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The anti-craving drug acamprosate reduces c-*fos* expression in rats undergoing ethanol withdrawal

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Abstract

Acamprosate (Ca salt of *N*-acetylhomotaurine) is a novel anti-craving substance which a double-blind placebo-controlled study has proven to be therapeutically useful in the prevention of relapses in weaned alcoholics. In the present study the expression of the immediate-early gene c-*fos* in rat hippocampal and cerebellar neurons was used to monitor the modulatory effect of acamprosate on neuronal excitability during ethanol withdrawal. Several hybridization techniques were employed to investigate the effect of acamprosate on c-*fos* expression. Acamprosate (200 mg/kg; intraperitoneally) reduced the elevated c-*fos* mRNA levels in the hippocampus and the cerebellum following 24 h of ethanol withdrawal, or the application of the convulsant pentylenetetrazole. The effect of ethanol withdrawal on c-*fos* expression was more pronounced in the cerebellum than in the hippocampus. In the hippocampus (CA1) and the cerebellum acamprosate alone induced a significant increase in c-*fos* expression in drug-naive animals. Only in the hippocampus did co-administration of pentylenetetrazole during ethanol withdrawal induce a further increase in c-*fos* expression. The present findings support the notion that acamprosate elicits its preventive effect on relapse by reducing the hyperexcitability of central neurons during withdrawal, following long-term ethanol consumption.

Keywords: Acamprosate; Ethanol withdrawal; Pentylenetetrazole; Hippocampus; Cerebellum; c-*fos* mRNA; Northern and dot blotting; In situ hybridization

1. Introduction

The chronic effects of ethanol on central neurons involve the activation of a variety of pre- and postsynaptically located ligand- and voltage-gated ion channels and signal transduction systems (Littleton et al., 1991; Gonzales and Hoffman, 1991; Hoffman and Tabakoff, 1994). In particular, neurons exposed chronically to ethanol are believed to up-regulate NMDA receptors and, in addition, may alter the combination of their subunit assemblies (Lovinger, 1993; Putzke et al., 1995). Both effects are believed to contribute, at least in part, to hyperexcitability during ethanol withdrawal, which is observed in animals (Begleiter et al., 1980; Brownstein and Usdin, 1994) as well as in humans (Tabakoff and Rothstein, 1983). Furthermore, a reduction of γ-aminobutyric acid (GABA)-mediated inhibition and an increase in the density of voltage-activated Ca^{2+} channels probably contribute to the hyperexcitability observed following cessation of alcohol consumption (Samson and Harris, 1992; Gupy and Littleton, 1994).

It has been shown previously that, in animals undergoing ethanol withdrawal, the expression of the immediate-early gene c-*fos* among others is increased in various brain regions (Wilce et al., 1994) and most markedly in the hippocampus (Dave et al., 1990). There is evidence that NMDA receptor activation plays a major role in this action (Morgan and Linnoila, 1991; Morgan et al., 1992; Wilce et al., 1993). The convulsant drug pentylenetetrazole inhibits GABAergic inhibition after binding to the picrotoxin binding site on the $GABA_A$ receptor and, thus, increases the neuronal excitability and the expression of c-*fos* (Morgan et al., 1987) as well as various late response genes (Yount et al., 1994) in the brain of rodents. Ethanol reduces this pentylenetetrazole-induced c-*fos* expression (Le et al., 1990, 1992).

Acamprosate, the calcium salt of acetylhomotaurine,

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reduces the incidence of relapse into drinking in weaned alcoholics (Lhuintre et al., 1985, 1990; Moore and Libert, 1991). A recent placebo-controlled, double-blind study, performed in 10 European countries and involving more than 3600 patients, has established the therapeutical potential of this novel anti-craving drug (Paille et al., 1995; Whitworth et al., 1996; Sass et al., 1996) which has recently been approved by the authorities of the European Community. Despite this clinical use little is known about the basic mechanism underlying the anti-craving action of acamprosate.

There is evidence from our previous studies that acamprosate may elicit at least some of its therapeutic effect by reducing the hyperexcitability of central neurons mediated via the activation of L-glutamate-operated ion channels. This compound reduced the action of iontophoretically applied excitatory amino acid receptor agonists in neocortical neurons (Zieglgänsberger and Zeise, 1992; Zeise et al., 1993) without altering the responses to iontophoretically applied GABA (cf. Daoust et al., 1992). In contrast, it was previously postulated that acamprosate may affect central neurons through an activation of GABAergic transmission (Boismare et al., 1984; Lhuintre et al., 1985; Daoust et al., 1992). Recent studies, performed in human embryonic kidney (HEK) 293 cells carrying native or transfected recombinant $GABA_A$ receptors, suggest that acamprosate does not interact with $GABA_A$ binding sites nor does it, in contrast to benzodiazepines, influence Cl^- currents triggered by the activation of $GABA_A$ receptors containing the α_1-subunit (Zieglgänsberger et al., 1995). These findings are further supported by behavioural observations which showed that acamprosate suppresses some physical signs of withdrawal from ethanol (Gutierez et al., 1987; Gewiss et al., 1991; Spanagel et al., 1996a).

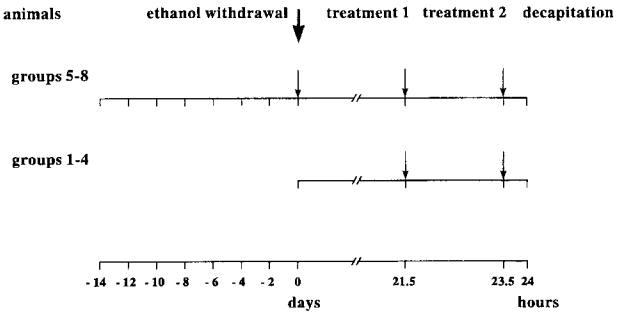
Both the glutamate and the GABA receptor system are involved in hyperexcitation and both systems are discussed as possible targets for the acamprosate action. In the present study the actions of acamprosate on both ethanol withdrawal- and pentylenetetrazole-induced increases in c-*fos* mRNA levels in the hippocampus and the cerebellum were investigated.

2. Materials and methods

2.1. Animals and treatment schedules

Male Wistar rats (200–300 g) were group-housed under controlled environmental and lighting conditions (12 : 12 h light/dark cycle with the light phase commencing at 7.00 a.m.), with food and water available ad libitum. For chronic ethanol treatment, drinking water was replaced by a 20% (v/v) ethanol solution which was given as the sole drinking fluid for the following 14 days. For the different treatments, (listed in Table 1), the animals were divided into 8 groups consisting of 6 or 7 animals. After a period of 14 days of continuous ethanol ingestion it was withdrawn. Since the behavioural signs of withdrawal peaked 24 h after discontinuing ethanol drinking in this animal model (Spanagel et al., 1996a) the animals were injected 21.5 h after ethanol withdrawal intraperitoneally with either saline or 200 mg/kg acamprosate, *treatment 1*; followed by a second injection with either saline or pentylenetetrazole (30 mg/kg) 2 h later, *treatment 2*. The animals were killed by decapitation 0.5 h after the last treatment. Groups 1–4 (see Table 1) served as control groups. All drugs were dissolved in ultrapure, filtered distilled water and injected intraperitoneally (i.p.) in a volume of 1 ml/kg body weight.

Table 1
Treatment schedules for the different drug treatment groups. Animal groups 5–8 underwent ethanol withdrawal; groups 1–4 served as controls



2.2. RNA isolation and hybridization procedures

The hippocampus and the cerebellum were rapidly dissected and immediately frozen on dry ice. For each group the tissue from either 5 or 6 animals was collected and used for the preparation of total RNA by the method of Chomczynski and Sacchi (1984). After ethanol precipitation, RNA was dissolved in diethylpyrocarbonate-treated water and stored at −20°C until used. Quantitation and purity were assessed by measurement of the absorbance at 260 nm and 280 nm, and by agarose gel electrophoresis.

Northern blot analysis was performed using a buffer-puffer system and Hybond-N-nylon membranes (Hybaid, Teddington and Amersham Buchler, Braunschweig, Germany). Approximately 40 μg pooled total RNA were heat-denatured with glyoxal, separated by electrophoresis in a 1.5% (w/v) agarose gel and transferred onto the membranes. The membranes were prehybridized for 2 h at 50°C in the prehybridization buffer and then hybridized with ^{32}P-labelled oligonucleotide probes for 18 h at 50°C in hybridization buffer. After washing in 1 × SSC (saline-sodium citrate) at 55°C the membranes were exposed to MP-film (Amersham Buchler) for several days.

For dot blotting, group-pooled RNA samples (40 μg per dot, corresponding to 8 or 6.7 μg/animal from 5 or 6

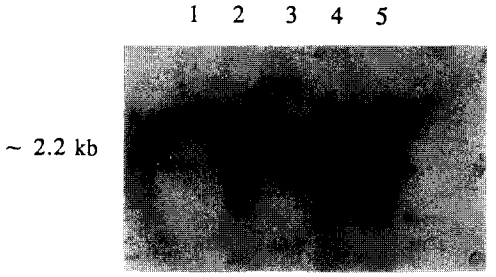


Fig. 1. Effect of acamprosate on pentylenetetrazole-treatment with or without ethanol withdrawal in the rat hippocampus analyzed by Northern blot analysis. Total pooled ($n = 5–6$ animals) RNA samples (40 μg) isolated from control (lane 1), pentylenetetrazole-treated (lane 2), pentylenetetrazole- and acamprosate-treated (lane 3), pentylenetetrazole and ethanol withdrawal (lane 4), and pentylenetetrazole, ethanol withdrawal and acamprosate-treated animals (lane 5) were fractionated by electrophoresis, blotted onto a nylon membrane and hybridized with ^{32}P-labelled oligonucleotide probe specific for c-*fos*.

animals per group, respectively) and RNA samples (10 μg/dot) from single animals were heat-denatured with glyoxal at 55°C for 1 h and applied on a commercially available manifold. For Northern blotting and dot blotting, the same membranes and hybridization conditions were used. Several blots, containing the RNA isolates from all single animals, were prepared and hybridized at least twice for the c-*fos* oligonucleotide probe, and once for the β-actin probe, and used for quantitative analysis as described in Section 2.4.

For in situ hybridization one brain per group was quickly removed after decapitation and frozen on dry ice. Horizontal slices (14 μm) from the thalamic and hippocampal regions were prepared and fixed with paraformaldehyde. The slices were hybridized for 12 h with ^{35}S- and terminal transferase-labelled oligonucleotide probes for c-*fos* and β-actin mRNA at 42°C. After washing with 1 × SSC for 30 min the slices were dehydrated with ethanol and exposed for 4 weeks on Hyperfilm β-max (Amersham Buchler). ^{14}C-micro-scales (Amersham Buchler), which were co-exposed with the in situ hybridization slides, were used to reveal a log-log linear relationship between β emission and optical density.

2.3. Oligonucleotide probes

A synthetic 45-mer oligonucleotide probe with a sequence complementary to bases 1–45 of the rat c-*fos* mRNA, and a synthetic 40-mer oligonucleotide probe for

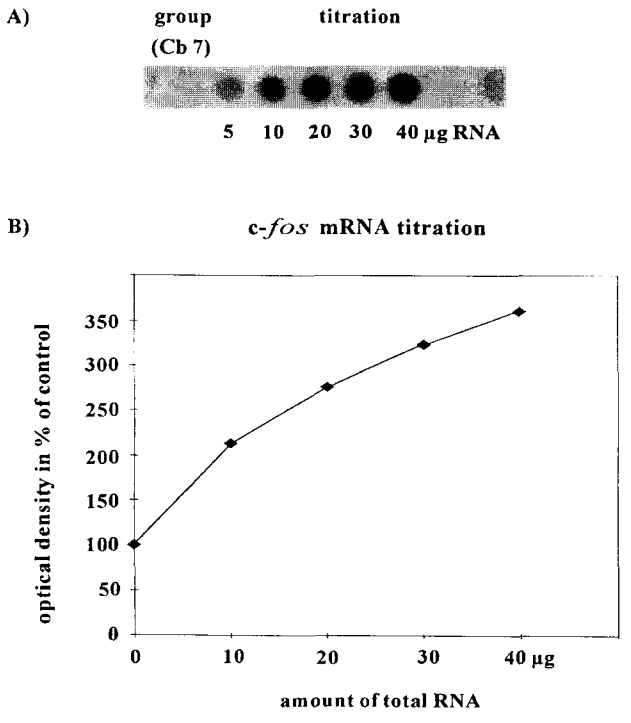


Fig. 2. (A) Evaluation of c-*fos* signal (cerebellum) with total RNA samples (5–40 μg) from one animal of group 7 (Cb 7; ethanol withdrawal plus pentylenetetrazole). (B) The relationship between optical density (as percentage of control: 0 μg RNA = 100%), and the amount of total RNA.

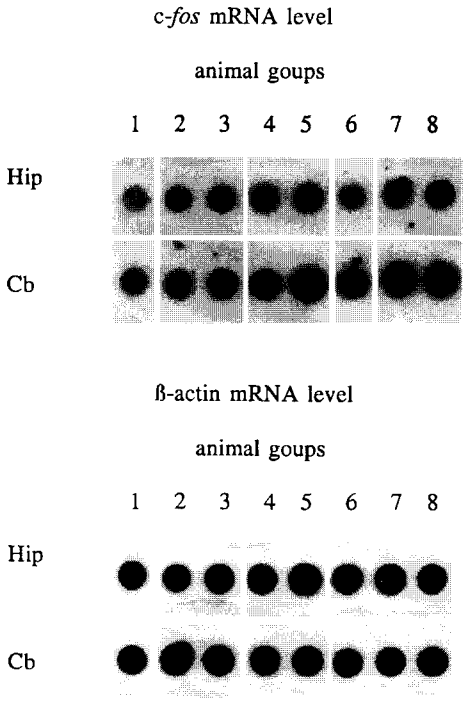
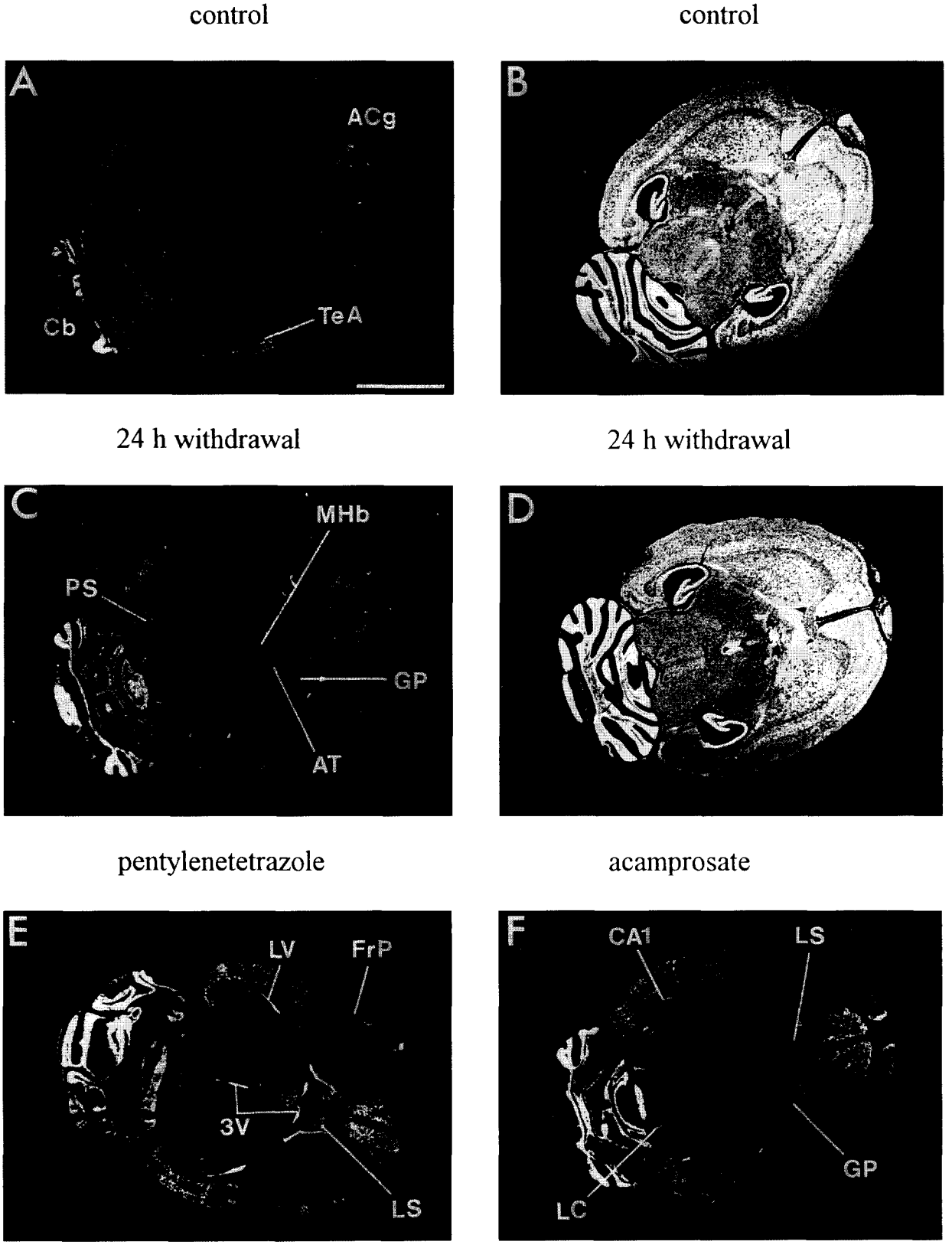


Fig. 3. Dot blot analysis of c-*fos* mRNA expression in the hippocampus (Hip) and cerebellum (Cb). Each dot (1–8) contains 40 μg heat-glyoxal denatured pooled total RNA ($n = 5–6$ animals). The membranes were washed and rehybridized with a β-actin probe as an internal standard (below). Control (group 1), acamprosate (group 2), acamprosate plus pentylenetetrazole (group 3), pentylenetetrazole (group 4), 24 h of ethanol withdrawal after 2 weeks of ethanol consumption (group 5), group 5 plus acamprosate (group 6), group 5 plus pentylenetetrazole (group 7), group 5 plus acamprosate plus pentylenetetrazole (group 8) (for details see Section 2). The figure depicts examples of at least 2 or 1 blots of pooled RNA samples per region, hybridized with the c-*fos* or β-actin probe, respectively.

β-actin with a sequence complementary to bases 394–434 of the rat β-actin mRNA, were purchased from MWG-Biotech (Ebersberg, Germany) and Dianova (Hamburg, Germany), respectively. Oligonucleotide probes were 3'-labelled (37°C, 10 min) to specific activities of 2×10^5 cpm/pmol (for dot or Northern blotting) or 2×10^6



cpm/pmol (for in situ hybridization) using terminal deoxynucleotide transferase (Boehringer Mannheim, Germany) and [α-^{32}P]dATP (DuPont NEN, Dreieich, Germany, 6000 Ci/mmol) or [α-^{35}S]dATP (NEN, 1000–1500 Ci/mmol), respectively, according to the manufacturer's protocol.

2.4. Quantification and statistical analysis

Optical densities of blot autoradiographs obtained with single animal blots (10 μg total RNA for each animal were loaded per dot) were digitized and measured by a computer-assisted image analysis system (Optimas/BIOScan, Edmonds, USA), equipped with a CCD camera (Sony). For imaging the c-*fos* signals, the optical densities (with background subtraction) and the areas covered by the dots were measured in a reproducible way with the same procedure of imaging. In addition, the rehybridized membranes were also analyzed for β-actin signals and both measurements resulted in grey value/area ratios. Afterwards these values were calculated as percentage of control in order to grade differences which occurred after several hybridizations with different blots. The percentage values were expressed as c-*fos*/β-actin ratios and used for an analysis of variance (ANOVA). The differences between the various drug-treatment groups and the control group were tested for significance for both brain regions with the Duncan post-hoc test. $P < 0.05$ was accepted as the level of significance.

3. Results

The size of the mRNA, detected by the probe employed in this study, corresponded to that reported previously for rat c-*fos* mRNA (Morgan et al., 1987). Northern blot analysis (Fig. 1) and competition hybridizations (data not shown) confirmed the specificity of the c-*fos* oligonucleotide probe. In addition, titration of the amount of total RNA from one animal established the relationship between optical density and the amount of RNA (Fig. 2). A constitutive expression of c-*fos* was observed in the hippocampus, the cerebellum, the anterior cingulate cortex and the auditory area of the temporal cortex (Fig. 3 and Fig. 4A). The effects of acamprosate on expression of c-*fos* in pentylenetetrazole-treated, ethanol withdrawal and ethanol withdrawal plus pentylenetetrazole-treated (E) animals are shown in Fig. 5. All percentage values indicated in the following are depicted as c-*fos*/β-actin ratios, as given in Fig. 6.

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 (Spanagel et al., 1996a). Animals which underwent ethanol withdrawal displayed markedly enhanced c-*fos* expression in the hippocampus (+113%) and the cerebellum (+260%; Fig. 3). In situ hybridization also revealed a moderate increase in c-*fos* expression in the anterior cingulate cortex, globus pallidus, medial habenular nucleus, anterior thalamic nucleus, auditory area of the temporal cortex, locus coeruleus and the para- and presubiculum (Fig. 4C).

The injection of pentylenetetrazole in subconvulsive concentrations (30 mg/kg, i.p.) markedly enhanced c-*fos* expression within 30 min in the hippocampus (+321%), and in the cerebellum (+117%) (Figs. 1 and 3). The in situ hybridization experiments showed increased c-*fos* mRNA levels following pentylenetetrazole stimulation in the lateral septal nucleus, medial habenular nucleus, caudate putamen, somatosensory area of the frontoparietal cortex, auditory area of the temporal cortex and the anterior cingulate cortex (Fig. 4E). A pronounced elevation of the c-*fos* mRNA level could be observed in pentylenetetrazole-treated animals in structures outlining the lateral and the third ventricles (groups 3, 4, 7, 8, according to Fig. 5A, B, E, F and Fig. 7). Pentylenetetrazole, administered during withdrawal, further increased c-*fos* expression in the hippocampus (+420%) but not in the cerebellum (+232%) (Figs. 3 and 5E).

Acamprosate (200 mg/kg; intraperitoneally) reduced the ethanol withdrawal-induced c-*fos* expression in the hippocampus significantly, (48%) and in the cerebellum (10%) (Figs. 3 and 5C, D). Acamprosate also reduced pentylenetetrazole-induced c-*fos* mRNA levels in the hippocampus (37%) and in the cerebellum (23%) (Figs. 3 and 5A, B). c-*fos* mRNA levels were decreased by 16% in the hippocampus and by 17% in the cerebellum in rats which had been in withdrawal for 24 h and which had additionally received pentylenetetrazole, and those animals that had received an acamprosate injection 2 h before the convulsant drug (Figs. 3 and 5E, F).

It has to be noted that, in both structures, acamprosate alone induced a significant increase in c-*fos* expression (hippocampus, mainly in the CA1 region: +72%; cerebellum: +49%; Figs. 3 and 4F). In situ hybridization re-

Fig. 4. X-ray film autoradiographs depicting the expression of c-*fos* and β-actin mRNA in horizontal sections of the rat brain. c-*fos* mRNA expression of control (A), 24 h of withdrawal after 2 weeks of ethanol ingestion (C), pentylenetetrazole-treated (E) and acamprosate-treated (F) rats are shown. (B) and (D) show the β-actin mRNA expression obtained from one animal of the control group and one animal of the ethanol withdrawal group, respectively. Scale bar in A, 5 mm. ACg, anterior cingulate cortex; AT, anterior thalamic nucleus; CA1, field CA1 of Ammon's horn; Cb, cerebellum; FrP, somatosensoric area of frontoparietal cortex; GP, globus pallidus; LC, locus coeruleus; LS, lateral septal nucleus; LV, lateral ventricle; MHb, medial habenular nucleus; PS, pre- and parasubiculum; TeA, auditory area of temporal cortex; 3V, third ventricle.

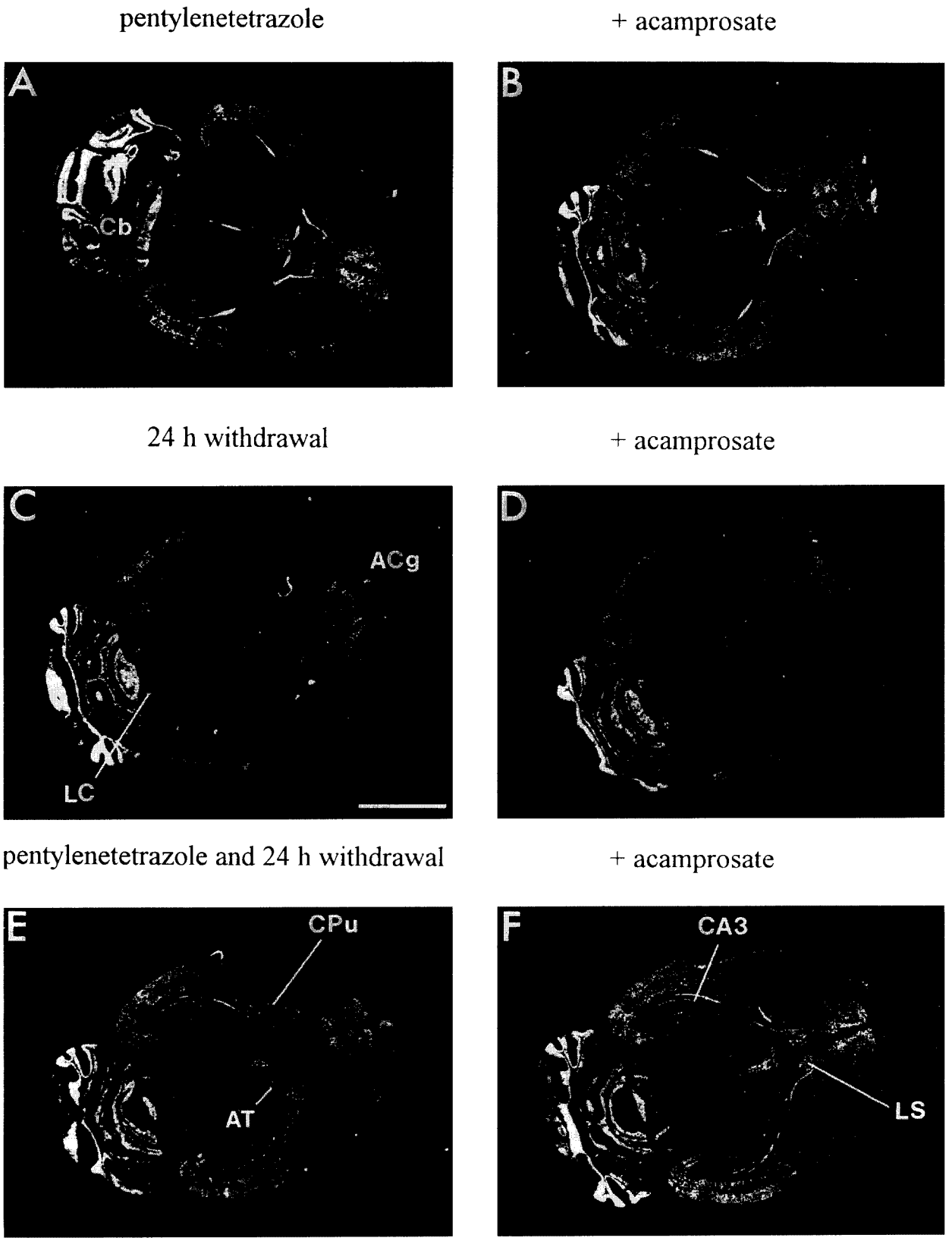


Fig. 5. X-ray film autoradiographs depicting the expression of c-*fos* mRNA in horizontal sections of the rat brain. The effects of acamprosate (B), (D), (F) on pentylenetetrazole-treated (A), ethanol withdrawal (C) and ethanol withdrawal plus pentylenetetrazole-treated (E) animals are shown. Scale bar in C, 5 mm. ACg, anterior cingulate cortex; AT, anterior thalamic nucleus; CA3, field CA3 of Ammon's horn; Cb, cerebellum; CPu, caudate putamen; LC, locus coeruleus; LS, lateral septal nucleus.

vealed a slight induction of c-*fos* mRNA expression by acamprosate alone in the anterior cingulate cortex, lateral septal nucleus, globus pallidus, auditory area of the temporal cortex, locus coeruleus and the subiculum (Fig. 4F).

In none of the experimental groups were significant alterations of β-actin mRNA levels induced by these procedures (Figs. 3 and 4B, D).

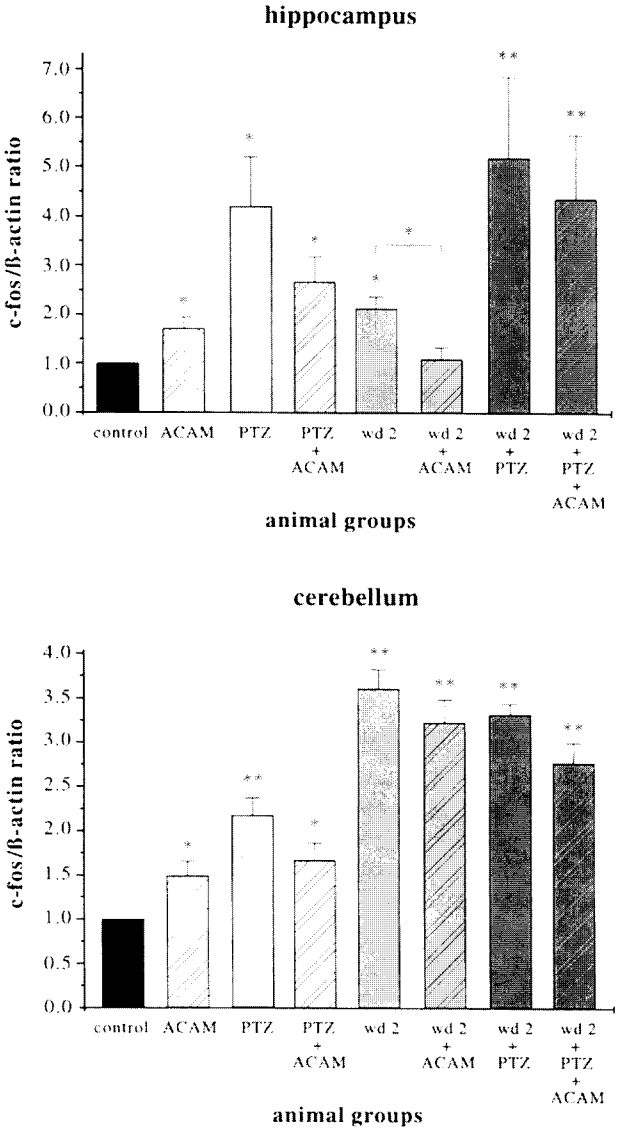


Fig. 6. Quantitation of dot blot signals by densitometry. Values give ratios of the c-*fos* and β-actin RNA expression (optical densities were measured, the backgrounds of the autoradiographic films were subtracted, the obtained values were transformed into percentage of control and calculated as c-*fos*/β-actin ratios) in the hippocampus (upper panel) and in the cerebellum (lower panel). Several dot blots (10 μg total RNA) with single animal ($n = 5–6$ animals) RNA isolates were prepared and hybridized at least twice with the c-*fos* oligonucleotide probe, and once with the β-actin probe under the same experimental conditions. Bars show mean ± S.E.M. of percentile changes. Asterisks indicate significant differences from control (* $P < 0.05$; ** $P < 0.01$) or between distinct groups. ACAM, acamprosate; PTZ, pentylenetetrazole; wd 2, 24 h of withdrawal after 2 weeks of ethanol consumption.

Fig. 7. Distribution of c-*fos* mRNA in a coronal section at the thalamic level of a pentylenetetrazole-treated animal, killed 30 min after 30 mg/kg i.p. The accumulated c-*fos* mRNA adjacent to the ventricle is clearly visible. Scale bar: 5 mm. CA1, field CA1 of Ammon's horn; FrP, somatosensoric area of frontoparietal cortex; LV, lateral ventricle.

4. Discussion

The present study demonstrates that: (1) acamprosate reduced both ethanol withdrawal and pentylenetetrazole-induced c-*fos* mRNA levels in neurons in the rat central nervous system; (2) these effects are different in the hippocampus and the cerebellum; and (3) acamprosate alone induced a moderate c-*fos* expression in these brain regions in drug-naive animals.

Behavioural experiments suggest an involvement of NMDA receptor activation in the development and the expression of ethanol withdrawal seizures (Grant et al., 1990; Morrisett et al., 1990). In these studies MK-801 and other NMDA receptor antagonists reduced the severity of withdrawal seizures when administered before and during the period of maximal seizure susceptibility during ethanol withdrawal (Grant et al., 1990; Morrisett et al., 1990), or prevented the increased c-*fos* expression during ethanol withdrawal (Morgan et al., 1992; Wilce et al., 1993). Furthermore, chronic ethanol consumption induced an increase in NMDA receptor function (Samson and Harris, 1992; Tabakoff and Hoffman, 1996). Both observations strongly support the concept that this excitatory amino acid receptor subtype is a major mediator in ethanol withdrawal hyperexcitability and the resulting activity-dependent immediate-early gene expression (Curran et al., 1990; Hunt et al., 1995).

Our present data are in accordance with the assumption that the anti-craving drug acamprosate decreases the ethanol withdrawal-induced c-*fos* expression in the rat brain, by reducing glutamatergic transmission. Studies of the regulation of gene expression by drugs of abuse have focussed on two families of transcription factors: cAMP response element-binding proteins and the products of immediate-early genes, such as c-*fos* and c-*jun*. A causal relationship between immediate-early gene expression and long-term changes in the expression of target genes coding for, e.g., ion channels, is still lacking (Morgan et al., 1987, 1992).

However, circumstantial evidence indicates that the suppression of c-*fos* might interfere with neuroplastic changes induced in neurons during chronic exposure to ethanol. Beside its effect on ethanol withdrawal, we have shown that acamprosate also reduced pentylenetetrazole-increased c-*fos* expression. Since non-competititve NMDA receptor antagonists (Sonnenberg et al., 1989; Le et al., 1992) and competitive antagonists (Zhu and Inturrisi, 1993) also reverse pentylenetetrazole-induced c-*fos* expression, it is feasible to assume that a reduction of GABAergic transmission by the antagonist at $GABA_A$ receptors unmasks NMDA receptor-mediated excitatory synaptic transmission. The assumption that acamprosate may elicit its therapeutic effect by reducing the hyperexcitability in central neurons mainly via the NMDA receptor system is supported by electrophysiological studies which have shown that acamprosate reduced glutamatergic transmission in neocortical pyramidal neurons of the rat (Zieglgänsberger and Zeise, 1992; Zeise et al., 1993; for review see references in Littleton, 1995). Acamprosate bears a structural relationship to the naturally occurring excitatory amino acid transmitter homocysteic acid, which has been suggested as an endogenous ligand for the NMDA receptor (Knöpfel et al., 1987). Initially the structural resemblance between acamprosate and taurin led to the speculation that acamprosate may affect central neurons through an activation of GABAergic transmission (Boismare et al., 1984; Lhuintre et al., 1985; Daoust et al., 1992) (see also Section 1).

The effect of ethanol withdrawal on c-*fos* expression was more pronounced in the cerebellum than in the hippocampus. The blocking effect of acamprosate on the expression was greater in the hippocampus than in the cerebellum. In contrast, pentylenetetrazole enhanced the c-*fos* mRNA expression in the hippocampus more than in the cerebellum. Moreover, the c-*fos* expression was moderately increased in the hippocampus but not in the cerebellum after withdrawal and additional treatment with pentylenetetrazole. The expression of different splice variants of the NMDA1 receptor in the hippocampus and the cerebellum (Laurie et al., 1995) or of different GABA receptor subunits (Wisden et al., 1992) could underly this tissue-specific sensitivity to ethanol, as well as pentylenetetrazole treatment.

The increase of c-*fos* expression by acamprosate in drug-naive animals in the cerebellum and the hippocampus may be due to an inhibition of inhibitory interneurons receiving glutamatergic synapses. Such a neuronal activation by disinhibition (Zieglgänsberger et al., 1979; Siggins and Zieglgänsberger, 1981) may also be reflected by the modest stimulating effect of acamprosate on motor behaviour observed in drug-naive animals (Spanagel et al., 1996a). Disinhibition may also cause the increase in c-*fos* mRNA expression following MK-801 administration in thalamic regions (Dragunow and Faull, 1990). However, it has been shown that MK-801 and not acamprosate generalizes for the ethanol cue in drug discrimination studies (Schechter et al., 1993; Spanagel et al., 1996b). These behavioural studies, therefore, rule out a common site of action at the phencyclidine binding site and suggest distinct mechanisms of action.

Unexpectedly, after pentylenetetrazole treatment an intense c-*fos* mRNA expression was found in ependyma cells outlining the lateral and third ventricles. Tests with a labelled sense (coding strand) oligonucleotide for c-*fos* and a 100-fold excess of the same unlabelled probe established this accumulation of c-*fos* mRNA within these cells as a specific signal. FOS-positive cells within the lamina terminalis, and the ependyma cells outlining the third ventricle, have previously been reported by Hamamura et al. (1992) after a hypertonic saline injection, and by Wessel et al. (1991) following global forebrain ischemia. It remains to be established as to whether a transient ischemia, which may result from pentylenetetrazole-induced seizure activity, is responsible for the increase in c-*fos* mRNA in these cells.

The present results suggest that the anti-craving drug acamprosate may elicit at least part of its therapeutic effect by reducing the hyperexcitability of central neurons generated by chronic ethanol ingestion. The site through which acamprosate modulates glutamatergic transmission at, e.g., NMDA receptors is not yet known. Acute ethanol (Le et al., 1990, 1992), competitive (Zhu and Inturrisi, 1993) and non-competitive (Sonnenberg et al., 1989; Morgan and Linnoila, 1991; Morgan et al., 1992; Wilce et al., 1993) NMDA antagonists, as well as acamprosate as a putative modulator at the NMDA receptor, decrease ethanol withdrawal-increased and pentylenetetrazole-enhanced c-*fos* expression in the mammalian brain. The anti-craving compound acamprosate may help to restore physiological discharge activity in neurons which have been altered by activity-dependent gene modulation during repeated seizure-like hyperactivity, following the cessation of long-term ethanol consumption.

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