Pharmacology Biochemistry and Behavior, Vol. 41, pp. 669-674, 1992

0091-3057/92 $5.00 + .00

Acamprosate Modulates Synaptosomal GABA Transmission in Chronically Alcoholised Rats

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Received 30 April 1991

DAOUST, M., E. LEGRAND, M. GEWISS, C. HEIDBREDER, P. DEWITTE, G. TRAN AND P. DURBIN. *Acamprosate modulates synaptosomal GABA transmission in chronically alcoholised rats.* PHARMACOL BIOCHEM BEHAV 41(4) 669-674, 1992. – Male Sprague-Dawley rats were pulmonary alcoholised for 30 days. Six were treated with acamprosate (400 mg/kg/day, PO) during alcoholisation. The control nonalcoholised group also received acamprosate (400 mg/kg/day, PO) during the 30 days. At the end of the experiment, brains areas (cortex, hippocampus, thalamus, striatum, and olfactory bulbs) were dissected for the study of synaptosomal ^{3}H-GABA uptake. In another experiment, GABA levels were determined in the same areas using HPLC with electrochemical detection. Blood ethanol levels were also measured during alcoholisation. Acamprosate treatment did not modify blood ethanol levels. In cortex and olfactory bulbs, alcoholisation increased ^{3}H-GABA uptake (V_{max}) with an increase in the affinity (K_m). ^{3}H-GABA uptake was not affected by alcoholisation in other brain areas. In hippocampus and thalamus, acamprosate treatment enhanced ^{3}H-GABA uptake (V_{max}) only in alcoholised rats. Moreover, in thalamus, alcoholisation enhanced GABA levels. The effect of alcohol and acamprosate on GABA presynaptic events is discussed and it is concluded that the action of ethanol and acamprosate on GABA transport could be, in part, responsible for the modulation by acamprosate treatment of ethanol behaviour.

Acamprosate Ethanol ^{3}H-GABA uptake GABA levels Pulmonary alcoholisation

SEVERAL lines of evidence suggested that γ-aminobutyric acid (GABA) plays a major role in the pharmacology of ethanol (EtOH) [for review, see (24)], but the molecular mechanism involved in the central action of alcohol is still unknown. Behavioural studies showed that EtOH potentiates the sedative properties of GABA mimetic drugs (3,19) and this effect was blocked by the GABA receptor antagonist picrotoxin (23). In biochemical studies, EtOH produced either no change (29) or an increase in brain levels of GABA (26). Data obtained by receptor binding techniques showed an increase in the number of low-affinity GABA receptors in rats and mice after acute injection of EtOH (14,31). On the other hand, no change in GABA receptor density was observed after exposure to EtOH in rats (20,30). Studies using ^{35}S-t-butylbicyclophosphonothionate (^{35}S-TBPS) clearly showed that chronic EtOH administration enhances the function of the $GABA_A$-coupled chloride channel (28). Moreover, a number of workers have shown that EtOH decreases $^{45}Ca^{++}$ uptake in synaptosomes (12,18) and that the decrease in calcium entry is paralleled by attenuation of depolarisation-dependent release of neurotransmitter (17). Taken together, these data support the idea that pharmacological, biochemical, and behavioural effects elicited by EtOH are mediated, in part, by an increase in the functioning of the GABA system. Consequently, it can be suggested that a drug that modifies EtOH behavior both in rats (1) and in men (18) could exert its action on EtOH intake via, in part, the GABA system. We previously showed that acamprosate decreased EtOH intake in rats (1,16). This effect was antagonised by the GABA antagonist bicuculline, and we showed that acamprosate possessed GABA properties (2). In rats, we also showed that GABAergic drugs were able to decrease EtOH intake (6). The success of clinical trials (25) in alcoholic patients with acamprosate encouraged us to study more deeply its pharmacological action on GABA synapse.

The purpose of the present study was to elucidate the action of acamprosate on GABA transmission in alcoholised

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rats. To evaluate modifications of GABA transmission, we studied synaptosomal GABA uptake in different brain areas and, on the other hand, GABA levels in these areas in rats treated with acamprosate, alcoholised or not.

METHOD

Alcoholisation (15)

Animals (male Sprague-Dawley rats), individually housed, were maintained for 4 weeks in an isolated plastic chamber (160 × 60 × 60 cm) in an alcohol-containing atmosphere. A mixture of alcohol and air was pulsed into the chamber via a mixing system allowing the quantity of alcohol to be increased every 2 days during the period of the experiment.

Two groups of six rats were kept for 4 weeks in the alcoholisation chamber. The first group received acamprosate (400 mg/kg/day PO), and the second group received tapwater (control group) in their drinking bottle. The ingestion of acamprosate reached a mean value of 7.27 ± 2.76 mg/ml/rat/day. Moreover, two groups of six nonalcoholised rats were submitted to the same treatment during a 4-week period outside the plastic chamber. At the end of the experimental procedure, both alcoholised and nonalcoholised rats were decapitated and their brains rapidly removed. The olfactory bulb, frontal cortex, striatum, thalamus, hypothalamus, and hippocampus were dissected out on an ice-cooled glass plate. Tissue samples from alcoholised and nonalcoholised rats were then placed into Eppendorf tubes and stored at −20°C awaiting studies of GABA levels. A second experiment was done and brain areas were immediately used for GABA uptake studies.

^{3}H-GABA Uptake (35)

Synaptosomes from brain areas were prepared in O.32 M cold sucrose and homogenised with a potter-type Elvejhem (850 rpm). After centrifugation (1000 × *g*, 10 min), the supernatant synaptosomal preparation was preincubated for 5 min in oxygenated Krebs medium (O_2 95%, CO_2 5%). At the end of preincubation, uptake was started with ^{3}H-GABA. Varying concentrations of GABA (0.1–5 μM, six concentrations) were used, keeping ^{3}H-GABA concentration constant (10 nM). Incubation was carried out at 37°C for 3 min in Krebs oxygenated medium containing $_B$alanine (1 mM) to prevent glial uptake. Incubation was stopped by filtration (Whatman GF/B filters, 45 μm pore sizes). Each filter was rinsed twice with 5 ml iced buffer and dried. Filters were soaked in a scintillation vial with scintillation cocktail (ACS II, Amersham). Radioactivity was then determined in a liquid scintillation counter (Kontron, efficiency 30%). Nonspecific uptake was done in parallel at 0°C and represented 20% of total uptake. The protein concentration was determined with crystalline bovine serum albumine as standard (22).

Determination of GABA Levels (35)

Brain areas were weighed and homogenised with an ultra Turrax in 1 ml cold methanol and centrifuged for 10 min at 8000 × *g*, 4°C, and stored at −20°C until assayed.

Chromatographic system. The HPLC system comprised a Beckman 110B pump with a Rheodyne 7125 injector (loop: 20 μl), a spherisorb column (C 18, 25 cm, 4.6 mm i.d., 3 μM particule size). The effluent was analysed with a Metrohm 686 electrochemical detector with a vitreous carbon electrode as working electrode, an Ag/AgCl reference electrode, and a gold auxiliary electrode. The potential used was 0.75 V and the sensivity 10 nA. The detector output was recorded and integrated on a Spectra Physics integrator SP 4290 with a 10-mV input.

Mobil phase. The mobil phase was a mixture (2/3, vol/vol) of 0.1 M KH_2PO_4 and methanol. The solution was adjusted to an apparent pH of 5.5 with 1 M H_3PO_4 and filtered through a 0.45-μm Millipore filter before use. Flow rate was 0.9 ml/min.

Reagents. The derivatising solution consisted of 27 mg *O*-phthaldehyde (OPA), 1 ml absolute ethanol, and 40 μl ethyl mercaptan (EMC) in 10 ml 0.1 M sodium tetraborate. The pH was adjusted to 9.17 with 5 N NaOH. The GABA stock solution (20.6 mg in methanol–water; 50/50) was stored at 5°C for up to 1 month.

Derivatisation. Tissue samples were derivatised by incubating 50 μl supernatant or diluted GABA with 200 μl OPA-EMC solution for exactly 2 min at 20°C. At the end of 2 min, 20 μl derivatisation mixture was injected into the HPLC column.

Blood Ethanol Levels

During alcoholisation, tail blood was sampled and blood ethanol levels were determined using enzymatic determination (NAD-NADH multiassay vial, Sigma).

Statistical Analysis

A Student's *t*-test was used to compare results (uptake parameters: K_m and V_{max} and GABA levels) in treated groups with those of respective controls.

RESULTS

Acamprosate treatment does not modify blood EtOH levels during alcoholisation (Fig. 1).

All V_{max} values in different brain areas are presented in Fig. 2.

In cortex and olfactory bulbs, alcoholisation increases ^{3}H-GABA uptake. In cortex, V_{max} is increased (1 ± 0.4 pmol/mg prot/3 min in control vs. 2.71 ± 0.5 pmol/mg prot/3 min in alcoholised rats, $p < 0.05$) without modifications in the affinity (Table 1). In olfactory bulbs, the increase in V_{max} (0.3 ± 0.08 pmol/mg prot/3 min in control vs. 12 ± 3 pmol/mg

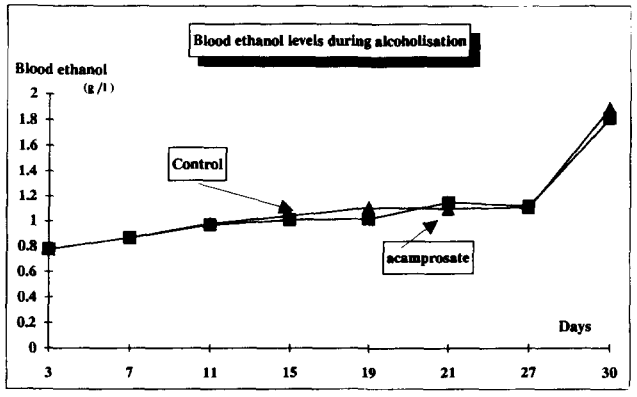
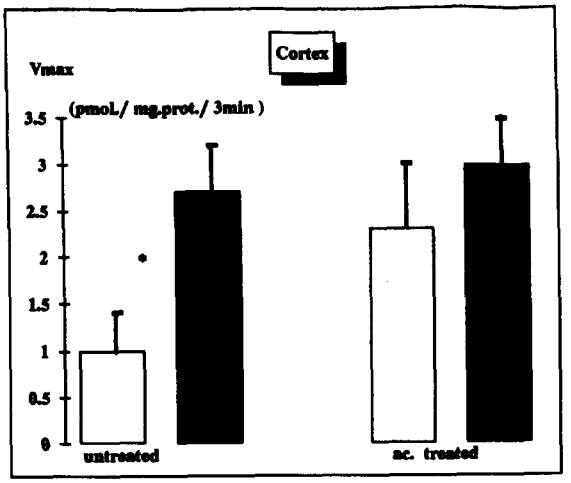
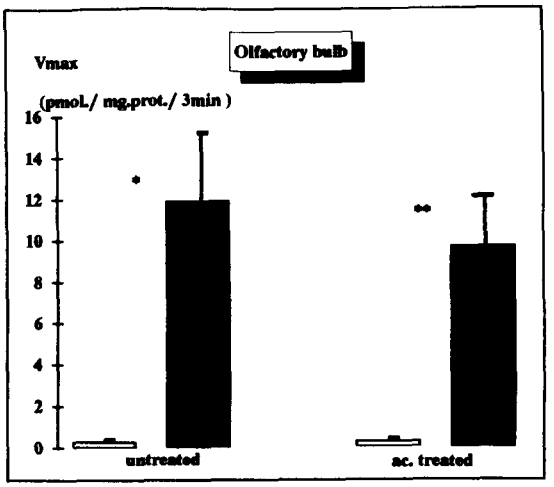
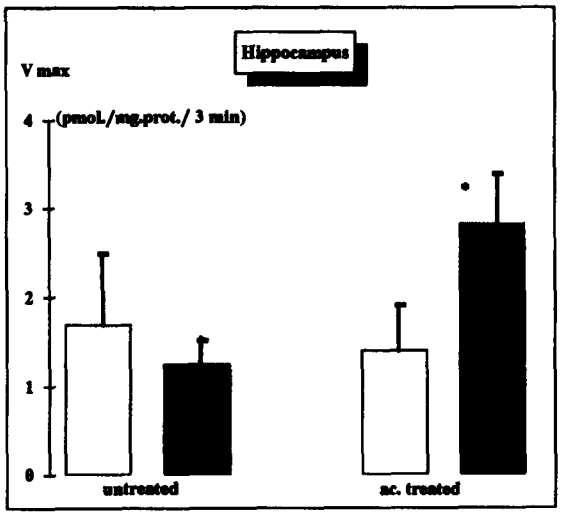
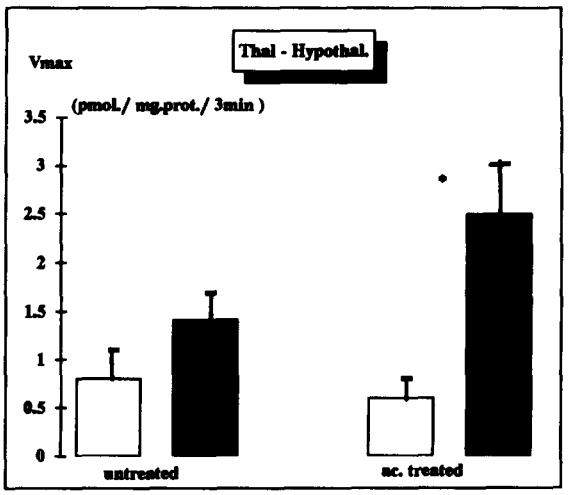


FIG. 1. Blood ethanol levels expressed as g/l in rats during alcoholisation period. Acamprosate group (n = 12) received acamprosate, 400 mg/kg/day, po. Control group n = 12.









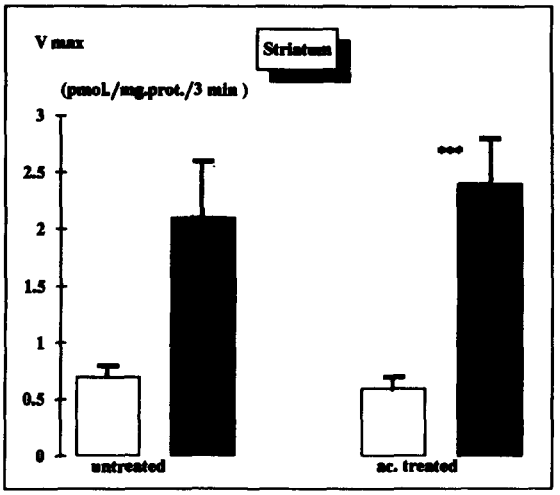


FIG. 2. V_{max} values (mean ± SEM) in different brain areas in control (white bars) or in alcoholised rats (black bars) receiving acamprosate (right) or not (left). Cortex, $*p < 0.05$, untreated rats, alcoholised vs. nonalcoholised. Olfactory bulbs, $*p < 0.05$, untreated rats, alcoholised vs. nonalcoholised; $**p < 0.01$, acamprosate-treated rats, alcoholised vs. nonalcoholised. Hippocampus, $*p < 0.05$, alcoholised rats, acamprosate treated vs. nonalcoholised. Thalamus, $*p < 0.05$, acamprosate-treated rats, alcoholised vs. nonalcoholised rats. Striatum, $***p < 0.001$, acamprosate-treated rats, alcoholised vs. nonalcoholised rats.

TABLE 1

SYNAPTOSOMAL UPTAKE OF ^{3}H-GABA; K_m VALUES (MEAN ± SEM) IN UNTREATED AND ACAMPROSATE TREATED RATS (ALCOHOLISED OR NOT)

	Untreated		Acamprosate Treated	
	Ethanol	Water	Ethanol	Water
Hippocampus	4.84 ± 1.5	8 ± 2	7.94 ± 1.6	8 ± 1.4
Cortex	8 ± 1.3	5.9 ± 1.6	9.4 ± 2.3	8.7 ± 1.8
Thalamus-hypothalamus	5.18 ± 1.05	7.7 ± 3	13 ± 2.8*	4.5 ± 1
Olfactory bulbs	1.5 ± 0.27	5.3 ± 1.3	1.98 ± 0.57*	4.7 ± 1
Striatum	7.6 ± 1.7	5.7 ± 1	8.2 ± 1.9	11 ± 2

K_m values are expressed as μM. Uptake studies are described in the text (Method section). Acamprosate was po given during alcoholisation period.

*$p < 0.05$ in acamprosate-treated alcoholised rats vs. acamprosate-treated nonalcoholised rats (t-test), $n = 6$.

prot/3 min in alcoholised rats, $p < 0.05$) is associated with an increase in carrier affinity (K_m decreased: 5.3 ± 1 μM in control vs. 1.5 ± 0.27 μM in alcoholised rats, $p < 0.01$). Synaptosomal ^{3}H-GABA uptake is not affected by alcoholisation in other areas.

In hippocampus, acamprosate treatment increases ^{3}H-GABA uptake only in alcoholised rats (V_{max} = 1.24 ± 0.27 pmol/mg prot/3 min in alcoholised control rats vs. 2.82 ± 0.56 pmol/mg prot/3 min in treated alcoholised rats, $p < 0.05$).

In olfactory bulbs, acamprosate does not modify GABA uptake, and the increase observed in alcoholised rats is not modified by treatment (acamprosate treated rats: alcoholised vs. nonalcoholised).

In thalamus, acamprosate increases GABA transport only in alcoholised rats (2.49 ± 0.53 pmol/mg prot/3 min in alcoholised treated rats vs. 0.6 ± 0.1 pmol/mg prot/3 min in nonalcoholised treated rats, $p < 0.05$). In this area, acamprosate increases the affinity for the carrier only in alcoholised rats (K_m = 13.15 ± 2.8 μM in alcoholised rats vs. 4.5 ± 1 μM in treated nonalcoholised rats, $p < 0.05$).

In striatum, alcoholisation increases GABA uptake only in acamprosate-treated rats (0.6 ± 0.1 pmol/mg prot/3 min in nonalcoholised rats vs. 2.4 ± 0.4 pmol/mg prot/3 min in alcoholised rats, $p < 0.01$), the increase observed in nontreated alcoholised rats being nonsignificantly different from control rats (0.7 ± 0.1 pmol/mg prot/3 min in control vs. 2.1 ± 0.5 pmol/mg prot/3 min in alcoholised rats).

EtOH and acamprosate do not modify brain area GABA content (Table 2) except in thalamus, where alcoholisation enhances GABA levels.

DISCUSSION

The aim of this study was to evaluate the effect of acamprosate, PO administered, on GABA transmission in alcoholised rats. The first result to be discussed is the action of chronic alcoholisation on the GABA uptake system. We studied here the high-affinity synaptosomal transport of GABA (34) with K_m values of 5 μM. This transport system is believed to be responsible for the inactivation of GABA transmission (13,33), and its functioning is dependent on a sodium-potassium pump and on the integrity of the transport site. Chronic alcohol exposure increases GABA transport, increasing V_{max}, except in olfactory bulbs, where the affinity of tritiated GABA for the carrier is also enhanced. It cannot be argued that modifications in GABA levels are responsible for these increases since GABA levels are not modified by EtOH. It is well known that chronic alcoholisation alters GABA transmission at the postsynaptic level. Ticku and Burch (31) showed that in mice chronic EtOH decreases the binding capacity of the low-affinity GABA binding site. Moreover, de Vries et al. (9) showed that chronic alcohol inhalation induced an increase of benzodiazepine binding to mouse brain membranes by GABA. Studies using ^{35}S-TBPS binding to the $GABA_A$ receptor-coupled chloride channel (28) demonstrated that EtOH, like benzodiazepines or GABA mimetics, reduces the binding of ^{35}S-TBPS, reducing the apparent affinity of these sites for their ligand.

Presynaptic events also seem to be altered by alcoholisation. Chronic EtOH treatment alters ATP-dependent Ca^{++} uptake (8,10,27). It has been argued that sensitivity to Ca^{++} at the presynaptic terminal was increased during tolerance as a possible adaptative mechanism. Although no relation between Ca^{++} uptake and GABA uptake has been demonstrated, the cotransport Na^+/Ca^{++} can be altered during alcoholisation and Na^+-dependent uptake systems modified. In previous work, we also showed an increase in the affinity of ^{3}H-nipecotic acid (an inhibitor of GABA uptake) for its binding sites (7). The enhancement of GABA transport by alcoholisation and by acamprosate demonstrated in this work suggests a facilitation of the inactivation of GABA in the synapse. We showed that only the synaptosomal internalisation of GABA was increased, without significant modification of the affinity for the carrier. This may be interpreted as the reflection of an acceleration of uptake-release-coupling mechanisms. These results are in agreement with data supporting an ethanol-induced facilitation of GABA transmission (28,31).

On the other hand, no changes in GABA accumulation were found after chronic alcoholisation by Frye and Fincher (11). This work studied the role of ethanol (chronically and acutely administered) on the presynaptic availability of GABA. The study was carried out by measuring GABA concentrations in synaptosomes. It is possible that ethanol modifies the GABA transporter function without alterations in the steady state of total synaptic GABA. The specific action of alcoholisation on uptake systems could be suggested; we showed that serotonin uptake was also increased by chronic alcoholisation in men (5) and in rats (4), both in synaptosomes and in platelets. Not all brain areas are affected by alcoholisa-

TABLE 2

SYNAPTOSOMAL GABA LEVELS (MEAN ± SEM) IN UNTREATED AND ACAMPROSATE-TREATED RATS (ALCOHOLISED OR NOT)

	Untreated		Acamprosate Treated	
	Ethanol	Water	Ethanol	Water
Hippocampus	0.34 ± 0.13	0.58 ± 0.11	0.48 ± 0.03	0.61 ± 0.1
Cortex	0.8 ± 0.2	0.4 ± 0.05	0.4 ± 0.09	0.6 ± 0.13
Thalamus-hypothalamus	0.4 ± 0.11	1.28 ± 0.5	0.4 ± 0.09	1.3 ± 0.45*
Olfactory bulbs	0.9 ± 0.18	0.9 ± 0.16	1.5 ± 0.5	0.6 ± 0.2
Striatum	0.51 ± 0.07	0.54 ± 0.11	0.82 ± 0.28	0.7 ± 0.25

Values are expressed as $\mu g/g$ fress structure. GABA levels were determined as described in the text (Method section). Acamprosate was po given during alcoholisation period.

*$p < 0.05$ in acamprosate-treated nonalcoholised rats vs. acamprosate-treated alcoholised rats (t-test), $n = 6$.

tion, showing differences in the sensitivity to EtOH. These results agree with those of Virmani et al. (32) suggesting that EtOH dependance was associated with changes in calcium binding activity in some areas of rats' brains.

Acamprosate PO administered does not significantly modify GABA uptake in nonalcoholised rats. We previously showed an increase in GABA uptake in rats treated with acamprosate (2). In this first study, acamprosate was intraperitoneally administered and the treatment stimulated GABA uptake. In the present study, the inability of acamprosate to alter GABA uptake in naive rats can be attributed to the administration route. There are no other indications concerning the action of acamprosate on GABA system. In a recent study (21), it was shown that acamprosate acted on the excitatory amino acid system. In bovine adrenal chromaffin cells in culture withdrawn from EtOH, glutamic acid, NMDA, and homocysteic acid released a higher percentage of stored catecholamines from EtOH-withdrawn cells than from controls. The response to excitatory amino acids could be inhibited by the NMDA receptor antagonist MK 801, by dihydropyridine calcium channel antagonists, but also by acamprosate. This result suggests a potential action of acamprosate on amino acid systems in relation to alcoholism.

Acamprosate increases GABA uptake in alcoholised rats only in hippocampus. In this area, EtOH does not affect GABA uptake, suggesting a facilitation by alcoholisation of acamprosate effects. In thalamus and striatum, acamprosate potentiates the effects of alcohol (acamprosate alcoholised vs. acamprosate nonalcoholised). Among all mechanisms involved in the modulation of GABA transport, at least one seems to be asensitive to both EtOH and acamprosate. A competition between the two drugs on the GABA uptake system could explain the ability of acamprosate to decrease EtOH intake in dependant rats.

The effect of both drugs on GABA levels in the thalamus is not explained, and studies in GABA turnover have to be carried out.

Finally, our present data show that the effect of acamprosate on EtOH behavior in rats can be associated with an action on the GABA system. Although the exact nature of these two actions has yet to be demonstrated, its effect on another system (excitatory aminoacid and/or serotonergic) may also be suggested.

In summary, the most interesting finding is that, on the one hand, acamprosate, PO administered, is able to modify brain GABA transmission in alcoholised rats and, on the other hand, that not all brain areas present the same sensitivity to the treatment.

The action of both treatments (alcohol and acamprosate) seems to be specific to GABA internalisation since the affinity of the carrier and the substrate itself (GABA) is not modified. These ethanol and acamprosate effects on GABA transport may be partly responsible for the modulation of ethanol intake observed in acamprosate-treated rats.

ACKNOWLEDGEMENT

The authors are grateful to M. Simkins for English translation of the manuscript.

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