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ORIGINAL ARTICLE

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Local acamprosate modulates dopamine release in the rat nucleus accumbens through NMDA receptors: an in vivo microdialysis study

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Abstract The effects of acamprosate on the in vivo dopamine extracellular levels in the nucleus accumbens and the involvement of N-methyl-D-aspartate (NMDA) receptors in these effects were investigated. Microdialysis in freely moving rats was used to assess dopamine levels before and during simultaneous perfusion of acamprosate and/or different agonists or antagonists of NMDA receptors. Perfusion with acamprosate at concentrations of 0.5 and 5 mM provoked a concentration-dependent increase in extracellular dopamine in nucleus accumbens. The lowest concentration of acamprosate assayed (0.05 mM) had no effect on dopamine levels. Infusion of NMDA (25 and 500 μM) and the glutamate uptake blocker, L-trans-pyrrolidine-2,4-dicarboxilic acid (PDC) (0.5 mM) into the NAc caused a significant increase in DA, whereas acamprosate (0.05 mM) co-infusion with these compounds blocked or attenuated the NMDA and PDC-induced increases in DA levels. Co-infusion of the selective antagonist of NMDA receptors, DL-2-amino-5-phosphonopentanoic acid (AP5) (400 μM) with acamprosate (0.5 mM), did not reduce the increase of DA levels induced by acamprosate. These results demonstrate that acamprosate is able to modulate DA extracellular levels in NAc via NMDA receptors and suggest that acamprosate acts as an antagonist of NMDA receptors.

Keywords Acamprosate · Dopamine · Nucleus accumbens · NMDA receptors · Microdialysis · Ethanol

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Introduction

There is broad evidence from animal to human studies suggesting that acamprosate can be useful in relapse prevention in detoxified alcoholics (see Spanagel and Zieglgänsberger 1997 for review). In animal models of ethanol self-administration, acamprosate (usually at doses higher than 200 mg/kg i.p.) decreased ethanol intake but not food or fluid intake (Boismmare et al. 1984; Stromberg et al. 2001) or intake of other reinforcers such as sucrose (Czachowski et al. 2001). Acamprosate also reduced the severity of relapse (Spanagel et al. 1996; Hölter et al. 1997; Heyser et al. 1998). The neurochemical mechanisms underlying the effects of acamprosate in these animal models are not known but may involve an interaction with the mesolimbocortical neurotransmission.

The mesolimbocortical dopaminergic pathway, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), prefrontal cortex and other limbic areas, has been strongly implicated in reward-related processes (Koob 1992; Spanagel and Weiss 1999). Dopamine (DA) transmission in the NAc plays a key role in the reinforcing action of many drugs of abuse including ethanol (Hoffman and Tabakoff 1996; Weiss and Porrino 2002). Oral self-administration of ethanol in dependent rats dose-dependently elevates extracellular DA in the NAc (Weiss et al. 1993, 1996). Moreover, the pharmacological manipulation of DA neurotransmission in NAc modifies ethanol-reinforced operant responding and the ethanol preference in rats. For example, drugs like the opioid antagonist naltrexone that are able to reduce the ethanol-stimulated dopamine release in NAc (Acquas et al. 1993) reduce the ethanol intake in dependent rats and in humans (Gonzales and Weiss 1998; Heyser et al. 1999). Although not fully understood, interactions between opioid systems and NAc DA transmission are clearly implicated (Herz 1997).

In spite of the well documented importance of the NAc DA neurotransmission in rewarding-related processes, there has been only one study of the effects of acamprosate on

DA efflux in NAc. Recently, Olive et al. (2002) have shown that acamprosate, like naltrexone, attenuates ethanol-stimulated DA release in NAc. Since in this study acamprosate was given intraperitoneally, the precise mechanism by which acamprosate exerted this action was not explored, but these and other authors (Zeise et al. 1993; Al Qatari et al. 1998; Berton et al. 1998; Naassila et al. 1998) point out a possible interaction of acamprosate with N-methyl-D-aspartate (NMDA) receptor mediated neurotransmission.

It is important to bear in mind that besides the dopaminergic innervation from the VTA, the NAc receives dense glutamatergic inputs from several brain areas (Kelley and Domesick 1982; Kelley et al. 1982; Phillipson and Griffiths 1985; Groenewegen et al. 1987). Numerous neuropharmacological studies have shown a modulatory function of the glutamatergic system on DA levels in NAc (Imperato et al. 1990a, 1990b; Youngren et al. 1993; Taber et al. 1996; Kretschmer 1999) and these interactions have been shown to be critical for the reinstatement of drug-seeking behaviors (Cornish et al. 1999; Cornish and Kalivas 2000).

The aim of the present study was to investigate the effects of acamprosate on NAc extracellular DA levels. To this end, we used local administration by reverse dialysis of acamprosate in the NAc and measured the changes in DA efflux in this brain area. Additionally, we performed several experiments using different agonists and antagonists of NMDA receptors to explore the possibility that the observed effects of acamprosate on accumbal DA efflux were mediated through NMDA receptors. Intracerebral microdialysis in freely moving rats was used to apply directly into NAc all compounds tested.

Materials and methods

Animals. Male Wistar rats (300–330 g) were used for the experiments. The rats were housed in groups of six in a humidity and temperature (22°C) controlled vivarium on a 12/12 h light/dark cycle (on 08:00, off 20:00) with free access to food and water. All procedures were conducted in strict adherence to the EEC Council Directive 86/609 and Spanish laws (RD 223/1988) and policies on protection of animals. Experiments were approved by the Animal Care Committee of the Faculty of Pharmacy of the University of Valencia.

Drugs and chemicals. The following drugs were kindly provided by or obtained from the sources indicated: Acamprosate (Lipha, Lyon, France); N-methyl-D-aspartic acid (NMDA) (Tocris Cookson, Bristol, UK); L-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) and DL-2-amino-5-phosphonopentanoic acid (AP5) (Sigma, St. Louis, MO, USA).

All reagents for the HPLC mobile phase and the perfusion fluid were analytical grade and were obtained from Sigma, Merck (Darmstadt, Germany) or JT Baker (Philipsburg, NJ, USA).

Surgery and microdialysis. Rats were anaesthetized intraperitoneally with 400 mg/kg of chloral hydrate and placed in a stereotaxic apparatus. An incision (8–10 mm) was made on the skin over the skull and the wound margin was infiltrated with lidocaine (10%). Three holes were drilled; two were used for the skull screws and the other for the microdialysis probe. A vertical concentric-type microdialysis probe with 2 mm of active membrane (Hospal AN69; molecular cutoff 60,000 Da; Bologne, Italy) was implanted in the NAc. Coordinates relative to bregma and skull surface (Paxinos and Watson 1986) were as follows: A/P: +2.0 mm; L: +1.6 mm; D/V: –8.1 mm at the ventral extent of the active membrane. Following surgery, rats were housed in individual cages (25×25×35 cm) in which they had free access to food and water.

Microdialysis experiments were carried out 24 to 48 h after the implantation of the probe. The probe was perfused with 0.1 mM aqueous sodium phosphate buffer containing 147 mM NaCl, 3.0 mM KCl, 1.3 mM $CaCl_2$ and 1.0 mM $MgCl_2$ (pH=7.4) at a rate of 3.5 µl/min. Fractions of dialysate were on-line analyzed for DA content every 20 min. Drug delivery and sample collection time were corrected for the lag time resulting from the dead volume of the inlet and outlet tubes used to connect the rat to the HPLC system.

Chromatographic analysis. Dialysates were on-line analyzed for DA content using an HPLC system with electrochemical detection. A Waters 510 series pump was used in conjunction with an electrochemical detector (Mod. Intro, Antec, Leyden, The Netherlands). The applied potential was +0.7 V (vs. Ag/AgCl). Dialysates were injected onto a 5 µm RP-18 column (LiCrhoCART 125–4, Merck, Darmstadt, Germany) via a VALCO valve fitted with a 65 µl sample loop. The mobile phase consisted of a sodium acetate/acetic acid buffer (0.1 M, pH=4.2) containing 61 mg/l of 1-octanesulfonic acid, 100 mg/l of EDTA and 7.5 ml/l of methanol. Mobile phase was pumped through the column at a flow rate of 0.6 ml/min. Chromatograms were integrated, compared with standards run separately on each experimental day, using a LCI-100 integrator (Perkin-Elmer, Norwalk, CT, USA). Detection limit was defined by a signal to noise ratio of 2:1 and it was approximately 4 fmol/sample.

Experiments. Experimental treatment was initiated after the establishment of a baseline that was defined as four consecutive samples with less than 10% variation in DA content. All drugs used on the experiments were dissolved in the perfusion fluid and infused via retrograde dialysis into the NAc. Treatment involved the application of acamprosate, NMDA, PDC or AP5 alone or a combination of these drugs. The concentrations of NMDA (25 and 500 µM), PDC (0.5 mM) and AP5 (400 µM) used here were in the range of those used in previous microdialysis studies that examined excitatory amino acid-dopamine interactions in the mesolimbocortical system (Youngren et al. 1993; Taber et al. 1996; Karreman et al. 1996; Westerink et al. 1996, 1998) The duration of drug infusions varied for each drug and is shown as an horizontal bar in figures.

Histology. Microdialysis probe placement was histologically examined after completion of the experiments. Rats were anaesthetized with chloral hydrate and the brains were fixed with 4% paraformaldehyde via intracardiac perfusion. Serial coronal sections (20 µm thick) were cut on a cryostat and stained with cresyl violet. All rats used in the study were confirmed to have the dialysis probe located both in the shell and in the core regions of the NAc. We pooled the results of all the experiments independently of the localization of the probe (shell or core).

Data analysis. All values given are expressed as percentages of controls. The average concentration of four stable baseline samples (less than 10% variation) was considered as the control and was defined as 100%. Data were analyzed by one-way ANOVA with repeated measures across time followed by the Dunnett's multiple comparisons test when appropriate. Individual time-points of two time-effect curves were compared by one-way ANOVA. The level of significance was set at $P<0.05$. The individual time-points of the three time-effect curves of acamprosate experiments were analyzed by one-way ANOVA followed by the Dunnett's multiple comparisons test, assuming as control group the rats perfused with the lowest dose of acamprosate (0.05 mM).

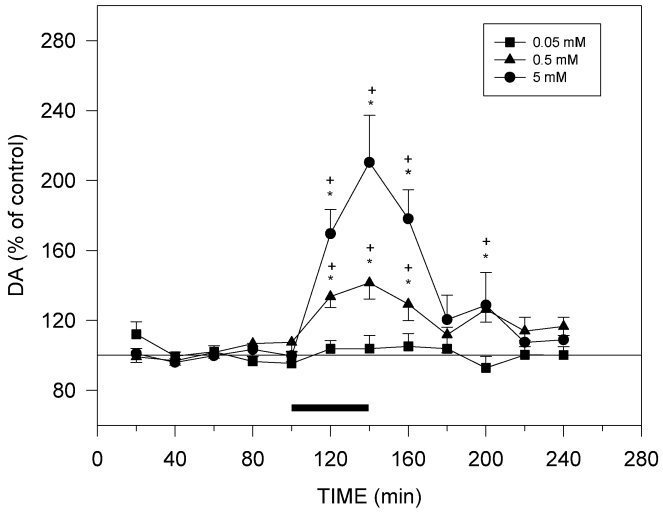

Fig. 1 Effects of local infusion of acamprosate on DA extracellular levels in the nucleus accumbens (NAc). Acamprosate (0.05, 0.5 and 5 mM in perfusion fluid) was administered via the dialysis probe into the NAc during 40 min (indicated by the *black bar*). Results are means ± SEM (n=5). *P<0.05 as compared with the control values (0–80 min) (one-way ANOVA with time as repeated measure followed by Dunnett's test). ^{+}P<0.05 as compared with the 0.05 mM group at the corresponding time points (one-way ANOVA followed by Dunnett's test)

Results

Basal levels of DA in NAc

The average basal values of DA in dialysates of the NAc for the different experiments did not differ significantly. The average basal values were 1.91±0.48 fmol/min.

Effects of local infusion of acamprosate on extracellular concentrations of DA in the NAc

In these experiments, three concentrations of acamprosate (0.05, 0.5, and 5 mM) were delivered into the NAc of three groups of rats via reverse microdialysis for 40 min. As can be seen in Fig. 1, local application of the two higher concentrations of acamprosate (0.5 and 5 mM) dose-dependently increased extracellular DA in the NAc (P<0.001 in both doses) whereas the lowest concentration (0.05 mM) had no effect (P=0.749). The maximum increases of DA produced by perfusion with 0.5 and 5 mM of acamprosate were 141% and 210% of baseline, respectively. DA concentration returned to baseline following removal of acamprosate.

Effects of acamprosate on the NMDA and PDC induced DA release in the NAc

To investigate whether acamprosate exerted its effects on DA levels in NAc through NMDA receptors, the agonist

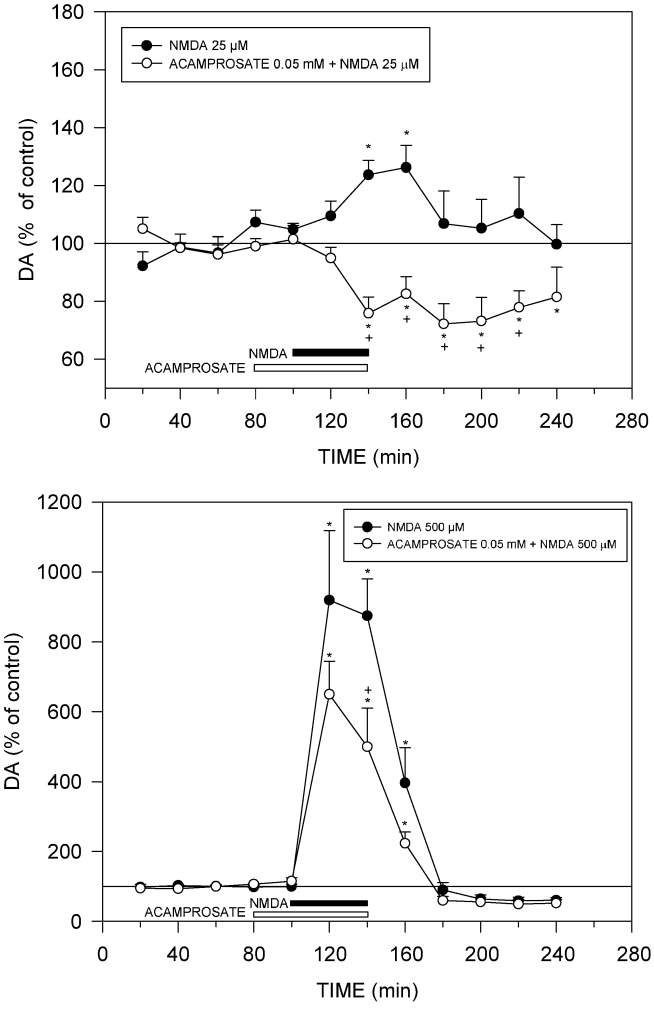

Fig. 2 Effects of acamprosate on the NMDA-induced DA extracellular levels in the NAc. NMDA (25 µM [*top*] or 500 µM [*bottom*]) was infused into the NAc of two groups of rats for 40 min (*black bars*). In two additional groups, 0.05 mM acamprosate was infused (*white bars*) into the NAc 20 min before and during (40 min) the application of NMDA. Results are means ± SEM (n=5). *P< 0.05 as compared with the respective control values (0–80 min) (one-way ANOVA with time as repeated measure followed by Dunnett's test). ^{+}P<0.05 as compared with the 25 µM or 500 µM NMDA group at the corresponding time points (one-way ANOVA)

NMDA and the glutamate uptake blocker PDC were perfused into NAc in absence and the presence of acamprosate in the perfusion fluid. In these experiments we used the 0.05 mM concentration of acamprosate which was below the threshold required to increase DA extracellular levels in the NAc (see above). Fig. 2 shows the effects of the two concentrations of NMDA on dialysate DA concentrations. The effect of NMDA was concentration-dependent; the lower concentration (25 µM, Fig. 2, top) increased DA concentration to 126% of controls (P<0.001) whereas the higher concentration (500 µM, Fig. 2, bottom) increased DA to 919% of controls (P<0.0001). Pretreatment with acamprosate (0.05 mM) 20 min before and during NMDA administration blocked the increase observed with the 25 µM dose of NMDA (Fig. 2, top) and at-

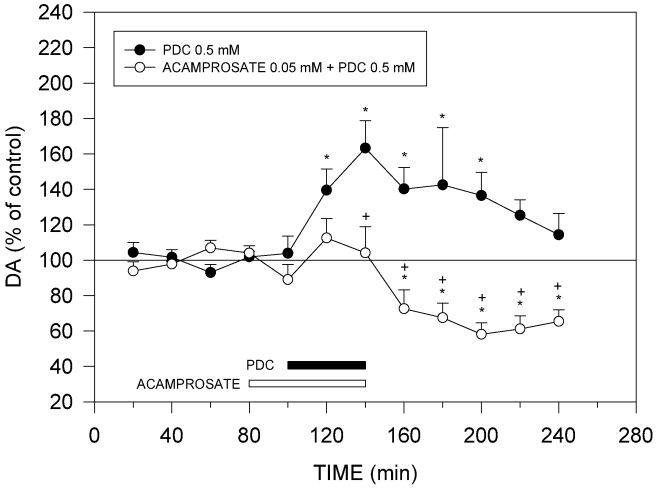

Fig. 3 Effects of acamprosate on the PDC-induced DA extracellular levels in the NAc. PDC (0.5 mM) was infused into the NAc in one group of rats for 40 min (*black bar*). In another group, 0.05 mM acamprosate was infused (*white bar*) into the NAc 20 min before and during (40 min) the application of PDC. Results are means ± SEM (n=5). *P<0.05 as compared with the respective control values (0–80 min) (one-way ANOVA with time as repeated measure followed by Dunnett's test). ^{+}P<0.05 as compared with the 0.5 mM PDC group at the corresponding time points (one-way ANOVA)

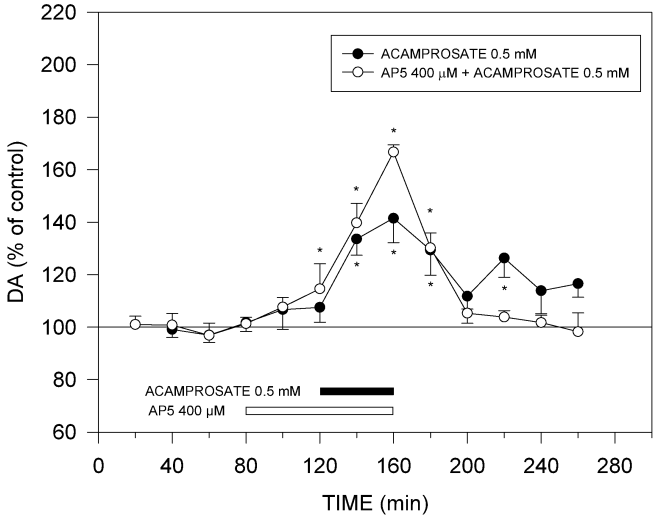

Fig. 4 Effects of AP5 on the acamprosate-induced DA extracellular levels in the NAc. Acamprosate (0.5 mM) was infused into the NAc in one group of rats for 40 min (*black bar*; taken from Fig. 1). In another group, 400 µM AP5 was infused (*white bar*) into the NAc 40 min before and during (40 min) the application of 0.5 mM acamprosate (*black bar*). Results are means ± SEM (n=5). *P<0.05 as compared with the respective controls (one-way ANOVA with time as repeated measure followed by Dunnett's test)

tenuated the increase observed with the 500-µM dose (Fig. 2, bottom). After the removal of the combination of NMDA (25 µM) and acamprosate, dialysate DA levels significantly decreased below controls (Fig. 2, top).

The glutamate uptake blocker PDC was used to asses the effect of increasing extracellular concentrations of endogenous glutamate on extracellular DA (Fig. 3). PDC (0.5 mM) caused a significant increase (P<0.0001) in extracellular DA. The maximum effect was an increase to 163% of control values. Pretreatment with 0.05 mM acamprosate, 20 min before and during PDC application completely blocked the observed increase in DA levels. After the removal of the combination of acamprosate and PDC, dialysate DA levels significantly decreased below baseline (Fig. 3).

Effects of AP5 on the acamprosate-induced DA release in the NAc

To investigate whether acamprosate acted as partial agonist or antagonist of NMDA receptors, we used the selective antagonist of NMDA receptors AP5. If acamprosate and AP5 share the same mechanism of action (antagonists of NMDA receptors) the effects on DA extracellular levels should be additive. On the contrary, if acamprosate acts as partial agonist, co-perfusion with AP5 would reduce the effects of the drug. We co-perfused AP5 with 0.5 mM acamprosate and we measured DA extracellular levels. We compared the results of this experiment with those obtained after perfusion of acamprosate 0.5 mM alone. We selected this acamprosate concentration because it provoked a moderate increase in DA levels (141% of the controls; see above). The concentration of AP5 (400 µM) was selected on the basis of previous experiments (data not shown) that showed that this concentration was below the threshold required to increase the extracellular levels of DA in NAc. Figure 4 shows that AP5 did not reduce the maximum in DA levels induced by 0.5 mM acamprosate: the maximum peak was 168% of the control values. Statistical comparison of the two time-effect curves showed in Fig. 4 did not detect any significant difference. Therefore, these results seem to discard the hypothesis that acamprosate acts as a partial agonist of NMDA receptors.

Discussion

In the present paper we demonstrate that acamprosate is able to modulate DA neurotransmission in the NAc through NMDA receptors. Acamprosate infused locally in NAc increased in a dose-dependent manner the extracellular levels of DA. Moreover, acamprosate attenuated or completely blocked the increases in DA levels provoked by local administration of NMDA or PDC in NAc. Finally, the specific antagonist of NMDA receptors AP5 was unable to reduce the effects of 0.5 mM acamprosate on DA NAc levels.

The projection of DA neurons from VTA to the NAc, the mesolimbocortical system, has been strongly implicated in reward-related processes of several drugs of abuse (e.g., Nestler 2001). It exists a well documented neuroanatomical evidence for a glutamatergic modulation of the DA neurotransmission in NAc (e.g., Sesack et al. 1989; Sesack and Pickel 1992; Wright et al. 1996). Some of the glutamatergic projections have been shown to synapse in

close apposition to dopamine terminal areas (Johnson et al. 1994). Numerous neuropharmacological studies also support the presence of presynaptic regulation by excitatory amino acids (EAA) of DA terminals in the NAc. Several in vivo reports, using microdialysis as experimental technique, have indicated that exogenous application of EAA directly in the NAc, at least at high concentrations, increases DA extracellular levels in this brain area (Imperato et al. 1990a, 1990b; Youngren et al. 1993; Taber et al. 1996). Also, it has been recently shown that electrical stimulation of the ventral subiculum of hippocampus (a brain area that sends a dense glutamatergic projection to NAc) induces a significant and prolonged increase in DA levels in NAc and this increase was completely blocked by reverse dialysis application of NMDA and non-NMDA antagonists (Taepavarapruk et al. 2000). Taken together, these results confirm the existence of EAA receptors located in the terminal area of DA neurons.

On the other hand, several experiments have shown that local perfusion of NMDA (and non-NMDA) antagonists did not reduce but, like administration of the agonists, also increases DA levels in NAc (Imperato et al. 1990b; Youngren et al. 1993; Taber and Fibiger 1995). In opinion of the authors of these three studies, the inability of antagonists to reduce DA levels in NAc suggests:

1. That EAA presynaptic receptors are not tonically activated (i.e., EAA do not exert a continuous facilitatory effect on DA release through these presynaptic receptors)
2. That there are additional EAA receptors in NAc GABA neurons that project back to VTA (Taber et al. 1996)

These receptors, unlike the EAA receptors located in the terminal area of DA neurons, should be continuously activated, exerting a tonic indirect inhibitory control (mediated by GABA projection neurons) on DA levels in NAc. So, although local application of competitive antagonists of glutamate receptors, as AP5, would block NMDA receptors located both in the terminal DA areas and in the GABA projection neurons, only the blockade of NMDA receptors tonically stimulated would have measurable effects on extracellular DA levels in NAc.

There is increasing evidence supporting the view that acamprosate modulates the activation of excitatory synapses operated by EAA. Recent in vitro studies suggest a possible interaction of acamprosate with NMDA receptors (Al Qatari et al. 1998; Berton et al. 1998; Naassila et al. 1998; Allgaier et al. 2000; Rammes et al. 2001), although whether acamprosate activates or blocks NMDA receptors is presently unknown.

As mentioned above, our results indicate that acamprosate modulates DA levels in NAc probably through interaction with NMDA receptors. In order to delineate whether it acts as an agonist or antagonist, we designed an additional experiment in which we simultaneously perfused 0.5 mM acamprosate (that induces an increase in DA levels to 141%) and AP5 (a competitive antagonist of NMDA receptors). As can be seen in Fig. 4, 400 µM AP5 did not significantly alter the increase in DA levels induced by 0.5 mM acamprosate. These latter results suggest that acamprosate acts as antagonist of the NMDA receptor.

Consistent with this antagonist action on NMDA receptors is the fact that acamprosate reduces several symptoms of acute ethanol withdrawal which are accompanied by neuronal hyperexcitability (Spanagel and Zieglgänsberger 1997). Ethanol blocks NMDA receptors and chronic ethanol treatment upregulates these glutamate receptors (for reviews see Hoffman and Tabakoff 1996; Lovinger 1996; Fadda and Rossetti 1998). The NMDA receptor mediated enhancement of EAA neurotransmission can be considered the cause of the excitatory syndrome that results upon withdrawal of chronic ethanol. In our opinion, acamprosate could attenuate this hyperexcitability by blocking the NMDA receptors. Interestingly, administration of NMDA receptor antagonists reduces the symptomatology of ethanol withdrawal (Liljequist 1991; Rossetti and Carboni 1995). Moreover, withdrawal of chronic ethanol is also associated with a significant decrease in DA release in NAc (Diana et al. 1993; Weiss et al. 1996). This effect seems to be linked to a reduction in neuronal activity of VTA neurons (Diana et al. 1993). Some authors suggest that this reduced activity is mediated, at least in part, through NMDA receptors since antagonists of NMDA receptors revert the reduction in DA levels during withdrawal (Rossetti et al. 1991). Thus, it seems that, during the withdrawal state, glutamate exerts an exacerbated inhibitory control on DA release, probably through local or distal inhibitory GABA interneurons that project to VTA-DA cells (Fadda and Rossetti 1998). An intriguing question is if acamprosate would be able to revert the DA decrease mediated by the NMDA receptors during withdrawal. Future studies are warranted to investigate this question.

In conclusion, we have demonstrated that acamprosate modulates DA levels in NAc through an interaction with NMDA receptors, and that these effects are compatible with a NMDA receptor antagonist-like action.

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