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Acamprosate and alcohol: III. Effects on alcohol discrimination in the rat

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Abstract

It has been shown that acamprosate (calcium-acetyl homotaurinate) decreases voluntary ethanol drinking in laboratory animals as well as relapse behaviour in human alcoholics. Although glutamatergic mechanisms have been implicated in several recent studies in the action of acamprosate, the basic mechanism remains unknown. In order to gain more insight into possible mechanisms underlying the ethanol intake-suppressing effects of acamprosate, we investigated this compound in an ethanol discrimination test. Male Wistar rats were trained in a two-lever operant drug discrimination paradigm to make differential responses for food following ethanol (1 g/kg i.p.; 12% v/v ethanol solution) or saline vehicle injections with a fixed ratio schedule of food reinforcement (FR 10) and a post-administration interval of 10 min. Once rats had acquired the discrimination, the criterion for stimulus control was set as at least 90% ethanol- or vehicle appropriate responding during ten consecutive sessions, an ethanol dose-response test (0.25–1.5 g/kg i.p.) was conducted. The effects of acamprosate on the discrimination were assessed in two ways: (i) Generalization test: acamprosate (25–250 mg/kg i.p.) was given either 30 or 120 min before the animals were put in the operant chambers. (ii) Antagonism test: acamprosate (25–250 mg/kg i.p.) was given 120 min before the animals were injected with ethanol, 10 min later the animals were tested. Data obtained in the dose-response test showed a dose-dependent effect with an ED_{50} of 0.53 g/kg ethanol. The results of generalization testing revealed that acamprosate failed to substitute for the ethanol cue, whereas, in comparison, dizocilpine (0.01–0.2 mg/kg i.p.) completely generalized for the ethanol cue ($ED_{50} = 0.05$ mg/kg). Acamprosate also had no effect when tested as an antagonist. Thus, neither the ethanol nor the saline discrimination was altered by acamprosate pretreatment. Furthermore, acamprosate had no effect on response latencies at all doses tested. In conclusion, acamprosate does not generalize for the ethanol cue, suggesting that it is not a substitution drug. Further, acamprosate does not seem to act via the dizocilpine binding site of the *N*-methyl-D-aspartic acid (NMDA) channel.

Keywords: Acamprosate; Ethanol; Drug discrimination; NMDA receptor antagonist; (Rat)

1. Introduction

Acamprosate (calcium-acetyl homotaurinate), a synthetic derivate of homotaurine, itself a structural analogue of γ-aminobutyric acid (GABA), decreases voluntary ethanol intake in alcohol-preferring as well as heterogenous Wistar rats (Boismare et al., 1984; Le Magnen et al., 1987; Gewiss et al., 1991). Furthermore, we showed that acamprosate diminishes the reinstatement behaviour of alcohol drinking in the alcohol-dependent rat, a model which mimics relapse behaviour in human alcoholics (Spanagel et al., 1995a). In humans, an initial single-center, double-blind study showed that the relapse rate in acamprosate-treated, weaned alcoholics clearly differed from that of placebo-treated patients (Lhuintre et al., 1985). This study was followed by numerous other clinical trials involving multi-center studies (Lhuintre et al., 1990; Moore and Libert, 1991; Ladewig et al., 1993; Sass et al., 1996). All of them revealed that acamprosate might be a promising anti-craving compound. However, the mechanism in the central nervous system by which acamprosate acts to elicit its anticraving effect is still unknown. An action of acamprosate on GABAergic systems has been discussed by several investigators (Chabenat et al., 1988; Daoust et al., 1992). However, Gewiss et al. (1991) demonstrated that the behavioural and cortical alterations following chronic ethanol consumption are differentially modulated by $GABA_A$ receptor agonists and acamprosate. Further electrophysiological studies showed that acamprosate reduces the activation of *l*-glutamate-operated ion channels

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but it does not alter the response to iontophoretically applied GABA (Zeise et al., 1993). Recent studies confirmed such findings by indicating that acamprosate neither acts as a modulator at the benzodiazepine or the GABA binding site at native or recombinant $GABA_A$ receptors nor influences Cl^- -currents in α_1-subunit transfected HEK 293 cells, in contrast to flunitrazepam (Zieglgänsberger et al., 1995).

In ethanol discrimination experiments it has been demontrated that several *N*-methyl-D-aspartic acid (NMDA) receptor antagonists substitute for the ethanol cue. Thus, in ethanol-trained pigeons, complete substitution has been obtained with PCP-like non-competitive NMDA receptor antagonists (Grant et al., 1991b). In mice and rats, both non-competitive and competitive NMDA receptor antagonists have produced either full or partial substitution for ethanol. However, the generalization was generally accompanied by substantial response latency decreasing effects (Grant et al., 1991a; Sanger, 1993; Schechter et al., 1993; Shelton and Balster, 1994). In view of these studies we developed the working hypothesis that acamprosate might generalize for the ethanol cue in a discrimination task.

2. Materials and methods

2.1. Animals

Male Wistar rats (Max Planck Institute of Psychiatry, Martinsried, Germany) weighing 250–270 g were housed individually with free access to water. Their weight was maintained at about 80% of those under free-feeding conditions during the experimental period by restricting their daily food consumption. Animals received water ad libitum and were kept in a climatically controlled room under a 12-h light/dark cycle, with the light phase commencing at 7.00 a.m.

2.2. Apparatus

Standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA, USA) were used. Each chamber was equipped with two levers, one on either side and equidistant from a food cup. The chambers were contained in ventilated, sound-attenuated cubicles equipped with a houselight. The experiments were controlled by a computer connected to the chambers through LVB interfaces (Med Associates, East Fairfield, VT, USA) using a modified version of the software package (Operant Package for the Neurosciences, OPN) described by Emmett-Oglesby et al. (1982) and Spencer and Emmett-Oglesby (1985).

2.3. Discrimination training

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

2.4. Discrimination testing

Tests were conducted twice weekly, with either ethanol or vehicle training during the intervening days. The day prior to testing all rats were trained with saline. Test sessions were terminated either after one completed fixed ratio (ten presses) or 5 min had elapsed. No responses were reinforced during these sessions. Testing commenced once the subjects were placed in the chambers, 10 min after either ethanol or saline administration. 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 against the total number of responses on both levers until the first fixed ratio was completed (first ten 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. For generation of the figures the graded measure was used. In addition to obtaining discrimination data, the time taken to complete the first ratio (selection latency) served as a measure of the of responding. The following tests were conducted. *(i) Ethanol dose-response test*: following acquisition of discrimination, generalization tests were conducted with four doses of ethanol (0.25–1.5 g/kg, i.p.) to obtain a dose-response relationship for discrimination. All doses were tested in a randomized order. *(ii) Acamprosate and dizocilpine generalization test*: animals were injected with either acamprosate (25–250 mg/kg i.p.) or dizocilpine (0.01–0.2 mg/kg i.p.) and were placed into the chambers after 30 and 120 min (for acamprosate) and after 15 min (for dizocilpine), respectively. The doses and pretreatment times employed in this study were those previously used (Le Magnen et al., 1987; Chabenat et al., 1988; Grant and Woolverton, 1989; Gewiss et al., 1991) and were further tested in pharmacokinetic studies (Lipha; personal communication) and in our own behavioural experiments (Spana-

gel et al., 1995a,b). *(iii) Acamprosate and antagonism test*: acamprosate administered intraperitoneally was tested against both drug and vehicle stimuli. For these tests, acamprosate was given 120 min prior to administration of either ethanol or saline. Ten minutes later the rats were placed in the chambers. Drugs and doses were injected in a randomized order in all three tests.

2.5. Statistics

The graded measure of discrimination was used to test for statistical significance of data. Each percentage score was transformed to an arcus-sine and a single-factor analysis of variance with repeated measures was employed. Post-hoc tests were the Student Newman Keuls test or, when applicable, Dunnett's test to identify significant differences between vehicle and drug pretreatment. The accepted level of significance was $P < 0.05$. A computer-generated formulation of Litchfield-Wilcoxon analysis (Tallarida and Murray, 1986) yielded ED_{50} values and confidence limits (C.L.; 95%) for ethanol dose-response curves.

2.6. Drugs

Dehydrated 99% ethyl alcohol was obtained from the hospital pharmacy (Schwabinger Hospital, Munich, Germany) and diluted to a 12% solution (v/v) with 0.9% saline.

Acamprosate (provided by Lipha, Lyon, France) and dizocilpine (RBI, Cologne, Germany) were dissolved in saline and injected i.p. in the same volume as used in the saline/ethanol training sessions.

3. Results

3.1. Acquisition of stimulus control

45 out of 56 animals trained to discriminate ethanol (1 g/kg) from saline acquired stimulus control by meeting the criterion of correct lever selection after approximately 80 training sessions.

3.2. Ethanol dose-response test

Data obtained for ethanol dose-response testing are shown in Fig. 1. Discrimination of the ethanol stimulus was dose-dependent. The lowest dose of ethanol which partly generalized to the ethanol training dose was 0.5 g/kg. The ED_{50} value for ethanol was calculated as 0.53 g/kg (C.L. 0.38–0.62 g/kg). Lever selection latencies with doses lower than 1 g/kg ethanol did not differ from those for the training dose. However, a dose of 1.5 g/kg ethanol significantly increased the time taken for rats to select the lever ($P < 0.05$; $n = 15$).

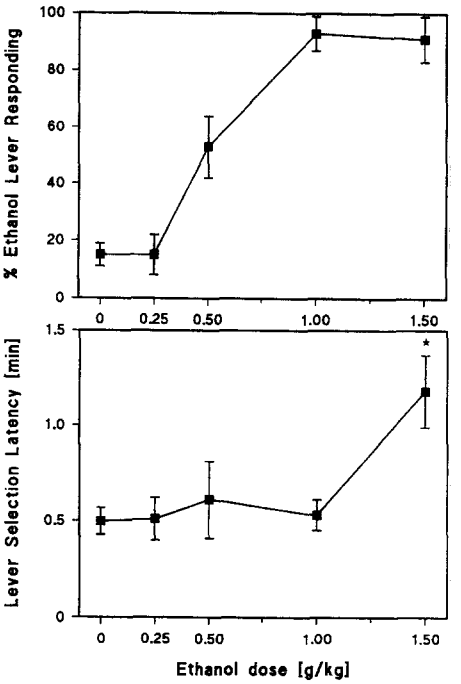

Fig. 1. Ethanol dose-response curve in rats trained to discriminate 1 g/kg ethanol from saline at a 10 min post-administration interval with a fixed ratio schedule of food reinforcement (FR 10).

3.3. Acamprosate and dizocilpine generalization test

The results of generalization testing are shown in Figs. 2 and 3. Acamprosate (25–250 mg/kg, i.p.), tested following an administration interval of 30 and 120 min, respectively, failed to substitute for ethanol at all test doses (Fig. 2). Further, acamprosate did not alter the time taken for rats to select the lever for the completion of a FR10 ratio. In contrast, the non-competitive NMDA receptor antagonist dizocilpine substituted for ethanol in a dose-dependent manner (Fig. 3). Complete generalization was obtained at a dose of 0.2 mg/kg i.p., a dose which also increased the lever selection latency significantly (Fig. 3). However, dizocilpine tested at lower doses did not alter the time taken for rats to select the lever. The ED_{50} value for generalization of dizocilpine for ethanol was 0.05 mg/kg (C.L. 0.03–0.10 mg/kg).

3.4. Acamprosate antagonism test

Fig. 4 shows the effects of vehicle and acamprosate (25–250 mg/kg, i.p.) pretreatment on ethanol/saline discrimination. Vehicle pretreatment did not affect the discrimination task. Acamprosate pretreatment, at all doses tested, failed to produce an effect on ethanol discrimination. It is important to note that discrimination of saline was not altered by acamprosate pretreatment. Lever selec-

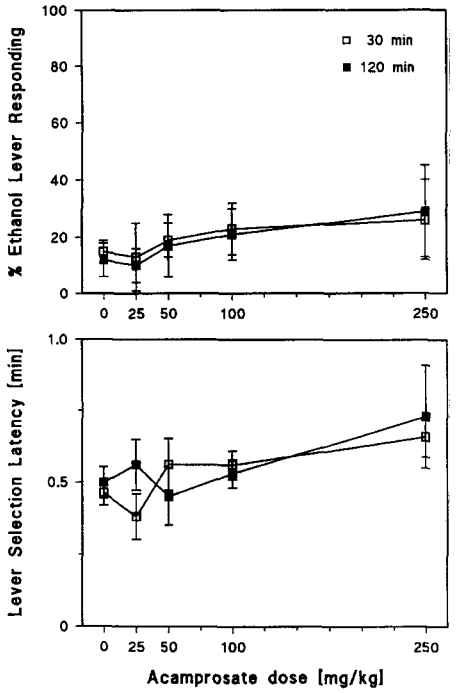

Fig. 2. Generalization curves for acamprosate in animals trained to discriminate 1g/kg ethanol from saline. Mean percentage (±S.E.) of ethanol-appropriate responding (top panel) and mean percentage (±S.E.) of lever selection latencies following various doses of acamprosate (25–250 mg/kg i.p.) and a 30 and 120 min post-administration interval are shown in the bottom panel.

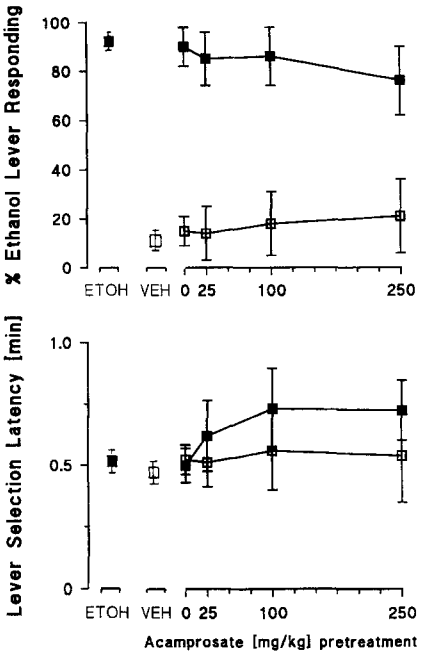

Fig. 4. Acamprosate (25–250 mg/kg i.p.) does not antagonize the discriminative effects of ethanol in rats trained to discriminate ethanol from saline. Acamprosate was injected in a randomized order 120 min prior to testing, and the percentage of responses made on the ethanol lever to complete the first ratio (FR 10) are given on the ordinate (top panel). Lever selection latencies following this treatment are shown in the bottom panel.

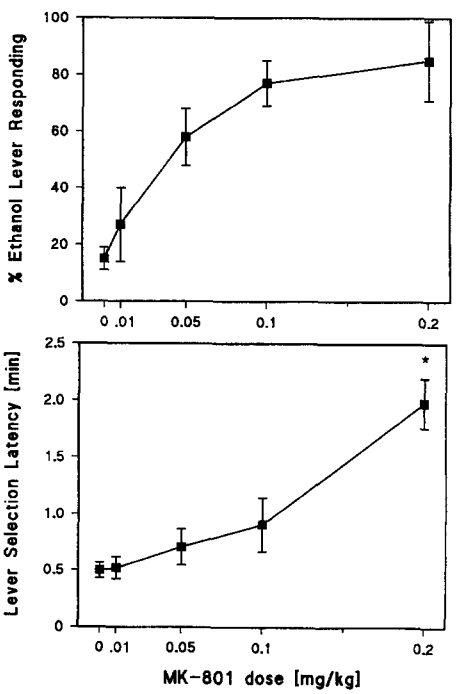

Fig. 3. Generalization curve of dizocilpine in animals trained to discriminate 1 g/kg ethanol from saline. Mean percentage (±S.E.) of ethanol-appropriate responding (top panel) and mean percentage (±S.E.) of lever selection latencies (bottom panel) following various doses of dizocilpine (0.01–0.2 mg/kg i.p.) given 30 min before the test session.

tion latencies after acamprosate injections did not differ from those after saline pretreatment.

4. Discussion

In the present study no similarities were found in the discriminative stimulus effects of ethanol and acamprosate in rats. Thus, acamprosate did not substitute for ethanol at doses which proved to be effective in other behavioural paradigms, e.g. suppressing voluntary alcohol drinking in rats (Boismare et al., 1984; Le Magnen et al., 1987; Gewiss et al., 1991), diminishing reinstatement of alcohol drinking in rats (Spanagel et al., 1995a), or suppressing withdrawal symptoms in physically alcohol-dependent rats (Gewiss et al., 1991). Further, acamprosate also failed to antagonize the ethanol discriminative stimulus cue.

Drug discrimination paradigms can be used to elucidate the neurochemical substrates which mediate at least some of the effects of ethanol. Thus, recent drug discrimination studies have examined the similarities between ethanol and drugs acting through the NMDA-receptor-complex. These studies showed in various species that both non-competitive and competitive NMDA receptor antagonists produce either full or partial generalization for ethanol (Grant et al., 1991a; Sanger, 1993; Schechter et al., 1993; Shelton and

Balster, 1994). The general conclusion from these findings is that ethanol may interact with the NMDA-receptor-complex, a view which is supported by numerous electrophysiological (Hoffman et al., 1989; Lovinger et al., 1989) and molecular biological data (for review see: Hoffman and Tabakoff, 1994). In line with the above-mentioned drug discrimination studies, we found that the non-competitive NMDA receptor antagonist dizocilpine fully generalized for ethanol in rats which were trained to discriminate ethanol (1 g/kg) from saline (Fig. 3). However, effective doses of dizocilpine resulted in increased lever-selection latencies, a finding which was also reported by other investigators (Grant et al., 1991a; Sanger, 1993; Shelton and Balster, 1994). Therefore, as claimed in electrophysiological experiments which show an interaction of acamprosate and glutamatergic synaptic transmission (Zeise et al., 1993), acamprosate might show a similar profile to that of NMDA receptor antagonists in an ethanol discrimination paradigm. However, acamprosate completely failed to substitute for the ethanol cue, a finding which leads to the conclusion that acamprosate might not act as an antagonist at the NMDA-receptor-complex.

In a second set of experiments we found that acamprosate did not antagonize the ethanol discriminative stimulus cue. Although acamprosate given in a dose range of 25–250 mg/kg did not influence lever response latencies, it influences other behaviours (see above) at these doses. Pharmacokinetic studies in rat brain tissue revealed that acamprosate levels peak 2 h after i.p. injection (Lipha; personal communication) and very recent studies from our laboratory show that a dose of 200 mg/kg acamprosate reduces pentylenetetrazole- (PTZ) as well as ethanol withdrawal-induced c-fos m-RNA levels in the brain (Putzke et al., 1995). Despite the evidence that administration of acamprosate at the doses tested in the present study leads to sufficient plasma and brain levels to influence CNS functions, acamprosate neither interfered with the ethanol cue nor influenced lever selection latencies.

In summary, acamprosate completely failed to generalize for the ethanol cue in the present drug discrimination paradigm. In view of the findings that acamprosate is neither self-administered by rhesus monkeys who reliably self-administered cocaine or pentobarbital (Grant and Woolverton, 1989) nor can be discriminated from saline by rats (Spanagel, unpublished data), one might suggest that acamprosate has, if at all, a very low abuse potential. Thus, it is very likely that acamprosate does not act as a substitution drug to elicit its anti-craving action, an assumption which is underlined by several clinical studies.

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