Naunyn-Schmiedeberg's Arch Pharmacol (2003) 368 : 324–327
DOI 10.1007/s00210-003-0795-3

SHORT COMMUNICATION

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Acamprosate blocks the increase in dopamine extracellular levels in nucleus accumbens evoked by chemical stimulation of the ventral hippocampus

Received: 4 June 2003 / Accepted: 28 July 2003 / Published online: 6 September 2003

Abstract Recently, we have shown that acamprosate is able to modulate extracellular dopamine (DA) levels in the nucleus accumbens (NAc) and may act as an antagonist of *N*-methyl-D-aspartate (NMDA) receptors. Neurochemical studies show that chemical stimulation (using NMDA) of the ventral subiculum (vSub) of the hippocampus produces robust and sustained increases in extracellular DA levels in the NAc, an effect mediated through ionotropic glutamate (iGlu) receptors. The present study examines whether acamprosate locally infused in the NAc of rats could block or attenuate the increase in NAc extracellular DA elicited by chemical stimulation (with 5 mM NMDA) of the ventral subiculum of the hippocampus. The stimulation of the vSub during perfusion of artificial cerebrospinal fluid in NAc induced a significant and persistent increase in NAc DA levels. Reverse dialysis of 0.05 mM acamprosate in NAc blocked the increase in DA evoked by the chemical stimulation of the vSub. These data support the possibility that the antagonism at the NMDA receptors in NAc can explain, at least in part, the mechanism of action of this drug.

Keywords Acamprosate · Ventral subiculum · Nucleus accumbens · NMDA · Dopamine · Microdialysis

Introduction

Acamprosate is currently used to prevent the relapse in detoxified alcoholics, although the precise neurochemical mechanisms underlying the effects of this drug are not fully known. We have recently shown (Cano-Cebrián et al. 2003) that acamprosate is able to modulate DA extracellular levels in NAc probably by exerting a NMDA (a subtype of iGlu receptor) receptor antagonist action.

The nucleus accumbens (NAc) receives an important glutamatergic projection from the hippocampus via the ventral subiculum (vSub) (Groenewegen et al. 1987). To this brain region also arrives a dense dopaminergic input proceeding from the ventral tegmental area (VTA). Neurochemical studies have shown that NAc dopamine (DA) levels are influenced by the projections from the vSub. Accordingly, chemical (by using NMDA infusions) (Brudzynski and Gibson 1997; Legault and Wise 1999; Legault et al. 2000; Floresco et al. 2001) or electrical stimulation of the vSub (Blaha et al. 1997; Taepavarapruk et al. 2000) induce persistent elevations in NAc DA. It has also been demonstrated that ionotropic glutamate receptors (iGlu) located both in the VTA (Legault et al. 2000) and in the NAc (Blaha et al. 1997; Taepavarapruk et al. 2000; Floresco et al. 2001) play an essential role in the increase observed in the NAc DA extracellular levels, since microinjections of iGlu antagonists in the VTA or in the NAc, are able to abolish vSub-stimulated increment in DA extracellular levels (Blaha et al. 1997; Taepavarapruk et al. 2000).

In order to confirm our previous results and to deeply explore the mechanism of action of acamprosate, we conducted the present experiments to asses whether acamprosate administered by reversed dialysis directly into the NAc is able to block or attenuate the increase in NAc DA extracellular levels elicited by chemical stimulation of vSub.

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Materials and methods

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.

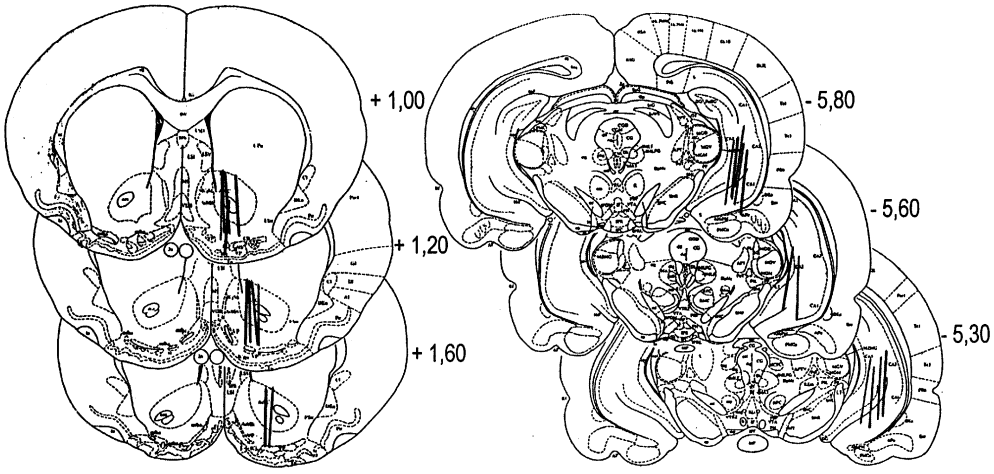

Fig. 1 Representation of the locations of dialysis membranes in the present study. Plates are taken from Paxinos and Watson (1986) and the numbers beside each plate correspond to millimeters from bregma

Acamprosate was kindly supplied by Lipha s.a, (France) and *N*-methyl-D-aspartic acid (NMDA) was obtained from Tocris Cookson Ltd. (Bristol, UK). All other reagents for the HPLC mobile phase and the perfusion fluid were analytical grade and were obtained from Sigma Chemical Co. (St Louis, MO, USA), Merck (Darmstadt, Germany) or JT Baker Chemical Co. (Philipsburg, NJ, USA).

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%). Four holes were drilled; two were used for the skull screws and the others for the microdialysis probes. Two vertical concentric-type microdialysis probes with 2 mm of active membrane (Hospal AN69; molecular cutoff 60,000 Da; Bologne, Italy) were implanted in the right NAc and in the ipsilateral vSub. 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: –8.3 mm for the NAc probe at the ventral extent of the active membrane and A/P: –5.8, L: –5.0, V: –9.0 mm for the vSub probe. 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 probes. The probes were perfused with a solution of artificial CSF (aCSF) comprised 0.1 mM aqueous 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 by HPLC. Drug delivery and sample connection 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.

DA content in the samples was measured 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 7.5ml/l of methanol. Mobile phase was pumped through the column at a flow rate of 0.6 ml/min. Chromatograms were integrated and 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.

All drugs used on the experiments were dissolved in the aCSF fluid and infused via retrograde dialysis through the implanted probes. 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. Treatments involved the local application of 5 mM NMDA for 40 min through the dialysis probe located in the Vsub and, simultaneously, the infusion of aCSF fluid (control group) or 0.05 mM acamprosate (experimental group) through the probe implanted in NAc. The length of NMDA infusions is shown as a horizontal bar in figures.

All values given are expressed as percentage of baseline that 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 the two time-effect curves were compared with Mann-Whitney U test. The level of significance was set at $p<0.05$. Microdialysis probe placements were histologically examined after completion of the experiments. Rats were anaesthetized with chloral hydrate and the brain was 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 probes located in NAc and in the vSub. The locations of microdialysis membranes are represented in Fig. 1.

Results

Application of 5 mM NMDA into the vSub in the control group produced a significant increase in extracellular levels of DA in the ipsilateral NAc ($p<0.001$; Fig. 2). The response reached 143±8% of baseline at 60 min after the end of the NMDA administration ($p=0.01$) and continued at 147±2% 2 h after administration of the drug ($p=0.002$). On the other hand, infusion of 0.05 mM acamprosate in NAc of the animals from the experimental group abolished the NMDA-evoked increase in NAc DA extracellular levels ($p=0.755$). As can be seen in the Fig. 2, the profile of the time-effect curve was completely different to that obtained in the control group. The Mann-Whitney U test revealed significant differences between some of the individual time-points (from 180 to 240 min) of the two curves ($p<0.05$).

Discussion

In the present paper we demonstrate for the first time that acamprosate is able to attenuate the increase in extracellu-

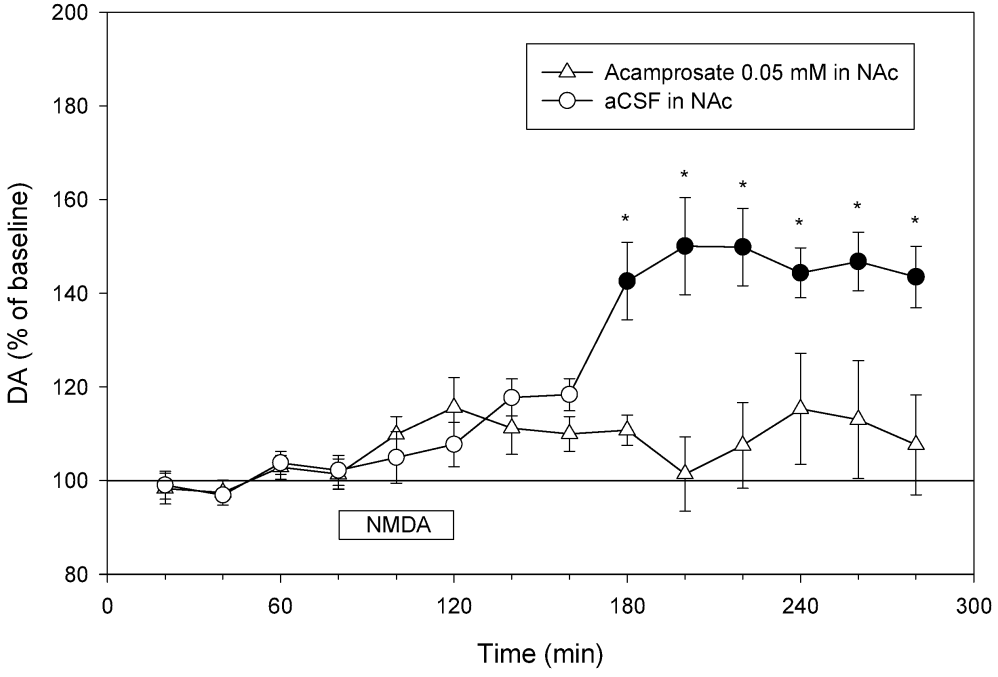

Fig. 2 Effect of *N*-methyl-D-aspartate (NMDA) infusion into the ventral subiculum (vSub) on nucleus accumbens (NAc) extracellular levels of dopamine (mean percentage of baseline ± SEM) during acamprosate perfusion (Δ n=6) or during aCSF perfusion (○ n=5) in NAc. The first four symbols represent baseline samples, and subsequent symbols represent samples collected after NMDA infusion (denoted by the *horizontal bar*). *Filled symbols* indicate significant differences from baseline (Dunnett's test, $p<0.05$). *$p<0.05$ as compared with the acamprosate group at the corresponding time points (Mann-Whitney U test)

lar DA in NAc elicited by chemical stimulation (by using NMDA infusions) of the vSub. In a previous paper, we reported that acamprosate modulates extracellular DA levels in NAc through interactions with NMDA receptors (Cano-Cebrián et al. 2003).

To develop the present experiments, we have selected two brain regions clearly implicated in the phenomenon of relapse to drug-taking behaviors in animal models, such as the ventral subiculum of the hippocampus and the nucleus accumbens. Two recently published papers (Vorel et al. 2001; Taepavarapruk and Phillips 2003) have shown that relapse in the consumption of several drugs of abuse can be triggered by activation of the subicular glutamatergic pathway to the NAc. Interestingly, in the Vorel et al. paper and in other papers (Cornish et al. 1999; Cornish and Kalivas 2000), it has been shown that iGlu antagonists microinjected in the NAc or in the VTA can block the relapse to drug-taking behaviors elicited by either re-exposure to the addictive drug or electrical stimulation of the vSub.

As indicated above, several neurochemical studies have shown that the electrical or chemical stimulation of the vSub is accompanied by a significant and persistent increase in extracellular DA levels in NAc, which reflects both the indirect activation of DA neurons in VTA (Legault et al. 2000) as well as presynaptic modulation of DA synapses in the NAc by glutamate (Blaha et al. 1997; Taepavarapruk et al. 2000). These previous studies have also shown that the evoked DA release can be completely blocked by intra-NAc administration of glutamate receptors antagonists. Our present results confirm these effects of the subicular stimulation on the DA levels in NAc. Moreover, our results indicate that acamprosate is able to block, as other iGlu receptor antagonists, the increase in DA extracellular levels in NAc after chemical stimulation of the vSub.

In conclusion, although our data do not prove directly that acamprosate administration in NAc can be able to block relapse in ethanol intake, the fact that acamprosate blocks the increase in extracellular levels of DA in NAc evoked by chemical stimulation of the vSub, (an event clearly associated with the reinstatement of drug-taking behaviors) supports the possibility that the antagonism, recently described by us (Cano-Cebrián et al. 2003), at the NMDA receptors in NAc can explain, al least in part, the mechanism of action of this drug.

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