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Pharmacology of Acamprosate: An Overview

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ABSTRACT

In the last years important advances have been made in the development of drugs for the treatment of alcohol addiction. Acamprosate (calcium bis-acetylhomotaurine) is one of the better established drugs in this field on the European market. This review focuses first on the pharmacokinetics of acamprosate. The published data and the recent advances in our knowledge on the mechanisms involved in the intestinal absorption and elimination of this drug are summarized. The importance of pharmacokinetics for the proper clinical use of acamprosate is highlighted. The anti-relapse as well as the well-known effects of acamprosate on ethanol intake are discussed. The recent experiments in animal models of conditioned withdrawal are reviewed. These experiments, explored for the first time the anticraving effect of the drug. Finally, the proposed hypotheses on the neuropharmacological mechanism of action of acamprosate are discussed. The discussion deals with the relative importance of various hypotheses as well as with the recent experiments that support them. It is pointed out that further research is necessary in order to clearly understand the mode of action of acamprosate as well as the neurobiological mechanisms involved in alcohol dependence.

INTRODUCTION

Chronic alcoholism continues to be a widespread and debilitating disorder, which places a tremendous burden on society in healthcare costs and personal suffering. The need for effective pharmacologic agents for this disorder cannot be overstated.

Acamprosate was introduced on the European market as a promising medication to treat alcohol dependence nearly fifteen years ago. Since then, acamprosate has been used clinically in several countries of the European Community for the prevention of relapse in

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alcoholics. In the USA the FDA has been reviewing the new drug application (NDA) for acamprosate since December 2001.

There is a substantial clinical evidence that acamprosate increases the abstinence rates in recently detoxified alcoholics and that this effect persists throughout one year of follow-up. This clinical evidence rests mainly on three pivotal European trials. These multicenter, double-blind randomized and placebo-controlled studies, have demonstrated the effectiveness of acamprosate, as compared to placebo, in the prevention of alcohol relapse in European patients (38,39,44,54,56,64). Despite its successful use in the treatment of alcoholism, some relevant aspects of its pharmacology (including some important pharmacokinetic and pharmacodynamic issues) remain unknown. For example, there are no published reports that would clearly explain the reason for the low oral bioavailability of acamprosate or the mechanism involved in its renal elimination. There is also no clear explanation of its mode of action. These aspects are clearly relevant, especially those concerning the effects of this drug on the central nervous system (CNS). A better understanding of the mechanism of the anticraving effect of acamprosate could be useful in the design of new drugs for the treatment of alcoholism. If we know more precisely how acamprosate acts, we might be able to design novel agents with anti-relapse effect and improved biopharmaceutic and pharmacodynamic profiles.

In this manuscript, we have reviewed the literature on the pharmacology of acamprosate, including recent studies on the mechanisms involved in its absorption and elimination. In addition, we have reviewed recent studies on the effects of this drug in animal models of alcoholism as well as current advances in our understanding of the neuropharmacological mechanism of acamprosate action.

PHARMACOKINETICS

Most of the pharmacokinetic studies with acamprosate are unpublished; they are mainly internal Lipha company reports that were briefly summarized in a review by Saivin et al. in 1998 (55). Although these reports are relevant to pharmacokinetics of acamprosate, some other issues, that are relevant to the mechanisms involved in the absorption and elimination of the drug, remained, until recently, completely unknown. The same situation exists with the drug distribution. Although, it has been demonstrated that acamprosate is not bound to plasma proteins (65) and is able to cross the blood-brain barrier in rats (21,57), little is known about its levels in the selected brain areas or the mechanism of its transport through the blood-brain barrier.

First, we will review the published data on absorption and elimination of acamprosate and discuss some aspects of its action that are important for the proper clinical use of this drug. Most of the data presented below have been obtained in our laboratory.

Absorption

Acamprosate (calcium bis acetyl-homotaurine) is a structural analog of γ-aminobutyric acid (GABA) and can be viewed as an acetylated form of taurine (Fig. 1).

Currently, acamprosate is available on the European market as an enteric coated tablet containing 333 mg of active substance; its recommended dosage ranges between 1.3 and

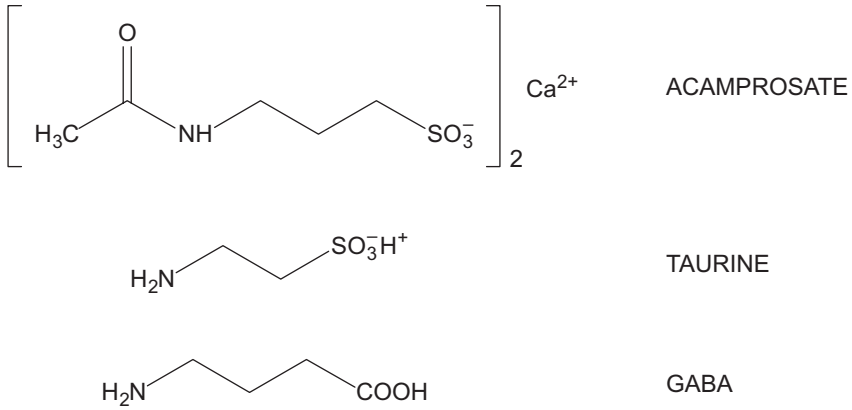

Fig. 1. Chemical structures of acamprosate, taurine, and GABA.

2 g/day. The drug is poorly absorbed and its bioavailability in humans is very low (11 ± 1%) (55).

To better understand how acamprosate is able to cross the intestinal barrier its physicochemical properties should be considered. The drug is highly hydrophilic and is freely soluble in water and most biological fluids. The calcium salt is almost completely dissociated (98%) in hydrophilic media and the acetylhomotaurinic acid is a strong, completely dissociated acid, which means that it has a strongly charged functional group (12). Accordingly, one can postulate that poor intestinal membrane permeability would be the cause of its low oral bioavailability.

Due to these properties, Chabenat et al. in 1988 concluded that acamprosate would cross the biological barriers, presumably with the help of a transporter (12). Several years later, other authors (55) postulated that acamprosate absorption might occur predominantly by the paracellular route.

In order to clarify the mechanisms and routes involved in the intestinal absorption of acamprosate, our research group (43) undertook a kinetic study in the rat using a broad range of acamprosate concentrations. Using *in vitro* methodologies, we showed that acamprosate is transported in the rat jejunum mainly by passive diffusion and, to a much lesser extent, by a carrier system, probably, an amino acid carrier. We did not determine whether diffusion occurred via transcellular or paracellular routes. Our results also demonstrated that chronic ethanol intake does not alter the absorption of the drug.

The knowledge of the mechanism of acamprosate absorption is important, if we want to increase its absorption and bioavailability. In this respect, the use of intestinal enhancers could be a valuable tool, not only to increase absorption, but also to explore the mechanism of its transport.

Several preliminary studies performed in our laboratory, using an *in situ* rat gut technique, confirmed that acamprosate absorption is very low. We also demonstrated that while the non-ionic surfactant, polysorbate 80 (P80) does not increase acamprosate absorption in the rat jejunum, the medium length chain fatty acid, sodium caprate (C10) enhances the intestinal transport of the drug (10,68) (Table 1). These results led us to postulate that the paracellular pathway would be the most probable, since it has been reported that sodium caprate facilitates the absorption of hydrophilic compounds via the paracellu-

TABLE 1. Influence of polysorbate 80 P(80) and sodium caprate (C10) on the absorption rate constant (k_a) of acamprosate (n = 6–10)

Concentration of P80 (mg/L)	$k_a \pm SD^1$ (h^{-1})	Concentration of C10 (mM)	$k_a \pm SD^1$ (h^{-1})
0	0.32 ± 0.02^a	0	0.27 ± 0.01^a
5	0.19 ± 0.06^b	13	0.51 ± 0.18^b
20	0.20 ± 0.05^b	16	0.48 ± 0.19^b
800	0.15 ± 0.04^b		

[1] SD, standard deviation.
[a,b] k_a values with different superscript are statistically different at a 5% significance level.
Adapted from refs. 10 and 68.

lar route (5,40). To confirm this hypothesis, we carried out several *in vitro* experiments with acamprosate in human intestinal cell monolayers (Caco-2 cells) using mannitol as a paracellular marker (unpublished results). The results showed that C10 enhanced the permeability of monolayers to either compound in a concentration-dependent manner. This finding was in agreement with the existence of a paracellular route for the transport of the drug. Additionally, these studies revealed that sodium caprate could be a good candidate for an excipient in acamprosate formulations to enhance drug absorption and, therefore, to improve oral bioavailability.

Excretion

Until recently the data on the excretion of acamprosate were scarce. As indicated above, the studies reviewed by Saivin et al. (55) are unpublished internal reports from Lipha, the manufacturer of the drug. Although these studies suggest that the drug is eliminated mainly by the renal route, there is no agreement on the percent of dose found unchanged in the urine. The results of some of the studies summarized in these internal reports suggest the involvement of a tubular secretion in the renal elimination of the drug. However, in spite of the importance of this aspect of acamprosate pharmacokinetics, until recently this finding has not been further explored.

In 2002, our group published the results of an extensive study on the disposition of acamprosate in the rat (69). Our main objective was to clarify some aspects of the distribution and, mainly, the mechanisms involved in the elimination of the drug. Our results clearly demonstrated that in the rat, pharmacokinetics of intravenous acamprosate is linear over a broad range of plasma concentrations. Moreover, we found that the drug is not metabolized and is excreted almost exclusively as an unchanged drug in the urine by glomerular filtration and tubular secretion. On average, 95% of the administered dose was excreted unchanged in the urine of the animals during 0–6 h after treatment. We also showed that the renal plasma clearance (measured at steady-state conditions) is clearly higher than the glomerular filtration rate in the rat. Since similar findings were reported by Saivin et al. in humans, we decided to investigate a possible interaction of acamprosate with probenecid (a well recognized inhibitor of the tubular secretion of several acidic drugs). We showed that probenecid competitively inhibits the renal tubular secretion of the drug and, therefore, increases in a dose-dependent manner the plasma levels of acamprosate (Fig. 2). This observation is of considerable importance since it indicates that acamprosate

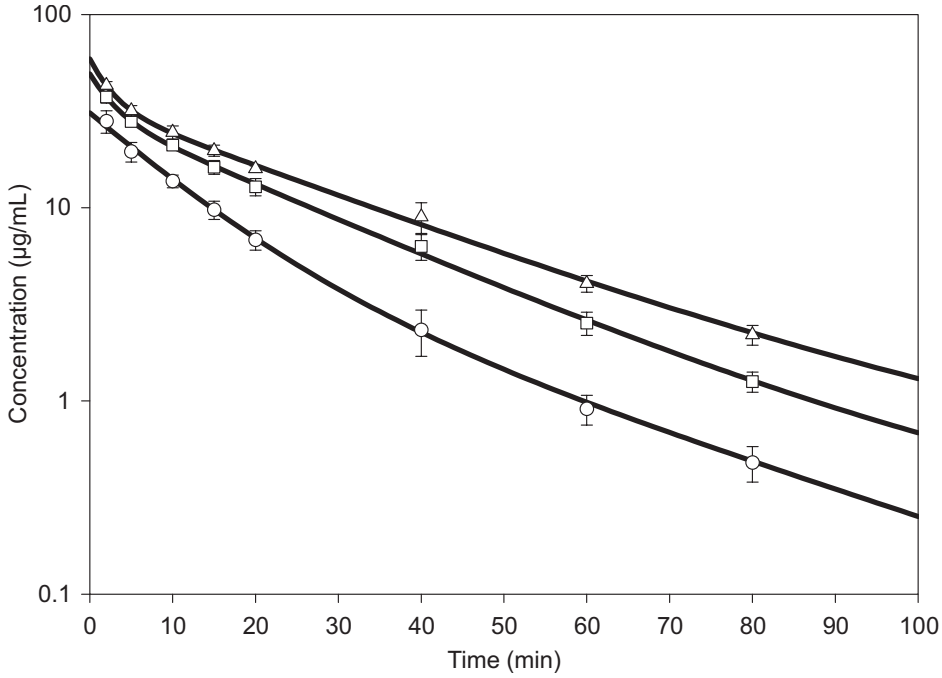

Fig. 2. Plasma level curves of acamprosate obtained after intravenous bolus administration of 9 mg/kg of acamprosate in absence (○) and presence of 32.3 mg/kg (□) or 64.6 mg/kg (△) of probenecid (n = 6). Adapted from ref. 69.

may interact with other acidic drugs, which are secreted by the same renal tubular mechanism.

Pharmacokinetics and Bioavailability of Oral Acamprosate

As with the absorption and excretion, the published studies on the oral pharmacokinetics and bioavailability of acamprosate are scarce.

It has been reported in internal reports from Lipha s. a. that in humans the absolute oral bioavailability of acamprosate is 11 ± 1% (55), a really low value. It is well known that low oral bioavailability leads to high variability in plasma concentrations and, therefore, in poor efficacy of the drug. We postulated that some of the therapeutic failures observed in the trials with acamprosate could have been be due to poor oral absorption.

At the same time, several internal Lipha reports stated that an average elimination half-life ($t_{1/2}$) after oral administration of acamprosate to humans is in the order of 32.7 ± 4.3 h, much longer than after i.v. infusion (3.2 ± 0.2 h) suggesting the existence of a flip-flop mechanism. However, this possibility has not been thoroughly investigated.

To explore this point and to learn more about the bioavailability of acamprosate, we performed several experiments involving oral administration of the drug at two differerent doses to several groups of rats (unpublished). We measured acamprosate plasma levels (Fig. 3) and the unchanged fractions of the drug in the urine (0–24 h) and in feces (0–48 h). Bioavailability was quantified as urinary recovery following oral administration

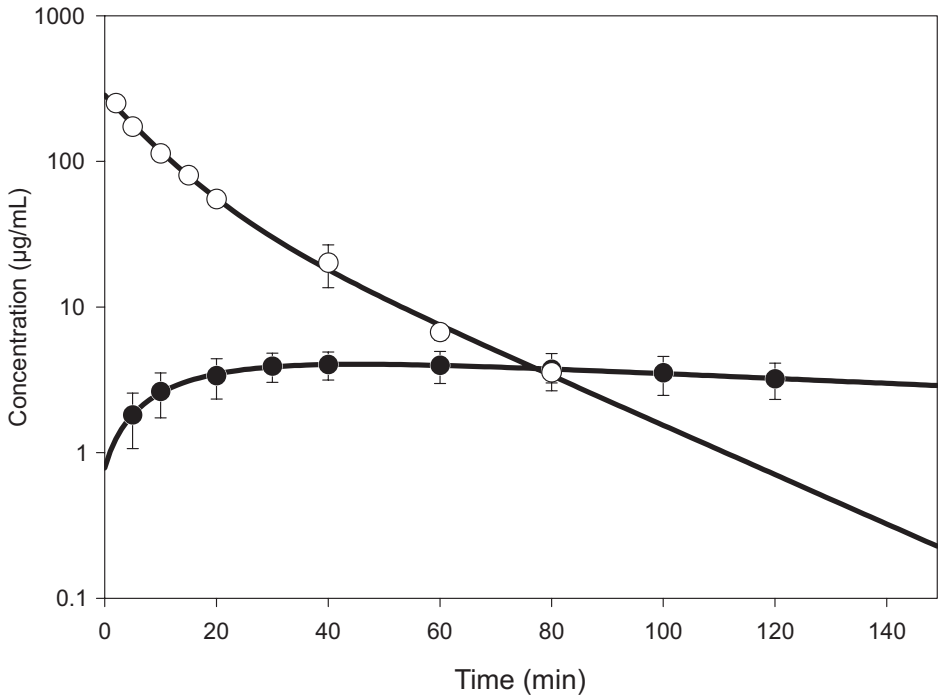

Fig. 3. Plasma level curves of acamprosate obtained after intravenous (○) and oral (as aqueous solution) (●) administration of 70 mg/kg of the drug to rats ($n = 6$).

since acamprosate is stable in plasma and, as we demonstrated previously, is excreted as unchanged drug in the urine. Pharmacokinetic parameters derived from plasma level curves clearly revealed the existence of a flip-flop phenomenon (24). The terminal rate constant obtained in our study (0.006 ± 0.001 min^{-1}) was considerably lower than that obtained after intravenous administration of the drug (0.036 ± 0.003 min^{-1}) (Fig. 3). These results demonstrate that the terminal slope of plasma level curves does not represent the elimination but the absorption process. In fact, this value of the terminal slope coincides with the absorption rate constant obtained in other *in situ* experiments from our laboratory. Hence, it is evident that the absorption of the drug is slower than its elimination.

On the other hand, according to our preliminary results, the mean bioavailability of acamprosate in the rat is approximately 20%. This low oral bioavailability is clearly related to the poor physicochemical characteristics of this drug, which leads to a poor intestinal permeation. Acamprosate intestinal absorption occurs, as indicated above, mainly by passive diffusion through the paracellular route. Therefore, the time of the drug residence in the gastrointestinal tract might be clearly insufficient to achieve a more complete absorption.

However the bioavailability of acamprosate in the rat (20%) is higher than that in humans. This finding seems surprising, since in general the bioavailability of drugs in rats tends to be lower than in humans (27). It is important to consider, however, that rats received the drug in an aqueous solution whereas humans as enteric coated tablets. This suggests that in order to be absorbed from the enteric coated tablets the drug must be first

dissolved. Saivin et al. (55) reported that in one study in human volunteers, the bioavailability of acamprosate in enteric coated tablets was 50% of that seen with the aqueous solution. It seems that the enteric coated tablets remain longer and possibly for variable periods of time in the stomach affecting the rate and extent of the acamprosate absorption. Clearly, this pharmaceutical formulation contributes to the flip-flop process and, more important, increases the variability of the absorption. In our opinion, a practical solution to improve the acamprosate absorption would be to change the dosage form using, for instance, individually coated granules (i.e., small microcapsules) that would empty gradually, but continuously, in the duodenum (24).

Recently, Mason et al. (45) reported the results of a pharmacokinetic and pharmacodynamic study in humans with a combination of acamprosate and naltrexone. It appears that naltrexone significantly increased the rate and the extent of acamprosate absorption. With this combination maximal plasma concentrations of acamprosate (C_{max}) were 33% higher than with acamprosate alone. The area under the plasma level curve (AUC) was also increased by 25%. This phenomenon has been recently confirmed by Johnson et al. (32), who have showed that naltrexone administration significantly enhanced acamprosate plasma levels.

Although Mason et al. (45) suggested a plausible explanation for the alteration of the gastrointestinal transit of acamprosate caused by naltrexone, they have not provided any experimental evidence. Further research on this interaction is, therefore, warranted.

BEHAVIORAL PHARMACOLOGY

Anti-craving Effects of Acamprosate

Several pharmacological and behavioral studies suggest that acamprosate does not act by imitating the subjective or reinforcing effects of alcohol. Acamprosate does not alter the alcohol-induced hypothermia, motor impairment and taste aversion for ethanol (37). It has also been shown that acamprosate does not have any reinforcing or aversive effects on its own. Acamprosate is not self-administered by monkeys (25), and does not induce place preference in rats (33,42). Acamprosate neither antagonizes the discriminative stimulus properties of alcohol, nor substitutes for alcohol in a discrimination task (59).

Therefore, and by exclusion, Littleton suggested in 1995 (41) that acamprosate is an "anti-craving" drug. Since then, some reports have tried to provide support for this hypothesis. These reports are reviewed below.

Littleton suggested that acamprosate reduces the incidence and severity of relapse drinking, principally, by reducing the "conditioned withdrawal syndrome." This concept is not easy to explain, but its understanding is needed to interpret recently reported effects of acamprosate in some behavioral studies. The hypothesis is as follows: the repeated administration of alcohol would induce adaptive changes in the brain, which would oppose the acute effects of ethanol. As the drug administration is repeated in the same environmental setting, these adaptive and opposite responses may become conditioned to the environmental stimuli. This phenomenon would explain the so-called "context-dependent tolerance" or "environment-dependent tolerance" to ethanol. Animals treated repeatedly with ethanol in the same environmental setting, develop tolerance to

the drug. This phenomenon has been observed for most of the acute effects of ethanol in mice and rats.

However, when in the environmental setting in which the animals repeatedly received ethanol, external stimuli are followed by the administration of saline, the compensatory responses, conditioned to the external stimuli, may trigger the effects opposite to the acute effects of ethanol. Under these conditions the compensatory responses cannot be counteracted by ethanol. For example, the presentation of the experimental setting to a rat that habitually received ethanol in that setting could induce hyperactivity. This response is clearly opposite to the motor inhibitory effects of ethanol. This phenomenon can be easily extrapolated to the human context. In the case of a detoxified alcoholic the compensatory responses could be evoked, for example, by entering into a familiar bar; the setting would trigger a strong adaptive response that would include anxiety, tremor, sweating, and so on, i.e., the so-called "conditioned withdrawal syndrome. "

The hypothesis for conditioned withdrawal assumes that some of the adaptative neurochemical mechanisms that cause the true withdrawal syndrome are shared with those involved in the conditioned syndrome. Therefore, any drug that prevented alcohol withdrawal could be useful to prevent craving and, therefore, relapse in alcohol drinking.

As we will describe in another section (see, *Neuropharmacological studies*) several authors reported that acamprosate reduces the acute neuronal hyperexcitability during ethanol withdrawal. Recently, several studies have been published suggesting that acamprosate could also reduce the conditioned withdrawal to ethanol. In 1999 Cole et al. (13) described an animal model useful for the study of the conditioned withdrawal to ethanol. This model was used in 2000 (14) to describe the effects of acamprosate and naltrexone on the behavior that best characterizes this conditioned abstinence model, namely the increase in stretched-attend postures. Acamprosate reduced the conditioned compensatory responses induced by ethanol-paired cues. In contrast, naltrexone did not show a significant effect on any of the behaviors exhibited by mice in this model.

In another recent study, Quertemont et al. (50), explored the effects of acamprosate in context-dependent tolerance to ethanol. Chronic acamprosate treatment totally abolished environment-dependent tolerance to ethanol. In this study, authors also observed an ethanol-opposite response (hyperactivity) when the external stimuli were presented in the absence of ethanol, although it was attributed to non-associative processes instead of conditioned withdrawal.

Therefore, the studies presented above seem to indicate that acamprosate is able to reduce the negative reinforcement of alcohol drinking by attenuating the severity of the conditioned withdrawal, supporting the initial hypothesis of Littleton.

However, acamprosate could also reduce the positive reinforcing effects of ethanol. In a recent paper (42) it was shown that acamprosate inhibits the development of ethanol conditioned place preference, suggesting that the ability of acamprosate to reduce ethanol consumption and relapse rates may also be attributable, at least in part, to its ability to reduce the acute rewarding effects of ethanol. In fact, Olive et al. (48) have shown that acamprosate attenuated the ethanol-induced increase in dopamine levels in the nucleus accumbens of rats, an event clearly associated with the rewarding effects of ethanol consumption.

Effects on Alcohol Intake and Relapse

Appropriate animal models of alcohol relapse are needed to study the neurobiological and molecular mechanisms of action of acamprosate. During the last few years several such models have been developed and used to study the mechanism of action of this drug.

Initially, acamprosate effects on alcohol intake were studied in several models of alcohol self-administration and alcohol preference in Wistar rats. Acamprosate dose-dependently decreased ethanol intake in animal models of ethanol self-administration without affecting food or fluid intake. Since the initial work of Boismare et al. in 1984 (8), several studies using distinct experimental procedures have confirmed this effect of acamprosate on total alcohol intake. At single acute doses acamprosate, at 200 or 400 mg/kg i.p., selectively reduced ethanol intake (15,29,37,48,61).

The anti-relapse effect of acamprosate has been studied by several authors in the alcohol-deprivation model. In this model, the intake of alcohol is determined after prolonged periods of forced abstinence in drug experienced rats. This model is based on the quantification of the alcohol deprivation effect (ADE). The ADE is the temporary increase in alcohol consumption after periods of abstinence. In general, animals are first trained to consume alcohol. When alcohol consumption is stabilized, animals are subjected to forced abstinence. If ethanol is then reintroduced, animals transiently increase their alcohol intake over the pre-deprivation period.

The ADE can be observed in two (or more) bottle choice studies or in operant alcohol administration paradigms (34) and both methods have been used to study the anti-relapse effect of acamprosate.

The first published study on this subject was conducted by Spanagel et al. in 1996 (58). These authors, using a three bottle choice procedure, reported that acamprosate, administered twice daily by i.p. route, significantly decreased, in a dose-dependent manner. the ADE in Wistar rats. Moreover, given at 200 mg/kg, acamprosate reduced alcohol intake to below baseline drinking.

The same group (30) studied the effects of acamprosate on the ADE in an operant two-lever free choice paradigm with concurrent water intake. In this study, acamprosate reduced in a dose-dependent manner lever pressing for ethanol and, consequently, the ethanol consumption and the ADE. Maximal effects were observed at the 200 mg/kg i.p. dose, during the first hour of the session.

In 1998, Heyser et al. (29) reported similar results to those described above. In this paper, authors described the effects of acamprosate on ADE using an animal model of oral ethanol self-administration in a limited access paradigm. By chronic administration acamprosate, at 100 and 200 mg/kg i.p., significantly blocked ADE. More recently, the efficacy of the acamprosate/naltrexone combination in the prevention of ADE was compared with that of naltrexone alone (28). In this publication, authors reported that acamprosate, combined with naltrexone, prevents ADE not only on post-deprivation day 1, but also on post-deprivation day 2. On that day the authors observed an increase in ethanol consumption in rats treated with naltrexone alone. Taken together, these studies show that acamprosate alone or in combination with other drugs, such as naltrexone, can reduce ADE.

NEUROPHARMACOLOGICAL STUDIES

In spite of the well documented efficacy of acamprosate in reducing alcohol consumption and the severity of relapse in humans and in animal models of alcoholism, the mechanism of acamprosate action remained unclear. During the last few years several authors have studied this problem using several methodologies.

Initial studies postulated that acamprosate might affect central neurones through an activation of the GABA neurotransmission (19,20). However, this hypothesis has been practically ruled out. Acamprosate does not seem to affect $GABA_A$ receptor mediated transmission. *In vitro* studies have shown that acamprosate neither interacts with $GABA_A$ receptors nor influences Cl^- currents triggered by activation of these receptors (6,66,67).

Since approximately 1997, investigators re-directed their efforts and explored three possible mechanisms of action of acamprosate (31,60):

— interaction of acamprosate with N-methyl-*D*-aspartate (NMDA) receptors;
— blockade of voltage-dependent Ca^{2+} channels;
— changes in NMDA receptor subunit composition.

In this section we review data that support these three hypotheses.

Interaction with NMDA Receptors

This hypothesis has received substantial attention by investigators. The interaction of acamprosate with NMDA receptors has been shown using several *in vitro* to *in vivo* techniques. In general, there is a consensus about the interaction of acamprosate with these receptors. However, it is not clear whether acamprosate enhances or inhibits NMDA receptor function. Probably, as suggested by Allgaier et al. (1), the differences in the NMDA receptor subunit expression in different brain regions, may account for the differences observed with the effects of acamprosate on NMDA receptor function. Zeise et al. (66), using *in vivo* electrophysiological recordings and *in vitro* techniques, reported in 1993 that acamprosate reduces the activation of synapses controlled by L-glutamate (L-Glu) in the rat neocortex. These authors showed, for the first time, that acamprosate potently and reversibly reduces depolarizing responses induced by several excitatory amino acids, including L-Glu and NMDA.

Since the publication of this paper, several authors have reported data on this topic and, in some cases, with opposite results. So, Berton et al. (1998) (6) using *in vitro* electrophysiological techniques showed that in nucleus accumbens slices acamprosate enhances, rather than inhibits NMDA-mediated neurotransmission.

In vitro binding studies (2,47) suggested that acamprosate allosterically interacts with a polyamine site on the NMDA receptor complex. In the opinion of these authors the effects of acamprosate are compatible with a partial agonist action at the NMDA receptors, i.e., acamprosate may have excitatory or inhibitory effects on these receptors depending on its concentration and receptor activity.

The interaction of acamprosate with NMDA receptors has also been studied using other *in vitro* techniques. So, Allgaier et al. (1) studied the effect of the drug on the Ca^{2+} influx evoked by NMDA in cultured neurons. The results obtained by these authors clearly show that acamprosate significantly reduces NMDA-induced elevation in free intracellular Ca^{2+} concentration. Similarly, Al-Qatari et al. (3) showed that acamprosate inhibits glutamate-induced calcium entry in cultures of neocortical neurons without affecting glutamate

neurotoxicity. Curiously, when cultures were previously exposed to ethanol, acamprosate blocked, in a concentration-dependent manner, not only glutamate-induced calcium entry but also the subsequent neurotoxicity. Authors concluded that, although it is unlikely that acamprosate directly affects NMDA receptors at the glutamate binding site or at the receptor-operated calcium channel, this drug could exert its inhibitory effects on NMDA receptor function through other receptor sites. In fact, Mayer et al. (46) reported that acamprosate interacts with an NMDA receptor site that is activated by several polyamines such as spermine and spermidine. In their experiments, acamprosate reduced the spermidine-induced neurotoxicity and calcium entry in ethanol-withdrawn cultures from neonatal rat hippocampus but was ineffective against NMDA-induced toxicity or calcium entry.

The neurochemical consequences of the interaction of acamprosate with the NMDA receptors in selected brain areas have been recently explored using *in vivo* microdialysis. One important brain area is the dopaminergic mesolimbic system. Over the course of chronic ethanol exposure, adaptations develop in the mesolimbic dopaminergic function that tend to counteract sustained stimulation of this system by ethanol (63).

There is broad evidence supporting the findings that the glutamatergic control of the activity of dopaminergic neurons in the mesolimbic system is affected by chronic exposure to ethanol (22). This control is mediated, at least in part, through NMDA receptors located both in the nucleus accumbens (NAc) and in the ventral tegmental area (VTA). We have recently studied (11) the effects of acamprosate on extracellular dopamine (DA) levels in NAc of conscious rats. We have shown, using microdialysis *in vivo*, that the effects of acamprosate in this brain area are compatible with an antagonist action at the NMDA receptors. Effects of agonists and antagonists of NMDA receptors in the mesolimbic system are complex and concentration-dependent. Local administration in NAc of several agonists of these receptors (such as NMDA) or substances that increase endogenous levels of L-Glu [as L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC)], induces significant elevations in NAc DA levels. Acamprosate, applied locally to NAc by reverse dialysis inhibits this effect, probably by blocking NMDA receptors.

Consistent with this antagonist action at the NMDA receptors, recent preliminary experiments in our laboratory (unpublished results), have shown that local application by reverse dialysis of acamprosate in NAc is also able to suppress elevation in NAc DA, induced by chemical stimulation of the ventral subiculum of hippocampus. The ventral hippocampus sends a dense glutamatergic input to NAc and its stimulation (by chemical or electrical procedures) induces persistent elevations in NAc DA levels (9,23,35,36). These elevations can be blocked by intra-NAc application of an antagonist of NMDA receptors (7,62). The antagonist action of acamprosate at the NMDA receptors could explain the suppression of elevation in NAc DA evoked by stimulation of the hippocampus.

Other neurochemical studies also support the existence of an interaction between acamprosate and the NMDA receptors. Thus, several studies have shown that alcohol withdrawal is associated with increases in extracellular L-Glu levels in NAc (16,53). Interestingly, acamprosate not only reduced the increase in L-Glu during withdrawal but also was able to reduce the hypermotility during ethanol withdrawal syndrome (16–18) in a way similar to that observed with other well recognized NMDA antagonists (52). Curiously, the neuronal hyperexcitability during the withdrawal state is also accompanied by an increase in the expression of the immediate-early gene c-fos in various brain regions. Acamprosate suppressed the elevations in c-fos expression in several rat brain structures such as hippocampus and NAc (49).

Taken together, it seems clear that acamprosate reduces glutamatergic neurotransmission. At this moment it is not clear whether these effects are a result of a direct interaction with the NMDA receptor channel or with the polyamines-sensitive binding site at the NMDA receptor. Acamprosate seems to act as a functional antagonist at the NMDA receptor system counterbalancing chronic ethanol-induced changes. This would lead to a reduction of neuronal hyperexcitability during the true and the conditioned ethanol withdrawal.

Blockade of Voltage-Dependent Ca^{2+} Channels

In addition to the above described interaction with the NMDA receptors, acamprosate has been found to block the voltage-gated Ca^{2+} channels. This effect could also be responsible for the reduction of neuronal hyperexcitability by acamprosate during alcohol withdrawal.

Initially, it has been reported that acamprosate displaces Ca^{2+} channel antagonists from a low-affinity site on brain membranes and inhibits the upregulation of Ca^{2+} channels in alcohol-withdrawn rats (4). More recently, Allgaier et al. (1) reported that in rat cultured mesencephalic neurons acamprosate antagonizes K^{+}-induced increase in intracellular Ca^{2+} concentration in a concentration-dependent manner.

Changes in NMDA Receptor Subunit Composition

In 2001, Rammes et al. (51) reported that acute acamprosate (and other well established NMDA antagonists such as memantine and MK-801) increased the expression of specific NMDA-receptor subunits in selected brain areas. This phenomenon could constitute a novel pharmacological explanation of the mechanism of action of this drug.

Importantly, upregulation has been selectively observed in the cortex and hippocampus and no changes were detected in the brainstem. In the cortex, the changes in protein expression were detected after a single i.p. dose of 200 mg/kg of acamprosate; they affected mainly the NMDAR1–3/1–4 and NMDAR2B subunits. In the hippocampus two injections of acamprosate, 200 mg/kg i.p., separated by a 12-h interval, increased protein expression of all subunits investigated (NMDAR1–1/1–2; NMDAR1–3/1–4; NMDAR2B).

These data strongly suggest that NMDA-receptor subunit expression can be rapidly and region-dependently increased after acute exposure to acamprosate. Provided that changes in NMDA-receptor subunits composition could induce changes in functional characteristics of these receptors in the brain regions affected, the modulation induced by acamprosate may constitute a novel explanation of the mode of action of this drug. However, more experiments are necessary to properly characterize the neurochemical consequences of these changes in the transcription of NMDA receptor subunits.

A new hypothesis to explain the mechanism of action of acamprosate has been recently proposed (26). According to this hypothesis acamprosate exerts its effects through interactions with the group I metabotropic receptors for glutamate (I mGluR). Harris et al. (26) found that acamprosate inhibits the binding of *trans*-ACPD (a well recognized agonist of the group I and II metabotropic glutamate receptors) to AP2 membrane preparations of several rat brain areas. Moreover, acamprosate was neuroprotective against *trans*-ACPD induced neurotoxicity in organotypic hippocampal slice cultures. Hence, in the opinion of

the authors, acamprosate's binding and functional characteristics are consistent with it being a group I mGluR antagonist.

SUMMARY

Exciting advances have been made in the pharmacology of acamprosate. Some pharmacokinetic aspects involving intestinal absorption and the renal excretion of this drug have been recently clarified. The oral absorption of acamprosate involves passive diffusion mechanism and paracellular pathway. The rate of its absorption is very low and appears to be responsible for its poor oral bioavailability. Recent experiments indicate that acamprosate is rapidly and completely eliminated by glomerular filtration and tubular secretion. This finding suggests the possibility of drug interactions with other acidic drugs that are secreted by the same tubular mechanism.

The development of appropriate animal models allowed us to explore the anti-craving and anti-relapse effects of this drug. During the last few years numerous publications described the effects of acamprosate on alcohol intake, relapse and conditioned withdrawal in animal models of alcoholism, supporting the efficacy of this drug in the treatment of alcoholism.

The precise neuropharmacological mechanism(s) of action of acamprosate remain(s) unknown, although several hypotheses have been proposed. The main hypothesis suggests that a reduction of glutamatergic hyperexcitability due to a weak antagonistic action at the NMDA receptors is responsible for the therapeutic effect of acamprosate. Other possible mechanisms of acamprosate action include blockade of voltage-operated calcium channels and changes in NMDA receptor subunit composition. Further investigations are needed to elucidate the validity and/or relative importance of the above hypotheses.

Clearly, the knowledge of the mechanism of action of this drug will facilitate not only the studies of the neurobiological mechanisms involved in alcohol addiction but also the development of new drugs for the treatment of alcohol dependence.

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