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Acamprosate and alcohol: II. Effects on alcohol withdrawal in the rat

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Abstract

The suppressing effect of acamprosate (calcium-acetyl homotaurinate) on alcohol drinking is well established; however, little is known about its effects upon the alcohol-induced withdrawal syndrome. Male Wistar rats received as a sole drinking fluid a 20% (v/v) alcohol solution for one week. Animals consumed on average 5.3 ± 0.3 g/kg per day alcohol, which resulted in blood alcohol levels of 38 ± 14 mg/dl. For the quantification of alcohol withdrawal we used a new radio-telemetric system which enabled us to monitor body temperature, locomotor activity, food and water intake patterns constantly during alcohol withdrawal. Although alcohol intake and the resulting blood alcohol levels were low, clear signs of withdrawal could be observed. Thus, hyperthermia and hyperlocomotion occurred 18 h after the termination of forced alcohol drinking. Food intake was initially enhanced but dropped significantly below basal food intake in control animals one day after the termination of forced alcohol drinking. Acamprosate given twice a day (200 mg/kg, i.p., 8 a.m. and 8 p.m.) reduced hyperlocomotion and food intake significantly in the alcohol withdrawal animals, however, it did not change withdrawal-induced hyperthermia. When acamprosate was given to alcohol-naive animals, it increased locomotor activity and body temperature transiently, in particular during the rats' active night phase. In summary, (i) the radio-telemetric system used in the present study proved to be a very sensitive method for quantifying alcohol-induced withdrawal symptoms; (ii) acamprosate reduced alcohol-induced physical signs of withdrawal, however, this effect could not be observed for all parameters measured, which might be explained by the fact that (iii) acamprosate exerts a slight, transient psychomotor stimulant effects by itself.

Keywords: Acamprosate; Alcohol; Withdrawal; Radiotelemetry; (Rat)

1. Introduction

Prevention of relapse in human alcoholics with acamprosate (calcium-acetyl homotaurinate) seems to be a promising therapeutical concept. This compound has proved to be a safe and effective medication in alcohol dependence to maintain abstinence (Lhuintre et al., 1985, 1990; Moore and Libert, 1991; Ladewig et al., 1993; Sass et al., 1996). In the multicenter studies patients received either acamprosate or placebo two weeks after alcohol withdrawal (detoxification phase). The efficacy of acamprosate during the detoxification phase has not yet been tested. Provided that acamprosate also effectively suppresses alcohol-induced withdrawal symptoms during the detoxification phase, this regimen could be expanded to improve the compliance. The aim of the present study was to investigate the efficacy of acamprosate treatment upon alcohol withdrawal in rats.

Most commonly, physical signs of alcohol withdrawal are assessed according to the criteria described by Hunter et al. (1972) and Majchrowicz (1975). Although such a subjective rating of severe physical signs of alcohol withdrawal is a reliable method and is often used, real quantification and inter-laboratory comparison are difficult. Furthermore, the assessment of mild physical signs of alcohol withdrawal according to these subjective rating scales is impossible (Pohorecky and Roberts, 1991). Therefore, we developed a fully automated, computerized system which enabled us to continuously monitor even mild physical signs of alcohol withdrawal. A radiotelemetric system monitored body temperature and locomotor activity. Food and water intake was recorded via a fully automated food pellet- and liquid-dispenser system.

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2. Materials and methods

2.1. Animals

Male adult Wistar rats (200–300 g), purchased from a local supplier (Max Planck Institute of Psychiatry, Martinsried, Germany), were housed in individual cages with free access to standard chow and drinking water, under conditions of constant temperature (21 ± 2°C) and a 12 : 12-h light/dark cycle with the light phase commencing at 8:00 a.m. Following one week of habituation drinking water was replaced by a 20% (v/v) alcohol solution, which was given as the sole drinking fluid for the following 7 days.

2.2. Measurement of physical signs of alcohol withdrawal

Core body temperature and locomotor activity (expressed in arbitrary units) were monitored in undisturbed rats with a radiotelemetric method using the Dataquest IV system (Data Sciences International, St. Paul, MN, USA). A battery-powered transmitter was implanted into the peritoneal cavity of each animal under halothane anesthesia. After surgery, animals were allowed to recover for two days. Rats were adapted to the cages for the measurements for 24 h. During this time rats still received alcohol as the sole drinking fluid. The frequency of the signal emitted by the transmitter is proportional to the animal's body temperature. This signal reaches a receiver underneath each cage and is transferred to and processed by an IBM personal computer. Body temperature was continually recorded at 5-min intervals. Means of these data were analyzed at 3-h intervals.

Activity of the animals was measured by continuously monitoring changes in the received signal strength from the transmitter which occur upon movement of the animal. Changes in signal strength generate a digital pulse which is counted by the Dataquest IV system. Above rates of motion of 1 cm/s the number of pulses depends strictly on the distance the animal moves. Locomotor activity was continually recorded at 5-min intervals. Means of these data were analyzed at 3-h intervals.

Licking rate (expressed in arbitrary units) was continuously monitored with lick sensors (Data Sciences International), which were connected to the drinking nipple and were common to each cage. Touching the nipple during fluid intake generates a pulse that is received by the Dataquest IV system. Licking rate was continually recorded at 5-min intervals. Means of these values were analyzed at 6-h intervals.

Rats could obtain food pellets (45 mg; Bioserve, Frenchtown, NJ, USA) from a food dispenser system by lever pressing (FR1). Lever pressing rate was continually recorded at 5-min intervals by the Dataquest IV system. Means of these values were analyzed at 6-h intervals.

Following 24 h of recording basal temperature, activity, food and liquid consumption the alcohol solution was replaced by water. Alcohol withdrawal was monitored for the following 48 h and compared to that of control animals which had only received water.

The influence of acamprosate upon alcohol withdrawal was examined by injecting acamprosate (200 mg; i.p.) twice a day at 8 a.m. and 8 p.m. The acamprosate dose and treatment schedule used in the present study proved to be most effective in other behavioral tests (Gewiss et al., 1991; Spanagel et al., 1995). Controls received saline injections at the same time points.

2.3. Blood alcohol concentrations

A separate group of rats ($n = 8$) were fitted with chronic i.v. catheters in the jugular vein five days following forced drinking of the 20% (v/v) alcohol solution. Two days after recovery blood samples were taken during the dark cycle at different time points (8 p.m.; 12 p.m.; 4 a.m.; 8 a.m.) and were collected in heperanized 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

Results are expressed as means + S.E. For statistical evaluation the values of each treatment group were submitted to a two-way analysis of vaiance (ANOVA) with repeated measures of the factor time, followed by a Neumann Keul's post-hoc test. A probability below 0.05 was considered as significant.

3. Results

3.1. Assessment of alcohol withdrawal

The forced alcohol ingestion schedule used in the present study resulted in an intake of ethanol of 5.3 ± 0.3 g/kg per day. Blood alcohol levels were in a range of 4–46 mg/dl (mean ± S.E.: 38 ± 14 mg/dl; blood alcohol levels at the different time points of collection were: 8 p.m.: 10–46 mg/dl, 12 p.m.: 4–35 mg/dl, 4 a.m.: 15–40 mg/dl; 8 a.m.: 12–45 mg/dl). Locomotor activity as well as body temperature, was significantly enhanced 18 h after the termination of forced alcohol drinking (factor withdrawal x time: locomotor activity: $F(15,256) = 9.8$, $P < 0.001$; body temperature: $F(15,256) = 13.7$, $P < 0.001$; Fig. 1). Both effects were most pronounced during the late night hours (activity phase). When alcohol was withdrawn, food intake was significantly different to that of the control group ($F(7,128) = 5.3$, $P < 0.001$; Table 1). Initially food intake was enhanced, however, one day after the termination of forced alcohol drinking food intake was significantly less than in controls. In licking rates (water intake)

Table 1
Food consumption during alcohol withdrawal and acamprosate treatment

Food intake	Time intervals [h]									
[lever presses/5 min]	−12	−6	0 6	12	18	24	30	36	42	48
Control group	3.1 ± 0.3	2.5 ± 0.2	0.9 ± 0.3	2.0 ± 0.4	3.4 ± 0.5	2.1 ± 0.5	0.7 ± 0.2	1.9 ± 0.3	2.7 ± 0.4	2.9 ± 0.2
Withdrawal group	2.9 ± 0.3	2.0 ± 0.2	2.9 ± 0.3 [a]	2.5 ± 0.2	2.1 ± 0.3 [a]	1.2 ± 0.3 [a]	0.7 ± 0.1	0.8 ± 0.3 [a]	1.4 ± 0.2 [a]	1.6 ± 0.2 [a]
Saline group	3.1 ± 0.2	2.3 ± 0.2	1.2 ± 0.3	1.5 ± 0.3	2.4 ± 0.5	2.6 ± 0.4	0.9 ± 0.4	2.4 ± 0.5	3.1 ± 0.2	1.4 ± 0.4
Acamprosate group	3.2 ± 0.3	2.6 ± 0.2	0.9 ± 0.2	1.3 ± 0.2	2.9 ± 0.3	3.0 ± 0.5	0.9 ± 0.3	1.9 ± 0.2	2.8 ± 0.3	1.8 ± 0.5
Withdrawal group + saline	2.6 ± 0.3	1.9 ± 0.3	2.9 ± 0.4	2.8 ± 0.4	2.0 ± 0.4	1.6 ± 0.3	0.5 ± 0.4	0.4 ± 0.5	1.8 ± 0.4	2.1 ± 0.5
Withdrawal group + acamprosate	2.8 ± 0.3	2.0 ± 0.2	1.0 ± 0.2 [b]	1.0 ± 0.4 [b]	1.5 ± 0.3	0.6 ± 0.2 [b]	0.6 ± 0.3	0.3 ± 0.3	0.8 ± 0.3 [b]	1.3 ± 0.3 [b]

Acamprosate (200 mg; i.p.) was given twice a day at 8 a.m. and 8 p.m. Food intake was measured by lever presses which resulted in the delivery of a food pellet (FR1; 45 mg; see Materials and methods). Values are given as the means from 8 animals ± S.E.M. [a] Significant differences between the control and withdrawal groups. [b] Significant differences between the withdrawal group and saline and withdrawal group + acamprosate. [a,b] $P < 0.05$ by the Newman-Keuls test.

an initial significant increase was observed which lasted for 12 h ($F(7,128) = 3.7$, $P < 0.01$; Table 2).

3.2. Effects of acamprosate in naive animals and on alcohol withdrawal

Acamprosate (200 mg/kg, i.p.), injected twice per day at 8.a.m. and 8.p.m., altered locomotor activity and body temperature in drug-naive animals. Thus, both parameters were significantly enhanced during the late night hours (activity phase) (locomotor activity: $F(15,256) = 4.9$, $P < 0.01$; body temperature: $F(15,256) = 5.2$, $P < 0.01$; Fig. 2). Acamprosate had no effect on food and water consumption (Tables 1 and 2).

Acamprosate (200 mg; i.p.) treatment twice per day at 8 a.m. and 8 p.m. had an inconsistent effect upon signs of alcohol withdrawal in comparison to saline. No effect on alcohol withdrawal-induced hyperthermia could be observed, whereas hyperlocomotion was significantly attenuated ($F(15,256) = 17.8$, $P < 0.001$; Fig. 3). Further, acamprosate-treated animals undergoing alcohol withdrawal had a significantly suppressed food intake in comparison to that of saline-treated control animals undergoing alcohol withdrawal ($F(7,128) = 4.7$, $P < 0.01$; Table 1). The effect of acamprosate treatment during alcohol withdrawal on licking rates was inconsistent. Thus, during some time intervals licking rates were decreased; at other time intervals water intake was increased (Table 2).

It is important to note that during baseline conditions no differences between the groups were found. Furthermore, following 48 h of alcohol withdrawal rats lost some weight, however, this effect was non-significant when compared to that of control animals (body weight after 48 h of alcohol witdrawal: control group: 320 ± 15 g, withdrawal group: 299 ± 14 g).

4. Discussion

In the present study we describe a new fully automated, computerized system for the quantitative assessment of even mild alcohol withdrawal symptoms. Using such a system we studied the effects of acamprosate, a new anti-craving drug, upon chronic alcohol-induced withdrawal symptoms. Acamprosate effectively diminished some signs of withdrawal, implying that acamprosate treatment may be used not only in the alcohol weaning phase, but also in the alcohol detoxification phase in human alcoholics.

Chronic ingestion of alcohol in laboratory animals leads to the rapid development of physical dependence. The assessment of physical signs of alcohol withdrawal is

Table 2
Water consumption during alcohol withdrawal and acamprosate treatment

Licking rate	Time intervals [h]								
[licks/5 min]	0 6	12	18	24	30	36	42	48
Control group	7 ± 4	34 ± 9	73 ± 16	68 ± 12	9 ± 4	21 ± 12	53 ± 16	62 ± 10
Withdrawal group	19 ± 8 [a]	58 ± 7 [a]	76 ± 8	77 ± 12	8 ± 4	22 ± 13	61 ± 9	77 ± 9
Saline group	7 ± 4	24 ± 10	75 ± 12	71 ± 11	12 ± 5	19 ± 9	51 ± 12	63 ± 8
Acamprosate group	6 ± 6	26 ± 11	65 ± 11	76 ± 14	16 ± 7	21 ± 10	70 ± 15	70 ± 9
Withdrawal group + saline	24 ± 12	45 ± 12	88 ± 12	71 ± 11	13 ± 7	20 ± 10	59 ± 11	63 ± 16
Withdrawal group + acamprosate	19 ± 7	55 ± 11	48 ± 7 [b]	51 ± 6 [b]	29 ± 6 [b]	34 ± 8	30 ± 7 [b]	59 ± 11

Acamprosate (200 mg; i.p.) was given twice a day at 8 a.m. and 8 p.m. Water intake was measured by licking rates at the water bottle. Values are given as the means for 8 animals ± S.E.M. [a] Significant differences between the control and withdrawal groups. [b] Indicate significant differences between the withdrawal group + saline and withdrawal group + acamprosate. [a,b] $P < 0.05$ by the Newman-Keuls test.

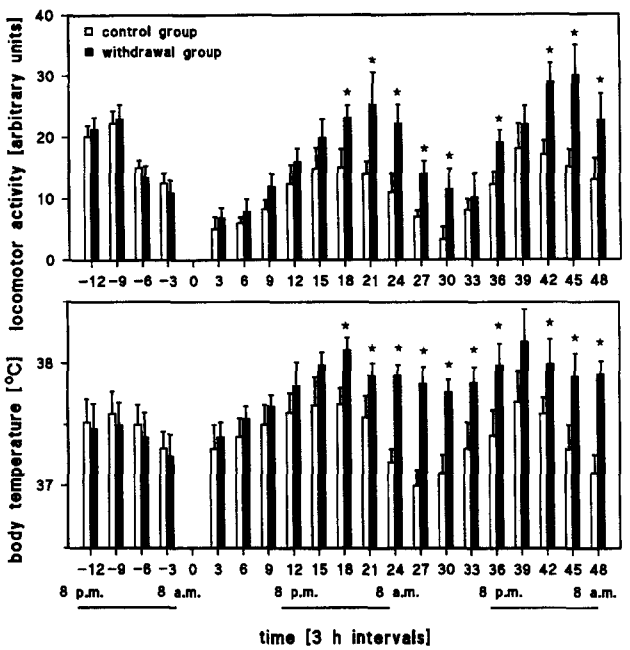

Fig. 1. Locomotor activity and body temperature in rats during alcohol withdrawal. The animals received either water or alcohol solution (20% v/v), as sole drinking fluid for 7 days. Locomotor activity and body temperature were recorded by radiotelemetry over a period of 48 h following alcohol witdrawal; mean values + S.E. of 3-h intervals are given. The lines indicate the dark phase. Asterisks indicate significant differences between the alcohol withdrawal and water control groups ($P < 0.05$).

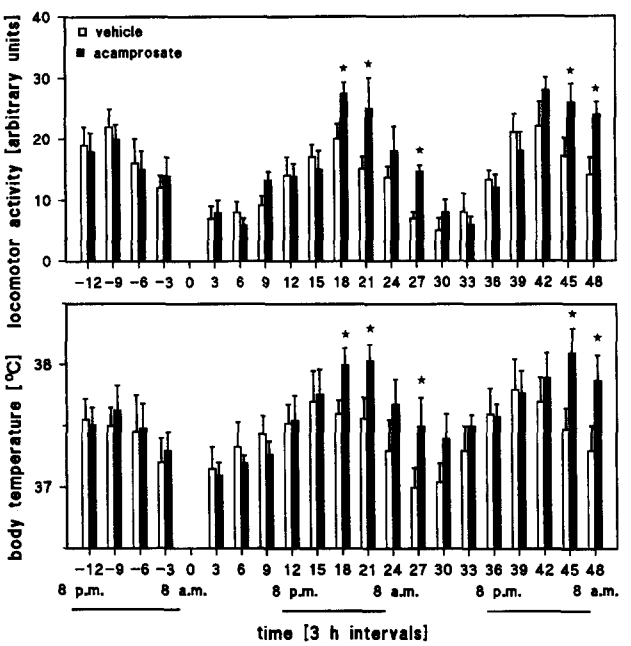

Fig. 2. Effects of acamprosate (200 mg/kg i.p.) given twice daily (8 a.m. and 8 p.m.) upon locomotor activity and body temperature in drug-naive animals. Locomotor activity and body temperature were recorded over a period of 48 h by radiotelemetry; mean values + S.E. of 3-h intervals are given. Lines indicate the dark phase. Arrows indicate either saline or acamprosate injections. Asterisks indicate significant differences between the alcohol withdrawal and water control group ($P < 0.05$).

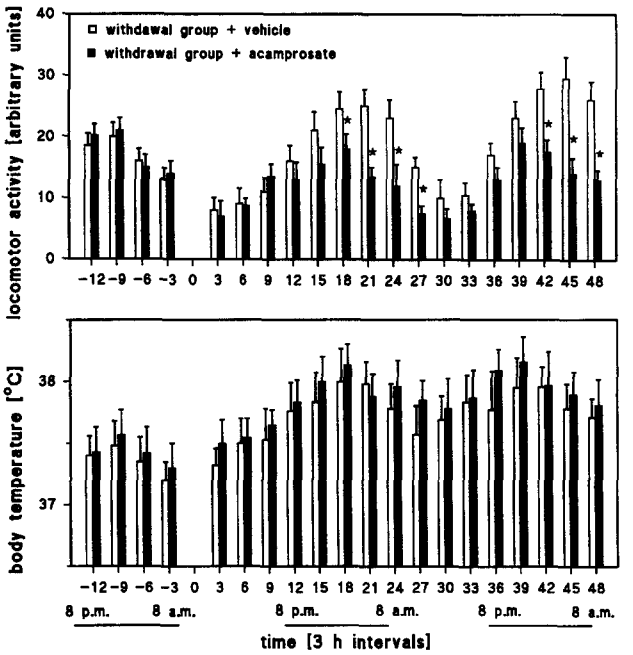

Fig. 3. Influence of acamprosate (200 mg/kg i.p.) given twice daily (8 a.m. and 8 p.m.) upon alcohol withdrawal. Locomotor activity and body temperature were recorded by telemetry over a period of 48 h following alcohol withdrawal; mean values + S.E. of 3-h intervals are given. Lines indicate the dark phase. Arrows indicate either saline or acamprosate injections. Asterisks indicate significant differences between the acamprosate-treated and the saline control groups ($P < 0.05$).

usually carried out with a subjective rating scale (Hunter et al., 1972; Majchrowicz, 1975). However, subjective rating is always difficult to compare and has several limitations (see Pohorecky and Roberts, 1991). In the present study we describe a sensitive telemetry system which measures locomotor activity and body temperature. Furthermore, food and water consumption was also continously monitored in this device. Although a mild, chronic alcohol ingestion schedule (i.e. the presentation of a 20% v/v alcohol solution as the sole drinking fluid) was used, resulting in blood alcohol levels of 38 ± 14 mg/dl, rats displayed clear signs of physical withdrawal after the cessation of chronic alcohol intake. Thus, hyperlocomotion, hyperthermia and a decrease in food consumption were observed. Hyperlocomotion or hyperactivity is a commonly observed sign of alcohol withdrawal (Mello, 1973; Liljequist et al., 1977; Waller et al., 1982). Although hyperthermia is a typical sign of withdrawal in severe alcoholic patients (Feuerlein, 1980), the observation of changes in thermoregulation in animals is controversial. One reason for this controversy might be the method by which body temperature was measured, especially in earlier studies. Thus, repeated or continuous measurement of rectal temperature always induce changes in core temperature (Gallaher et al., 1985), which in turn leads to misinterpretation of data. The use of implanted telemetry probes avoids this problem; using such a system, Gallaher and Egner (1987) described a rebound hyperthermia in rats

following acute ethanol treatment, an observation which was also made during alcohol withdrawal in the present study and by other investigators (Heyne et al., 1991). Further an initially enhanced food intake was observed after the termination of forced alcohol drinking. This observation should be seen in the context that the animals try to compensate for the caloric value of alcohol. At later time points, the animals exhibited a decrease in food consumption which paralleled other signs of alcohol withdrawal such as hyperlocomtion and hyperthermia. Our system takes advantage of telemetry/computer methods to observe the temporal course of several alcohol withdrawal signs and enables us to study behavioral patterns occurring during withdrawal in detail. Although the generally held view is that physical dependence in laboratory animals requires prior consumption of large amounts of alcohol and a continuous high blood alcohol level, the present study shows that physical signs of withdrawal occur even after a mild, chronic alcohol ingestion.

In the second part of this study we investigated the influence of acamprosate on alcohol-induced withdrawal signs. Acamprosate suppressed hyperlocomotion, such that locomotion in acamprosate-treated withdrawal animals was similar to that of control animals. This finding is in line with a study of Gewiss et al. (1991), who showed that acamprosate treatment completely prevented alcohol withdrawal-induced hyperactivity. In contrast, acamprosate treatment had no effect upon alcohol withdrawal-induced hyperthermia. Interestingly, food intake in withdrawal animals was diminished by acamprosate treatment, a finding which was also observed during reinstatement testing of alcohol drinking (Spanagel et al., 1995). Furthermore, acamprosate reduces alcohol withdrawal-induced c-fos expression in the hippocampus and cerebellum (Putzke et al., 1995). However, it is important to note that in control alcohol-naive animals, acamprosate treatment (200 mg/kg; i.p. twice per day) induced changes in locomotor activity. Thus, a transient hyperlocomotion, in particular during the rats' active night phase, was observed. Similar findings have been obtained for vigilance testing in humans; vigilance was enhanced when compared to that following placebo treatment (Lipha, unpublished data). Thus, a slight, however transient, psychomotor stimulant effect of acamprosate must be considered, although it reduces withdrawal-induced hyperactivity. Furthermore, acamprosate increased body temperature, an enhancement which paralleled acamprosate-induced hyperlomotion.

In contrast, Le Magnen et al. (1987) reported inconsistent body temperature changes following acute acamprosate injections. A very low dose of acamprosate (25 mg/kg; i.p.) slightly increased the rectal temperature whereas a dose of 50 mg/kg slightly decreased it. However, there are marked methodological differences between the two studies: (i) Rectal temperature versus core temperature measured by telemetry, (ii) low (25–50 mg/kg i.p.) versus high (200 mg/kg i.p.) doses and (iii) time course of temperature monitoring: in the Le Magnen study temperature was measured for two h following injection, whereas in our study only 18 h following the first acamprosate injection could a significant rise in temperature be observed during the active night phase.

In summary, although acamprosate produces slight effects in drug-naive animals, i.e. transient hyperlocomotion and hyperthermia, it reduces alcohol withdrawal-induced symptoms. These findings suggest that acamprosate may already be beneficial at the beginning of the detoxification phase in alcoholics.

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References

Feuerlein, W., 1980, Alcohol withdrawal syndrom, in: Psychopharmacology of Alcohol, ed. M. Sandler (Raven Press, New York) p. 215.

Gallaher, E.J. and D.A. Egner, 1987, Rebound hyperthermia follows ethanol-induced hypothermia in rats, Psychopharmacology 91, 34.

Gallaher, E.J., D.A. Egner and J.R. Sven, 1985, Automated remote temperature measurement in small animals using a telemetry/microcomputer interface, Comp. Biol. Med. 15, 103.

Gewiss, M., C. Heidebreder, L. Opsomer, Ph. Durbin and Ph. DEWitte, 1991, Acamprosate and diazepam differentially modulate alcohol-induced behavioural and cortical alterations in rats following chronic inhalation of ethanol vapour, Alcohol Alcoholism 26, 129.

Heyne, A., G. Slodkowska and J. Wolffgramm, 1991, Physical dependence on ethanol vs. rebound phenomena in an animal model of alcoholism, Naunyn-Schmied. Arch. Pharmacol. 344, R70.

Hunter, B.E., J.N. Riley and D.W. Walker, 1972, Ethanol dependence in the rat: a parametric analysis, Pharmacol. Biochem. Behav. 3, 619.

Ladewig, D., T. Knecht, P. Leher and A. Fendl, 1993, Acamprosat – ein Stabilisierungsfaktor in der Langzeitentwöhnung von Alkoholabhängigen, Ther. Umschau 50, 182.

Le Magnen, J., G. Tran and J. Durlach, 1987, Lack of effects of Ca-acetyl-homotaurinate on chronic and acute toxicities of ethanol in rats, Alcohol 4, 103.

Lhuintre, J.-P., N.D. Moore, C. Saligaut, F. Boismare, M. Daoust, P. Chretien, G. Tran and B. Hillemand, 1985, Ability of calcium bis acetyl homotaurine, a GABA agonist, to prevent relapse in weaned alcoholics, Lancet i, 1014.

Lhuintre, J.-P., N.D. Moore, G. Tran, L. Steru, S. Langrenon, M. Daoust, Ph. Parot, Ph. Ladure, C. Libert, F. Boismare and B. Hillemand, 1990, Acamprosate appears to decrease alcohol intake in weaned alcoholics, Alcohol Alcoholism 25, 613.

Liljequist, S., S. Ahlenius and J. Engel, 1977, The effect of chronic ethanol treatment on behaviour and central monoamines in the rat, Naunyn-Schmied. Arch. Pharmacol. 300, 205.

Majchrowicz, E., 1975, Induction of physical dependence upon ethanol and the associated behavioral changes in rats, Psychopharmacologia 43, 245.

Mello, N.K., 1973, A review of methods to induce alcohol addiction in animals, Pharmacol. Biochem. Behav. 1, 89.

Moore, N.D. and C. Libert, 1991, Acamprosate, citalopram, and alcoholism, Lancet 337, 1228.

Pohorecky, L.A. and P. Roberts, 1991, Development of tolerance to and physical dependence on ethanol: daily versus repeated cycles treatment with ethanol, Alcohol Clin. Exp. Res. 15, 824.

Putzke, J., R. Spanagel, T.R. Tölle and W. Zieglgänsberger, 1995, Acamprosate differentially alters PTZ- and PTZ plus ethanol withdrawal-induced c-fos expression in the rat brain, Alcohol Alcoholism 30, 550.

Sass, H., M. Soyka, K. Mann and W. Zieglgänsberger, 1996, Relapse prevention by acamprosate: results from a placebo controlled study in alcohol dependence, Arch. Gen. Psychiatry (in press).

Spanagel, R., S.M. Hölter, K. Allingham and W. Zieglgänsberger, 1995, Acamprosate and reinstatement behaviour in the alcohol-dependent rat, Alcohol Alcoholism 30, 551.

Waller, M.B., W.J. McBride, L. Lumeng and T.K. Li, 1982, Induction of dependence on ethanol by free-choice drinking in alkohol-preferring rats, Pharmacol. Biochem. Behav. 16, 501.