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Acamprosate and alcohol: I. Effects on alcohol intake following alcohol deprivation in the rat

Rainer Spanagel [a,*], Sabine M. Hölter [a], Karl Allingham [a], Rainer Landgraf [a], Walter Zieglgänsberger [b]

[a] *Max Planck Institute of Psychiatry, Clinical Institute, Department of Neuroendocrinology, Kraepelinstrasse 2–10, 80804 Munich, Germany*
[b] *Max Planck Institute of Psychiatry, Clinical Institute, Clinical Neuropharmacology, Kraepelinstrasse 2–10, 80804 Munich, Germany*

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Abstract

Acamprosate (calcium-acetyl homotaurinate) is a new compound in the treatment of alcoholism. Its efficacy has been proven in several clinical trials and registration is now pending in most European countries. The basic mechanisms by which acamprosate elicits its anti-craving action, thereby leading to reduced relapse rates, is not known at the moment. In the present study we describe a rat model of long-term alcohol-drinking which mimics relapse behavior in human alcoholics. The effect of acamprosate was studied in this model. Wistar rats had a free choice between water and alcohol solutions of different concentrations (5, 10, 20% v/v). After two months of continuous alcohol access, rats were deprived of alcohol for three days. Following this deprivation phase, all alcohol solutions were presented again. This procedure was repeated monthly for the following six months. The rats consumed 3.5 ± 0.3 g/kg alcohol a day. After alcohol deprivation, alcohol intake rose to 5.2 ± 0.3 g/kg per day resulting in blood alcohol levels of 30 ± 6 mg/dl. Interestingly, the addition of quinine to the alcohol solutions or the additional presentation of a 5% sucrose solution did not affect the alcohol-deprivation effect after eight months of this intermittent alcohol exposure. However, when acamprosate (50–200 mg/kg i.p.) was administered twice daily, alcohol-drinking following an alcohol-deprivation phase was decreased dose dependently. Given at the highest dose alcohol intake even dropped significantly below baseline drinking. Together, these results show that acamprosate effectively diminishes the alcohol-deprivation effect. Furthermore, the described model seems to be a suitable animal model to screen compounds for their anti-relapse properties and subsequently for their anti-craving action.

Keywords: Acamprosate; Alcohol; Alcohol-deprivation effect; Craving; Relapse; (Rat)

1. Introduction

Craving, the uncontrollable desire to drink alcohol even after a long period of abstinence, is considered to be an important trigger for relapse in human alcoholics. However, it should be mentioned that during drug craving, relapse occasionally does not occur despite drug availability (Hughes, 1987; Childress et al., 1988). Recent clinical trials suggest that acamprosate (calcium-acetyl homotaurinate), a synthetic derivative of homotaurine which is itself a structural analogue of γ-aminobutyric acid (GABA), is a most promising approach to the prevention of relapse. Single-center and multicenter double blind studies showed that the relapse-rate in acamprosate-treated, weaned alcoholics clearly differed from that of placebo-treated patients (Lhuintre et al., 1985, 1990; Moore and Libert, 1991; Ladewig et al., 1993; Sass et al., 1996).

The mechanisms by which acamprosate decreases alcohol ingestion and relapse rates in alcoholics is as yet unclear. The action of acamprosate on GABA-ergic systems (Chabenat et al., 1988; Daoust et al., 1992) and opioidergic systems (Le Magnen et al., 1987) has been discussed by several investigators and electrophysiological data suggest that acamprosate reduces the activation of excitatory synapses operated by L-glutamate (Zeise et al., 1993).

Some studies showed that acamprosate decreases voluntary alcohol intake in alcohol-preferring as well as heterogenous Wistar rats (Boismare et al., 1984; Le Magnen et al., 1987; Gewiss et al., 1991). In this article we describe the effect of acamprosate in a new long-term alcohol-drinking model in heterogenous Wistar rats.

* Corresponding author. Tel.: (49) 089 30622288; fax: (49) 089 30622569.

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2. Materials and methods

2.1. Animals and alcohol-drinking procedure

Male Wistar rats (Max Planck Institute of Psychiatry, Martinsried, Germany) weighing 180–200 g were housed individually. The animals received food and tap water ad libitum and were kept in a climatically controlled room (room temperature: 21 ± 2°C) under a 12 h light/dark cycle, with the light phase commencing at 6:00 a.m.. Tap water and alcohol solutions (5, 10, 20% v/v alcohol solutions) were offered in four bottles per cage. The alcohol solutions were obtained by diluting 96% alcohol (highest degree of purity, without additives) with tap water. Every third and fourth day (the measurement of a 24 h interval) the rats were weighed and the consumption of food and fluid was measured. To avoid conditioning, the positions of the bottles were changed once a week. Water and alcohol solutions were also completely replaced once a week. All fluids were offered in plastic bottles in which evaporation of alcohol was minimized. Control measurements in empty cages proved that the evaporation of alcohol during one week did not affect the results, therefore, the introduction of a correction factor was not necessary.

2.2. Manipulations of the alcohol-deprivation effect

After two months of continuous alcohol access rats were deprived of alcohol for three days. After this deprivation phase all alcohol solutions were presented again. This procedure was repeated monthly for the following six months. To monitor an alcohol-deprivation effect, basal drinking was measured for three days. Following alcohol deprivation alcohol intake was again measured for three days.

The alcohol-deprivation effect was manipulated by adding quinine hydrochloride (0.1 g/l) to the alcohol solutions, but not to the tap water. A second group of animals underwent a sucrose test – they had a free choice between a 5% sucrose solution and the 5, 10 and 20% alcohol solutions which did not contain sucrose. The quinine as well as the sucrose test was performed at two different time points, one after two months of continuous access to alcohol, the other one after eight months of intermittent alcohol access. In another set of experiments animals that already had experienced intermittent alcohol exposure for eight months underwent a 60-day period of abstinence. Following this long-term abstinence the alcohol-deprivation effect was measured.

The influence of acamprosate upon the alcohol-deprivation effect was tested by injecting the animals twice daily (8 a.m. and 8 p.m.) with various doses of acamprosate (0, 50, 100 and 200 mg/kg i.p.). Injections were given during the first 48 h after the alcohol presentation.

2.3. Blood alcohol concentrations

At the end of the experiments animals were killed during an alcohol reinstatement phase. Trunk blood samples were taken during the dark cycle and were collected in heparinized tubes. After centrifugation, the supernatant fractions were immediately used for alcohol determination. Alcohol was measured by a fully automated NAD-ADH enzyme spectrophotometric system (Hitachi).

2.4. Statistics

Alcohol and food intake were calculated in relation to body weight. Alcohol preference was calculated in the following way: (alcohol consumption in ml of the 5, 10 and 20% solution/total fluid consumption in ml) × 100. In addition, alcohol preference was calculated separately for each alcohol solution. For statistical evaluation, data were analyzed either by a two-way or a three-way analysis of variance (ANOVA) with either randomized block design or repeated measures (acamprosate treatment) followed by Tukey's *t*-test when appropriate. A probability below 0.05 was considered significant.

3. Results

3.1. Alcohol-deprivation effect

The alcohol-deprivation effect was monitored at two different time points. (i) Two months following continuous alcohol drinking, an alcohol deprivation phase for three days significantly enhanced alcohol intake ($F(1,18) = 56.5$; $P < 0.0001$) (Fig. 1a) as well as alcohol preference ($F(1,18) = 21.8$; $P < 0.001$). A preference for the 5% solution over higher alcohol concentrations (5% > > 10% > 20%) could be observed (factor concentration: $F(2,36) = 7.0$; $P < 0.01$) (Fig. 1b). This relation was not altered by the alcohol deprivation (concentration × deprivation: $F(2,36) = 0.2$; $P = 0.83$) (Fig. 1b). (ii) Alcohol deprivation following eight months of intermittent alcohol presentation raised baseline drinking (3.5 ± 0.3 g/kg per day) to levels of 5.2 ± 0.3 g/kg per day (Fig. 2a). Thus, significant changes in alcohol intake ($F(1,18) = 79.34$; $P < 0.0001$) as well as in the preference for alcohol ($F(1,18) = 11.12$; $P < 0.001$) could be observed. Blood alcohol levels after the alcohol-deprivation effect were in the range of 2–54 mg/dl (mean ± S.E. 30.2 ± 6.1 mg/dl blood alcohol concentration). A detailed analysis of preference for the various alcohol solutions revealed the following changes: during baseline drinking an initial preference for the 5% solution over the higher alcohol concentrations (5% > 20% > 10%) could be observed again (factor concentration: $F(2,36) = 3.67$; $P < 0.05$). However, this relation was altered by alcohol deprivation (concentration × deprivation: $F(2,36) = 16.19$; $P < 0.001$). Thus, the ani-

mals preferred the 20% alcohol solution to the lower alcohol concentrations (20% ≫ 10% > 5%). Following the alcohol deprivation phase food consumption was significantly less (35 ± 1 g/kg per day) than during baseline drinking (40 ± 1 g/kg per day; $P < 0.01$).

3.2. Manipulation of the alcohol-deprivation effect

In Fig. 3 the alcohol-deprivation effect is again compared at two different time points: after two months (top panel) and after eight months of intermittent alcohol exposure (bottom panel). Short term as well as long term alcohol-exposed rats drank significantly more alcohol on the first day following alcohol deprivation (paired t-test: for short term alcohol-exposed rats: $t(11$ d.f.$) = 6.06$; $P < 0.01$; for long term alcohol-exposed rats: $t(11$ d.f$) = 5.57$; $P < 0.01$). However, addition of quinine to all alcohol solutions, or the additional presentation of a 5% sucrose solution, revealed that the short-term alcohol-exposed rats drank less alcohol under these conditions than during baseline drinking. In contrast, despite quinine-induced taste aversion or the choice of a highly palatable sucrose solution, the long-term alcohol-exposed rats consumed amounts of alcohol which were significantly higher than those during baseline drinking (paired t-test: quinine test: $t(11$ d.f.$) = 4.01$; $P < 0.01$; sucrose test: $t(11$ d.f.$) = 4.23$; $P < 0.01$). Furthermore, alcohol intake after a long alcohol deprivation period (60 days) was of the same magnitude as it was after a three days' abstinence period; for technical reasons this experiment could not yet be conducted in short-term alcohol-exposed animals.

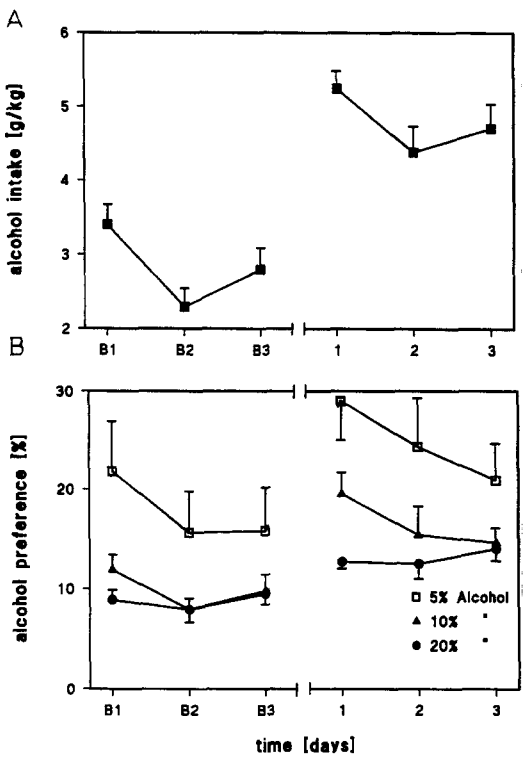

Fig. 1. Time course of the alcohol-deprivation effect in rats exposed to the free choice of water and three different alcohol solutions (5, 10, 20% v/v) for two months (the first alcohol deprivation phase). B1–B3 denote basal levels of alcohol intake (A; top panel) and alcohol preference (B; bottom panel) for the three alcohol solutions which were presented again in a free choice with water after three days of alcohol deprivation. Alcohol intake and preference were monitored for the following three days. Values are given ±S.E.M.

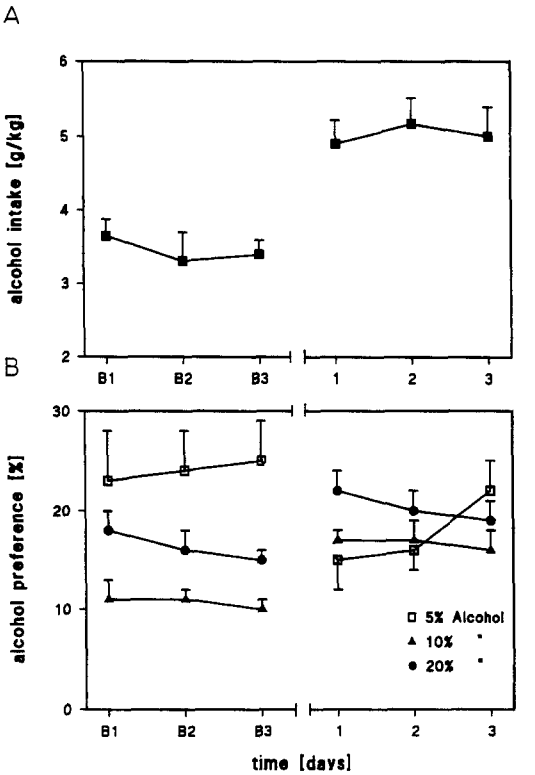

Fig. 2. Time course of the alcohol-deprivation effect in rats intermittently exposed to the free choice of water and three different alcohol solutions (5, 10, 20% v/v) for eight months. B1–B3 denote basal levels of alcohol intake (A; top panel) and alcohol preference (B; bottom panel) for the three alcohol solutions which were presented again in a free choice with water after three days of alcohol deprivation. Alcohol intake and preference were monitored for the following three days. Values are given ±S.E.M.

3.3. Influence of acamprosate on alcohol intake following alcohol deprivation

Fig. 4 shows the influence of acamprosate (50–200 mg/kg i.p.), given twice daily for two days, on the alcohol-deprivation effect. In comparison to the effect of saline in control animals acamprosate significantly diminished alcohol intake ($F(3,216) = 26.09$; $P < 0.0001$) and alcohol preference ($F(3,216) = 12.96$; $P < 0.001$) dose dependently. This effect was most pronounced at the highest dose (200 mg/kg), where alcohol intake even dropped below baseline drinking. Food consumption in acamprosate-treated animals was also suppressed. Thus, at the highest dose of acamprosate given basal food consumption

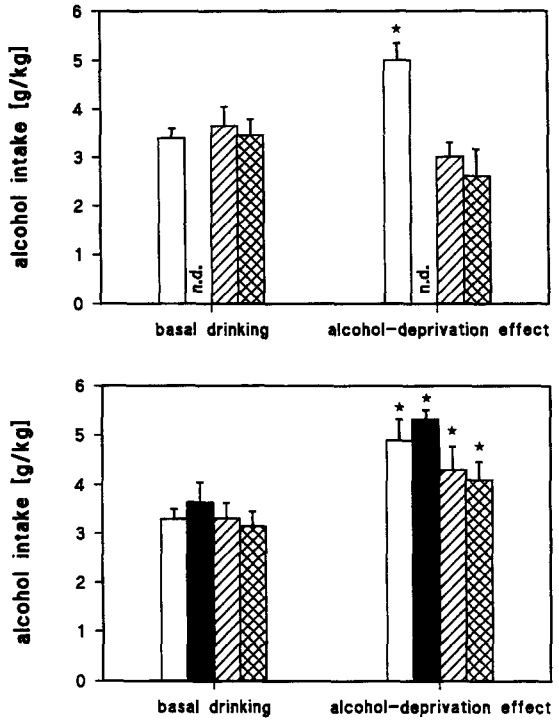

Fig. 3. The top panel shows the alcohol-deprivation effect after two months, the bottom panel after eight months of intermittent alcohol exposure. The bars always represent the last day of baseline drinking and the first day following abstinence. The open bars show the alcohol-deprivation effect after three days of abstinence, the closed bars after 60 days of abstinence (the 60 days abstinence phase was not tested in animals exposed to alcohol for only two months; not determined = n.d.). The striped bars show the alcohol-deprivation effect with alcohol solutions containing quinine, the crossed bars with the simultaneous presentation of a 5% sucrose solution. * Significant differences between basal drinking and the alcohol-deprivation effect ($P < 0.05$).

(40 ± 1 g/kg per day) dropped to 21 ± 3 g/kg on day 1 and 13 ± 2 g/kg on day 2 of treatment. However, within two days food consumption returned to basal levels.

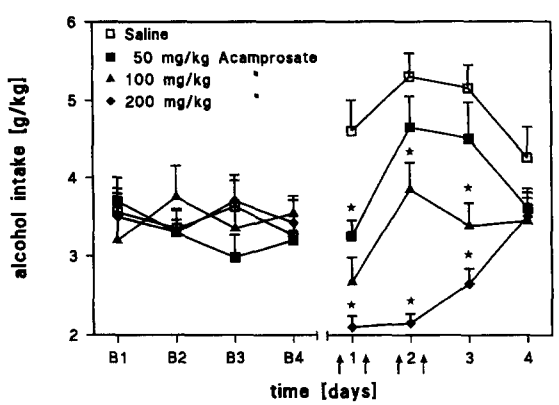

Fig. 4. The effects of different doses of acamprosate given twice daily (8 a.m. and 8 p.m.) on the first two days (indicated by the arrows) following alcohol abstinence in rats intermittently exposed to the free choice of water and three different alcohol solutions (5, 10, 20% v/v) for eight months. * denote significant differences to saline injections ($P < 0.01$).

4. Discussion

In the present study we describe the alcohol-deprivation effect in a rat model of long-term alcohol-drinking. The alcohol-deprivation effect was originally described by Sinclair and Senter (1967) and is characterized by a transient increase after abstinence in the amount of alcohol voluntarily selected. This robust phenomenon is found in rats (Sinclair et al., 1973; Pinel and Huang, 1976), monkeys (Sinclair, 1971) and human social drinkers (Burish et al., 1981). The alcohol-deprivation effect was monitored in our rats which had an intermittent free choice between different alcohol concentrations and water for at least eight months and were deprived of alcohol for three days once a month. The alcohol-deprivation effect after the last deprivation phase (after eight months intermittent alcohol drinking) in these animals was characterized by (i) a higher alcohol intake and preference than that seen with baseline drinking and blood alcohol concentrations similar to those found in rats selectively bred for high alcohol preference (Li et al., 1979). (ii) Changes in drinking patterns occurred where animals consumed larger amounts of more highly concentrated alcohol solutions (> 10% v/v) as compared to baseline drinking behavior. (iii) The alcohol-deprivation effect outlasted very long abstinence phases (up to two months), which is consistent with the findings reported from other studies (Wolffgramm, 1991; Wolffgramm and Heyne, 1995) using a similar experimental approach. They showed that the alcohol-deprivation effect in long-term alcohol exposed rats survived an abstinence period of nearly a third of the rats' lifespan without being attenuated. The authors claimed this effect to be irreversible, which seems plausible considering the nine months' abstinence period. (iv) The alcohol-deprivation effect remained unchanged even after presentation of alcohol solutions containing quinine hydrochloride, a substance which usually produces a strong taste aversion in rats (Aravich and Sclafani, 1980; Wolffgramm, 1991), or after presentation of a highly palatable sucrose (5%) solution.

In comparison, the alcohol-deprivation effect monitored in short-term alcohol-exposed rats that had free access to alcohol for two months was characterized only by higher alcohol intake and preference compared to baseline drinking. However, the addition of quinine to the alcohol solutions or the presentation of a sucrose solution had a strong influence on the alcohol-deprivation effect in these short-term alcohol-exposed animals. Thus, after these manipulations, alcohol intake and preference even dropped below those of baseline drinking following the alcohol-deprivation phase. These experiments led us to conclude that our long-term exposed animals show a different alcohol drinking behavior following alcohol deprivation from that of animals that had alcohol access for only two months. Thus, long-term exposed animals consume large amounts of a highly concentrated alcohol solution and continue to drink substantial amounts of alcohol, despite taste aversion or

the competitive presentation of a highly palatable fluid. These findings suggest a non-nutritional component of alcohol drinking (Dole, 1986). This non-nutritional component of alcohol drinking consists of an uncontrolled incentive demand for alcohol (Dole, 1986; Wolffgramm and Heyne, 1995) and, therefore, reflects an increase in craving for alcohol. However, it remains to be shown whether our model can serve as an animal model for all aspects of drug craving. At the present stage it is unknown whether the amount of drug consumed is an index of the degree of craving (Markou et al., 1993), and it should be noted that often drug craving does not lead to a relapse despite drug availability (Hughes, 1987; Childress et al., 1988).

The above described model was used to examine the influence of acamprosate on alcohol intake, preference and drinking behavior following an alcohol abstinence period. Acamprosate suppressed the alcohol-deprivation effect dose dependently. Thus, alcohol intake did not differ from that of normal baseline drinking. At the highest dose of acamprosate, alcohol intake even dropped below that of baseline drinking. These findings are in accordance with previous studies. Boismare and co-workers (Boismare et al., 1984) initially showed that acamprosate decreases voluntary basal alcohol intake in alcohol-preferring rats. Another study reported that acamprosate given at a high dose of 400 mg/kg per day completely abolished basal alcohol-drinking in a free-choice beverage paradigm (Gewiss et al., 1991).

The similarities between acamprosate-treated human alcoholics during a relapse phase and the animals treated with acamprosate are striking. There is evidence that if an acamprosate-treated patient relapses, the relapse behavior is shorter lasting and the patient stops drinking alcohol after less alcohol than he or she usually consumed previously during such phases (Lipha, unpublished data). In our study, acamprosate-treated rats also 'relapsed', following an alcohol abstinence period. However, their drinking behavior also seemed to be more controlled since they consumed even less alcohol than during baseline drinking.

The present study shows that our model of long-term alcohol drinking in heterogenous Wistar rats mimics quite closely relapse behavior in human alcoholics, and that the treatment with acamprosate alters the drinking behavior in these animals in a manner similar to that in alcoholic patients. These findings provide the basis for furher study of the mechanisms underlying the action of anti-craving drugs and the neuronal events triggering relapse.

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