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Discriminative Stimulus Effects of Glutamate Release Inhibitors in Rats Trained to Discriminate Ethanol

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HUNDT, W., S. HÖLTER AND R. SPANAGEL. *Discriminative stimulus effects of glutamate release inhibition with rats trained to discriminate ethanol*. PHARMACOL BIOCHEM BEHAV **59**(3) 691–695, 1998.—In a drug discrimination paradigm with rats trained to discriminate ethanol (1 g/kg IP) from saline we studied two substances, lamotrigine and riluzole, which are regarded as glutamate release inhibitors concerning their ability to substitute for ethanol. Both substances have been shown to act primarily on voltage-gated sodium channels; however, Lamotrigine dose dependently generalized to the ethanol cue, whereas riluzole did not. These results reflect the different high-dose effects of both sustances at voltage-gated calcium channels, where lamotrigine has inhibitory effects, but not riluzole, and provide further evidence for a role of voltage-gated calcium channels in the mediation of the effects of ethanol. © 1998 Elsevier Science Inc.

Drug discrimination Rats Ethanol Lamotrigine Riluzole Glutamate release inhibitors

THERE is accumulating evidence that glutamatergic mechanisms are involved in the mediation of the discriminative stimulus effects of ethanol. The effect of ethanol on glutamatergic neurotransmission is basically an inhibitory one. For example, noncompetitive and competitive NMDA-antagonists have been shown to substitute fully for the ethanol cue in drug-discrimination paradigms (6,8,27). Several in vitro studies further demonstrated that ethanol also decreases the release of glutamate (2,17,26), possibly via an opioid-dependent process (22,23), by blockade of NMDA receptors mediating gluta-mate release (2) or by activation of adenosine A1 receptors (4). Ethanol is also thought to enhance uptake and tissue concentration of glutamate (12).

However, it is unclear whether these neurochemical processes produce specific behavioral effects and how they contribute to the internal ethanol cue. To further elucidate the implications of glutamate release in relation to the behavioral effects of ethanol, we investigated the effects of riluzole and lamotrigine, which are both regarded as glutamate release inhibitors, in rats trained to discriminate ethanol from saline.

METHOD

Animals

Male Wistar rats (Max Planck Institute of Psychiatry, Martinsried, Germany; $n = 24$), weighing 250–270 g, were housed individually with free access to water. During the experimental period their weight was maintained at about 80% of that under free-feeding conditions by restricting their daily food consumption. Animals were kept in a climate-controlled room under a 12 L:12 D cycle, with the light phase commencing at 0700 h.

Apparatus

Standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) were used. Each chamber was equipped with two levers, one on either side of and equidistant from a food cup. The chambers were contained in ventilated, sound-attenuated cubicles equipped with a house light. The experiments were controlled by a computer connected to the chambers

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through LVB interfaces (Med Associates Inc., East Fairfield, VT) using a modified version of the software package as described by Spencer and Emmett-Oglesby (30).

Discrimination Training

The responding of the animals was shaped by reinforcement of lever pressing using food delivery according to an increased fixed ratio (FR). Once animals had reached a fixed ratio of 10 responses for each food pellet (FR10) (45 mg pellets, Bioserve, Frenchtown, NJ), drug and vehicle training sessions began. Training sessions began 10 min after an injection of either ethanol (1 g/kg IP; 12% v/v solution) or the appropriate volume of saline and ended after 15 min. Responses on the correct lever were reinforced and recorded, and those on the incorrect one were recorded only. The left lever was designated as the drug lever in 50% of the animals and the right one for the remaining animals. During each training session, the first 10 presses on either lever designated the "selected lever" were used to ascertain acquisition of stimulus control. Rats received a randomized sequence of training sessions (one session per day; Monday–Friday), with a maximum of three consecutive drug or vehicle training sessions. The criterion for stimulus control was set at 8 consecutive correct lever selections out of the last 10 sessions, with at least 90% drug- or vehicle-appropriate responses during these sessions.

Discrimination Testing

Tests were conducted twice weekly, with either ethanol or vehicle training on the intervening days. The day prior to testing all rats were trained with saline. Testing commenced after the rats had been placed in the chambers, which was 10 min after either ethanol or saline administration. Test sessions were terminated either after one FR (10 presses) had been completed or 5 min had elapsed. No responses were reinforced during these sessions. Two measures of discrimination were obtained. A quantal measure, which was derived from the percentage of animals tested that selected the ethanol lever, and a graded measure, which was calculated from the number of responses on the drug lever and the total number of responses on both levers until the first fixed ratio was completed (first 10 presses on either lever designated it as the selected lever). Thus, 19 possible responses could be elicited, and a percentage score was determined for each treatment. In the figures the graded measure is used. The time required to complete the first ratio (lever response latency) served as an additional measure of responding, indicating a possible impairment of responding by the injected substance itself. Animals that did not reach a minimum of 10 responses on either the saline or the ethanol lever within 5 min were excluded from the data analysis.

The following tests were conducted: (a) ethanol dose–response test: following acquisition of discrimination, generalization tests were conducted with four doses of ethanol (0.25–1.5 g/kg, IP) to obtain a dose–response relationship for discrimination. The doses were tested in a randomized order. (b) Generalization test: instead of ethanol, animals were injected IP with a vehicle solution of the substance to be tested and were placed into the chambers after a certain time interval that depended on the substance used (Table 1). The injected volumes were the same as during the ethanol training sessions. Drugs and doses were injected in a randomized order in all tests.

Drugs

Dehydrated 96% ethanol was obtained from the hospital pharmacy (Schwabing City Hospital, Munich, Germany) and diluted to a 12% solution (v/v) with 0.9% saline. The following drugs were used in the study: riluzole [2-amino-6-(trifluoro-methoxy)-benzothiazole] (RBI, Cologne, Germany) and lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] (generously provided by Wellcome Ltd., Beckenham, UK). Riluzole was administered at doses of 1.0, 4.0, and 6.0 mg/kg, lamotrigine at 5.0, 10.0, and 30.0 mg/kg. The doses administered were similar to those used in previous behavioral tests (13,28). As pretreatment time we chose 30 min for riluzole, because pharmacodynamic data show that glutamate release inhibition for riluzole is maximal after 40 min (3). Lamotrigine, which shows rapid bioavailability (24), was given 15 min before testing. Both substances were dissolved in saline.

Statistics

A statistical analysis of lever latency data was performed by using a one-way-ANOVA. Where significant main factor differences ($p < 0.05$) were found, a Student–Newman–Keuls test was performed as a post hoc test.

RESULTS

Discrimination of the ethanol stimulus from saline was dose dependent (Fig. 1). The lowest dose, which partly gener-

TABLE 1
RESULTS OF SUBSTITUTION TESTS WITH LAMOTRIGINE AND RILUZOLE IN RATS TRAINED TO DISCRIMINATE ETHANOL (1 g/kg IP)

Compound	Dose (mg/kg)	No. Tested/ No. Responding*	Quantal Measure†	% Substitution	Graded Measure‡
Lamotrigine	5.0	8/8	1/8	12.5	23.1
	10.0	12/12	3/12	25.0	52.2
	30.0	14/8	5/8	62.5	79.8
Riluzole	1.0	6/6	0/6	0.0	15.0
	4.0	8/8	0/8	0.0	21.5
	6.0	8/8	0/8	0.0	20.6

*The number of animals pressing at least 10 times on either the saline or ethanol lever.

†The quantal measure indicates the number of animals tested that selected the ethanol lever by completing 10 responses.

‡The number of ethanol appropriate responses divided by the total sum of responses until completing 10 responses on either the saline or the ethanol lever.

alized for the ethanol training dose, was 0.5 g/kg (Fig. 1). Lever selection latencies with doses lower than 1 g/kg ethanol did not differ from the training dose, and only a dose of 1.5 g/kg increased the time taken to press the lever, $F(4, 35) = 8.5$, $p < 0.05$ (Fig. 1).

Riluzole (1.0, 4.0, and 6.0 mg: $n = 6$, 8, and 8, respectively) failed to substitute for the ethanol cue at all given doses, an effect that seems to be independent of dosage, because even a high dose of 8.0 mg/kg, which was definitely effective because it drastically impaired responding (in six out of eight animals), did not show a tendency to generalization. The response latency increased dose dependently, $F(3, 26) = 7.2$, $p < 0.05$ (Fig. 2). However, lamotrigine (5.0, 10.0, and 30.0 mg: $n = 8$, 12, and 14, respectively) showed a dose-dependent, partial (quantal measure) and full (graded measure) generalization to the ethanol cue and did also result in a remarkable increase of the lever response latency at the highest dose, $F(3, 36) = 14.8$, $p < 0.05$ (Fig. 2) with only 8 out of 14 animals completing the test.

DISCUSSION

This study showed differing effects of the glutamate release inhibitors riluzole and lamotrigine in a drug discrimination experiment with rats trained to discriminate ethanol from saline: lamotrigine substituted for the ethanol cue, whereas riluzole did not. From a superficial point of view these contradictory results are somewhat surprising, as—assuming that ethanol acts as a glutamate release inhibitor—one would expect that both substances generalize to the ethanol cue. However, uptake and release of neurotransmitters is only the result of a variety of different extra- and intracellular processes. Consequently, if two substances decrease the release of a neurotransmitter this does not necessarily mean that they bind at the same sites, use the same second-messenger systems, and influence release to the same extent. Nevertheless, all these aspects may influence the interoceptive cues of these substances.

At present, few data are available on how ethanol inhibits the release of glutamate and to what extent voltage-sensitive cation channels are involved here. Ethanol is thought to inhibit primarily voltage-activated calcium channels (1,31), but also has an—although weaker—inhibitory effect on voltage-gated potassium and sodium channels [(1), for review see (16,21)]. As inhibition of voltage-activated calcium channels decreases the release of glutamate (10), one could speculate that the release inhibition of ethanol is also mediated via volt-

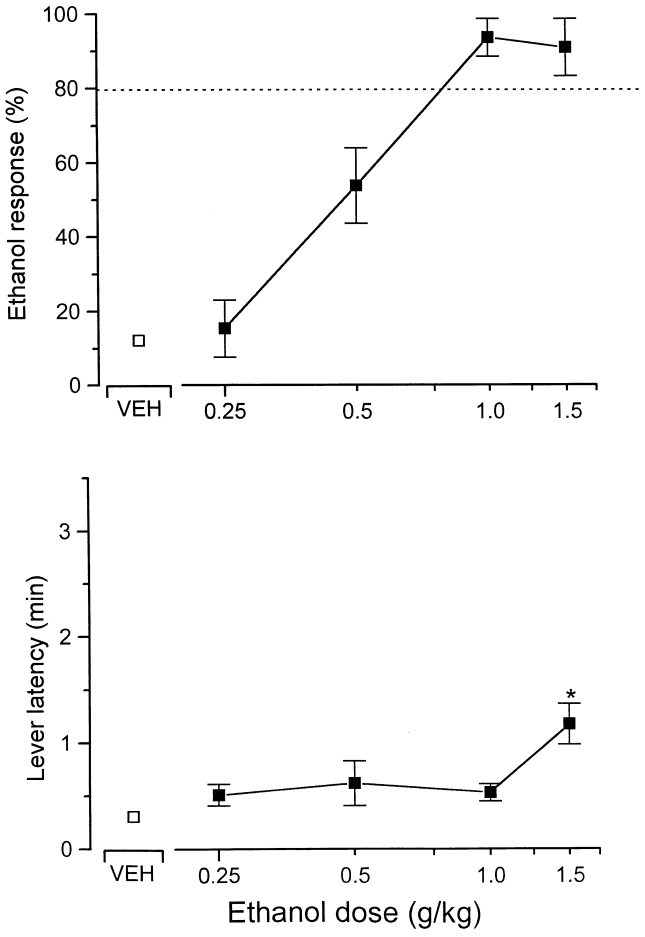

FIG. 1. Results of dose–response tests with increasing doses of ethanol (0.25, 0.5, 1.0, and 1.5 g/kg IP) and saline after consolidation of discrimination learning in rats trained to discriminate ethanol from saline. Values are means ± SEM. *$p < 0.05$.

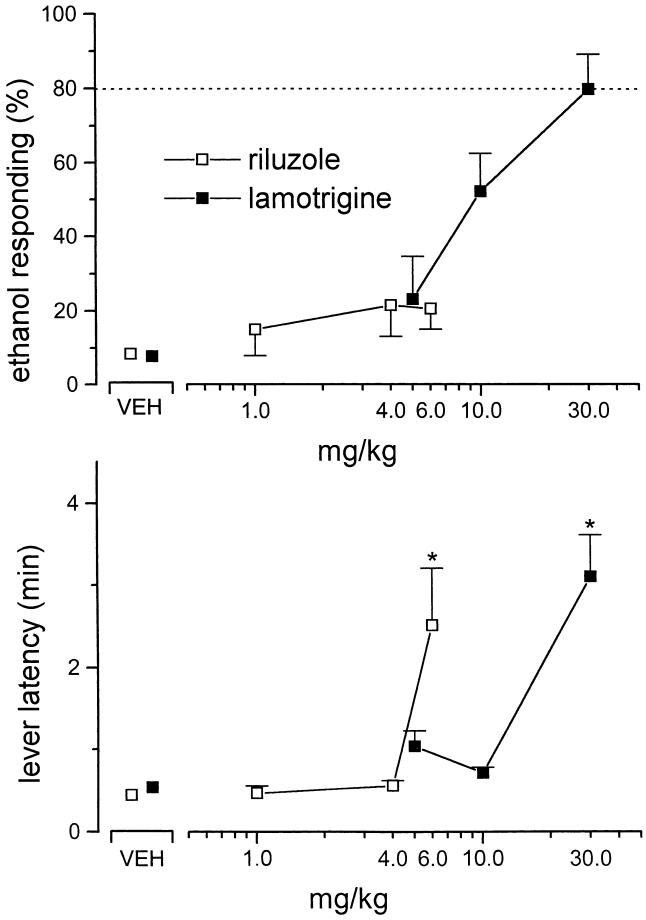

FIG. 2. Results of discrimination testing with increasing doses of lamotrigine and riluzole in rats trained to discriminate ethanol from saline: Ethanol-appropriate response is given as graded measure (%). The compounds were given IP before testing. Values are means ± SEM. *$p < 0.05$.

age-activated calcium channels. Interestingly, it could be shown by an in vivo study in rats using microdialysis that ethanol alters the release of glutamate from hippocampus and nucleus accumbens dose dependently in a biphasic manner with a stimulation of release at low and an inhibition at higher doses of ethanol (20), whereas other authors reported a pronounced glutamate release with increasing ethanol concentrations from in vitro studies (29).

There is evidence that lamotrigine inhibits presynaptic glutamate release primarily via interaction with voltage-activated sodium channels (14,19,32). In our study lamotrigine revealed a dose-dependent partial or full generalization—depending on the results of quantal and graded measurement (Table 1)—which would suggest that decreased glutamate release induced by voltage-activated sodium channels may be involved in the mediation of the subjective ethanol effects.

However, regarding the current literature there is little evidence for this possibility at the moment, as the activity of ethanol at voltage-activated sodium channels seems to be negligible compared to its activity at L-type calcium channels. Lees and Leach showed in an in vitro study that—in higher concentrations—lamotrigine also inhibits calcium currents by interference with calcium sequestration or calcium binding proteins (15), which could be an explanation for the results of our study. A previous drug discrimination study in rats trained to discriminate phencyclidine showed that lamotrigine does not generalize to the phencyclidine cue, indicating that it lacks direct activity at the postsynaptic NMDA receptor (14). As the doses of lamotrigine administered in this study can be regarded as similar to those of the aforementioned study, although a different way of administration was chosen, a direct effect of lamotrigine at the NMDA-channel seems to be unlikely (14).

Riluzole failed to substitute for ethanol in our study. Several in vitro studies show that riluzole, similarly to lamotrigine, selectively inhibits the release of glutamate and aspartate by interacting with certain subtypes of sodium channels (3,9,18). The failure of riluzole to generalize to the ethanol cue does not seem to be due to the usage of ineffective doses, because the highest dose administered had almost an hypnotic effect in the animals. In contrast to the action of lamotrigine at voltage-gated ion channels, riluzole does not act at calcium channels even in higher concentrations. This result is in line with various studies showing that, as mentioned above, voltage-gated sodium channels may not play an important role in the mediation of the effects of ethanol. Various L-type calcium channel antagonists have, however, been shown to potentiate the acute effects of ethanol (11,25) and the calcium channel antagonist israpidine has been shown to be involved in the mediation of the discriminative stimulus effects of ethanol by antagonizing the ethanol cue (5). Further, calcium channel antagonists effectively suppress voluntary ethanol intake and preference in rats (7).

In general, we do not know much about the relevance for behavioral effects of how glutamate release inhibition occurs. Further, we have to take into account that the effect of ethanol on glutamate release may be dose dependent (29) or possibly biphasic (20). The latter author reported a stimulation of glutamate release with 0.5 g/kg ethanol, a trend to decreased release after administration of 1.0 g/kg ethanol, which was the training dose applied in our study, and eventually a strong release inhibition at 2.0 g/kg. This raises the question whether the training dose of ethanol was appropiate to produce a constant and relevant inhibition of glutamate release.

We conclude that dose-dependent inhibition of glutamate release via an altered function of voltage-activated calcium channels may be involved in the mediation of the subjective ethanol effects, whereas voltage-activated sodium channels seem to play a minor role in this respect.

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