potential, they could prevent the development of ethanol dependence, expressed as a withdrawal seizures, in the above-described way.

In conclusion, our results support the data that NMDA receptors are involved in the development of ethanol dependence. However, the mechanism of development of ethanol dependence is probably much more complex, so we cannot exclude the involvement of γ -aminobutyric acid (GABA) or dopamine neurotransmission in the development of ethanol dependence and withdrawal (see [5] for review). On the other hand, although NMDA receptor antagonists are known to suppress several types of seizure activities (see [4] for review), and they are devoid of many side effects characteristic of competitive and non-competitive NMDA antagonist, the usefulness of both substances used in general prevention of the development of ethanol dependence needs more experiments.

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