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The NMDA Receptor Partial Agonist, 1-Aminocyclopropanecarboxylic Acid (ACPC), Reduces Ethanol Consumption in the Rat

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STROMBERG, M. F., J. R. VOLPICELLI, C. P. O'BRIEN AND S. A. MACKLER. *The NMDA receptor partial agoinist, 1-aminocyclopropanecarboxylic acid (ACPC), reduces ethanol consumption in the rat.* PHARMACOL BIOCHEM BEHAV **64**(3) 585–590, 1999.—The present studies assessed the effects of both systemic and intraaccumbens injections of 1-aminocyclopropanecarboxylic acid (ACPC), and NMDA partial agonist, on ethanol consumption in a limited access procedure in Wistar rats. Systemically administered ACPC reduced ethanol consumption in a dose-dependent manner, while a single dose of ACPC administered bilaterally into the nucleus accumbens also reversibly reduced ethanol consumption. Indirect measures of general appetitive behavior showed no effect of ACPC on weight or water intake, which suggests that this effect of ACPC may be specific to ethanol. These data are compatible with the role of NMDA receptors in modulating ethanol consumption and provide the first data showing that ACPC can reduce ethanol consumption. ACPC has neuroprotective effects and does not show the psychotomimetic effects observed with NMDA receptor agents. Thus, ACPC may be helpful in future clinical studies designed to reduce alcohol use. © 1999 Elsevier Science Inc.

Ethanol *N*-Methyl-D-Aspartate Glycine Nucleus accumbens Rats

ETHANOL is pharmacologically nonspecific, and has effects on many neurochemical systems that have been characterized to varying degrees. Ethanol's positive reinforcing effects are currently thought to be related to the release of dopamine (DA) in the nucleus accumbens of the mesolimbic DA system (7,18). Both experimenter (5,15) and self-administered (42) ethanol have been shown to produce increases in extracellular DA in the nucleus accumbens. Although ethanol's effects on DA appear critical for the emergence of its positive reinforcing properties, these effects can arise either directly, by its action on DA receptors [e.g., (5)], or indirectly, through its influence on other receptors whose action, in turn, serves to modulate DA release. One example is the glutamatergic *N*-Methyl-D-Aspartate (NMDA) receptor. NMDA receptors are ligand-gated ion channel receptor complexes that have been pharmacologically characterized as containing several interdependent domains or receptor subtypes that function to regulate activity of an ion channel. NMDA receptors are activated by glutamate and, after release from a voltage-dependent blockade by Mg^{++}, the ion channel is opened and allows the influx of Ca^{++}. This activation appears to be dependent upon the binding of the coagonist glycine (16).

Ethanol has been demonstrated to inhibit the function of the NMDA receptor (13,21) and, with chronic use, upregulate NMDA receptors in the hippocampus (8) and cortex and striatum (11). The upregulation of glutamatergic NMDA receptors has been suggested to account, in part, for neuronal hyperexcitability that accompanies alcohol withdrawal (20).

Glutamatergic NMDA receptors have also been identified in the nucleus accumbens, and glutamate afferents into the nucleus accumbens are received from the amygdala and hippocampus (40), the prefrontal cortex (35), and VTA (36). These glutamate inputs can modulate dopamine activity in the nucleus accumbens and thereby indirectly contribute to those neurochemical mechanisms maintaining ethanol consumption.

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Despite the findings suggesting that drugs acting on the NMDA receptor complex would be useful in the treatment of alcohol abuse and those effects associated with ethanol withdrawal, many of these agents possess serious side effects. For example, both competitive and noncompetitive NMDA receptor antagonists and channel blockers produce psychotomimetic and dissociative anesthetic effects. However, drugs that bind to the strychnine-insensitive glycine receptor subtype on the NMDA receptor complex appear to have a much better safety profile (6,17,22,39). One such drug is 1-aminocyclopropanecarboxylic acid (ACPC). Because ACPC exhibits few adverse effects in animal studies (22), the present experiments were designed to evaluate the effects of ACPC on ethanol consumption in a limited access animal model.

EXPERIMENT 1

The initial experiment was designed to evaluate the effects of a range of systemically delivered doses of ACPC on oral ethanol self-administration in rats using a limited access procedure.

Methods

Subjects. Thirty-six male Wistar rats were purchased from Ace Animals, Boyertown, PA, and arrived at the laboratory weighing between 250 and 300 g. The rats were housed in individual acrylic cages in a temperature-controlled (22°C) animal colony on a 12:12-h reverse light:dark cycle, with lights out from 0700 to 1900 h. Animals were provided with ad lib food and water for the entire experiment.

Procedure. Seven days after arriving in the laboratory, rats were weighed and handled daily and a bottle containing an ethanol solution was placed on the home cage in addition to the water bottle. An ascending series of ethanol concentrations were used as follows: 2% for 4 days, 4% for 4 days, and 6% for the balance of the experiment. Once consumption of the 6% ethanol solution stabilized at asymptote, the ethanol bottle was removed and presented on the following and subsequent days for 1 h between 1100 and 1200 h. Stability criterion was defined as no change in mean group consumption greater than 20% across 5 days. Bottles were weighed to the nearest 0.1 g, both prior to and following the 1 h limited access session. Once ethanol consumption stabilized across the limited access period, the rats were matched for ethanol consumption and randomly assigned to one of three ACPC groups or a vehicle control group. Rats in each group received intraperitoneal (IP) vehicle injections for 2 days prior to drug injection. Rats in group ACPC50. (n = 8) were injected with ACPC, 50 mg/kg, ACPC200 (n = 10) were injected with ACPC, 200 mg/kg, and rats in group ACPC400 (n = 8) were injected with ACPC, 400 mg/kg, 30 min prior to the limited access period for 4 days. Rats in the vehicle control group (n = 10) continued to receive vehicle injections on these days. Following the drug sessions all rats received vehicle injections 30 min before the limited access session for 2 additional days. All injections were administered IP in a volume of 3.0 ml/kg.

Drugs. ACPC, provided by Symphony Pharmaceuticals, Philadelphia, PA, was dissolved in a vehicle consisting of 70% saline and 30% deionized water. Both ACPC and vehicle were administered in a volume of 3.0 ml/kg.

Results

The results of Experiment 1 show that ACPC selectively and dose dependently reduced the consumption of an ethanol solution in a limited access paradigm. Figure 1 shows the consumption of ethanol across the last 10 days of baseline, the predrug saline period, the 4 days of drug administration, and the postdrug saline injections. A two-way repeated measures ANOVA (dose × treatment days) of the predrug saline days and the 4 drug days yielded a nonsignificant effect for dose, $F(3, 32) = 1.068$, $p = 0.377$, a significant effect for treatment, $F(4, 128) = 9.753$, $p < 0.001$, and a significant dose × treatment interaction, $F(12, 128) = 4.265$, $p < 0.001$. (For purposes of analysis, the data for the 2 predrug saline days were collapsed to a single value.) Subsequent one-way repeated-measures ANOVAs for each group across the predrug saline days and the 4 drug days revealed no significant effect for treatment days for group ACPC50, $F(4, 28) = 1.954$, $p = 0.129$, a significant effect for group ACPC200, $F(4, 36)$, $p > 0.001$, and a significant effect for group ACPC400, $F(4, 28) = 15.917$, $p < 0.001$. Subsequent Tukey's post hoc tests revealed that ethanol consumption following ACPC injections differed significantly from that following predrug saline injections for group ACPC200 on drug days 2 and 3, and for group ACPC400 on drug days 2, 3, and 4 (see Table 1).

Figure 2 shows consumption of water across the same period. A two-way repeated-measures ANOVA (dose × treatment days) of the 2 predrug saline days and the 4 drug days yielded a significant effect for dose, $F(3, 32) = 20.96$, $p = 0.001$, a significant effect for treatment, $F(4, 128) = 11.713$, $p < 0.001$, and a significant dose × treatment interaction, $F(12, 128) = 3.177$, $p < 0.001$. Subsequent one-way repeated-measures ANOVAs for each group across the predrug saline days and the 4 drug days revealed a significant effect for treatment days only for groups ACPC50 and ACPC400. As can be seen in Fig. 2, these effects were due to the increase in water consumption during the limited access period following vehicle injections rather than a reduction following ACPC injections. Most of this variance is attributable to the behavior of four rats in the ACPC50 group and three rats in the ACPC400 group. The fact that other rats in these groups and the rats in the ACPC 200 or control groups were not similarly affected suggests that this transient increase in water consumption was probably not due to any general effect related to experimental procedures or environment. This is indirectly confirmed by

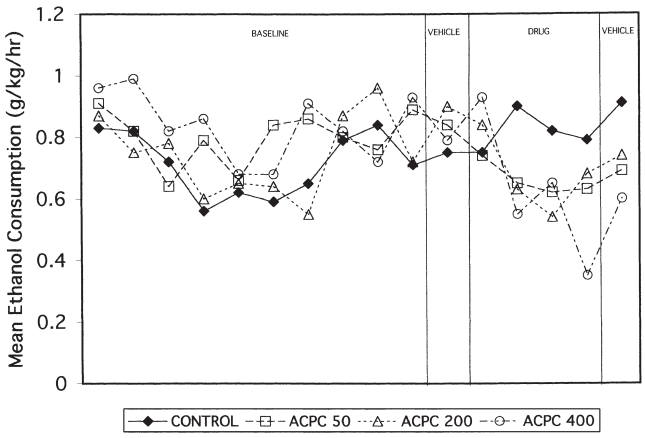

FIG. 1. The effects of systemically administered ACPC 0, 50, 200, and 400 mg/kg, on ethanol consumption (g/kg/h) across baseline, predrug saline, drug, and postdrug saline phases of Experiment 1.

TABLE 1

MEAN INTAKE OF ETHANOL FOLLOWING INJECTION OF VEHICLE OR ACPC

Group	Vehicle Baseline ETOH Consumption (g/kg/h) ± SEM	Drug Day 1 ETOH Consumption (g/kg/h) ± SEM	Drug Day 2 ETOH Consumption (g/kg/h) ± SEM	Drug Day 3 ETOH Consumption (g/kg/h) ± SEM	Drug Day 4 ETOH Consumption (g/kg/h) ± SEM
Vehicle	0.75	0.75	0.90	0.82	0.79
	0.066	0.065	0.086	0.071	0.080
ACPC 50 mg/kg	0.84	0.74	0.65	0.62	0.63
	0.072	0.072	0.090	0.100	0.052
ACPC 200 mg/kg	0.90	0.84	0.63*	0.54*	0.68
	0.091	0.095	0.089	0.070	0.069
ACPC 400 mg/kg	0.79	0.93	0.55*	0.65*	0.35*
	0.053	0.139	0.089	0.063	0.075

*Differs significantly from vehicle baseline ETOH consumption.

the animal weights, which did not show any variation across this same period.

EXPERIMENT 2

The second experiment utilized bilateral injection of ACPC into the nucleus accumbens to determine if ACPC's effects were related to NMDA receptors located there or rather form NMDA receptors in other regions.

Methods

Subjects. Eleven male Wistar rats that had previously served as saline control animals were matched for ethanol consumption and randomly assigned to either an ACPC (n = 6) or vehicle control group (n = 5). The limited access procedure utilized in this experiment was identical to that in Experiment 1.

Surgery and Microinjections. After limited access drinking was stable, the animals were prepared for surgery. Each rat was anesthetized with Nembutal (50 mg/kg, IP) and stereotaxically implanted with bilateral microinjection guide cannulae 1.0 mm above the nucleus accumbens. Stainless steel screws were inserted into the skull and the cannulae affixed to the skull and screws with dental acrylic cement. Chronic guide cannulae were positioned at the following coordinates: A/P 1.5 mm, M/L 3.0 mm, D/V 7.0 mm, relative to bregma with the nose bar at −3.0 mm (23). Rats were permitted a minimum 5-day recovery period from surgery prior to restarting ethanol self-administration. Rats were returned to limited access until drinking restabilized. On the morning of the injections, approximately 3 h before the scheduled limited access period, each rat was mildly restrained in the hands of the investigator. This time interval between intracranial injection and access to ethanol was used to minimize any stress-related effects that handling and injection could have had on drinking. The injection needles (33 ga) were inserted through the guide cannulae into the central region of the nucleus accumbens. Bilateral infusions (0.5 μl of 50 μg/μl ACPC in saline or 0.5 μl of saline) were delivered over 50 s. One minute after infusion the needles were removed and the stylets replaced. This volume of injection was chosen in part because it would not be expected to be limited to either the core or shell subregions of the accumbens.

Confirmation of the Microinjection Sites. Rats were given an overdose of pentobarbital at the completion of the experiment, and the brains fixed by intracardiac infusion of ice cold PBS, followed by 500 ml of 4% PBS–formalin. The brains were removed and stored in a sucrose solution overnight prior to making 40 μm-thick coronal slices on a vibratome. The slices were mounted on gelatin-coated slides, stained with cresyl violet, and the cannulae placement determined for each rat (24).

Results

The results of Experiment 2 show that ACPC infused bilaterally into the nucleus accumbens significantly reduced ethanol consumption compared to a vehicle control group. Figure 3 shows ethanol consumption across the last 4 days of baseline, the drug administration day and the postdrug day. A two-way repeated-measures ANOVA (treatment group × days) across the 4 predrug baseline days collapsed, and the drug day yielded a nonsignificant main effect for treatment group and days, but a significant treatment × days interaction, $F(1, 9) = 5.396$, $p < 0.05$. Subsequent pairwise comparisons revealed that the rats in group ACPC drank significantly less ethanol when compared to baseline, while rats in group saline showed a nonsignificant increase in ethanol consumption.

There was no evidence that ACPC affected water consumption either within the 1 h limited access period or across

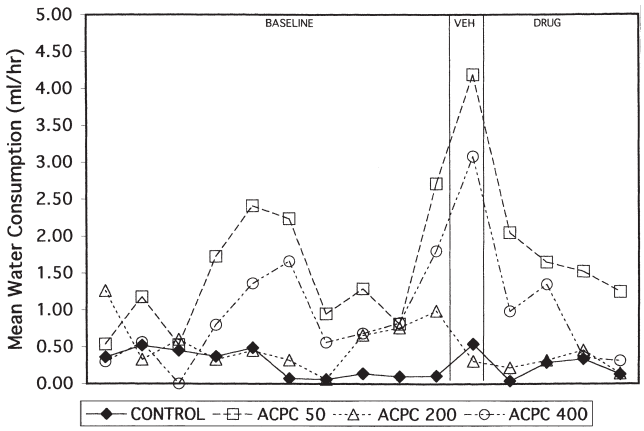

FIG. 2. The effect of systemically administered ACPC 0, 50, 200, and 400 mg/kg, on within-session water consumption across baseline, predrug saline, drug, and postdrug saline phases of Experiment 1.

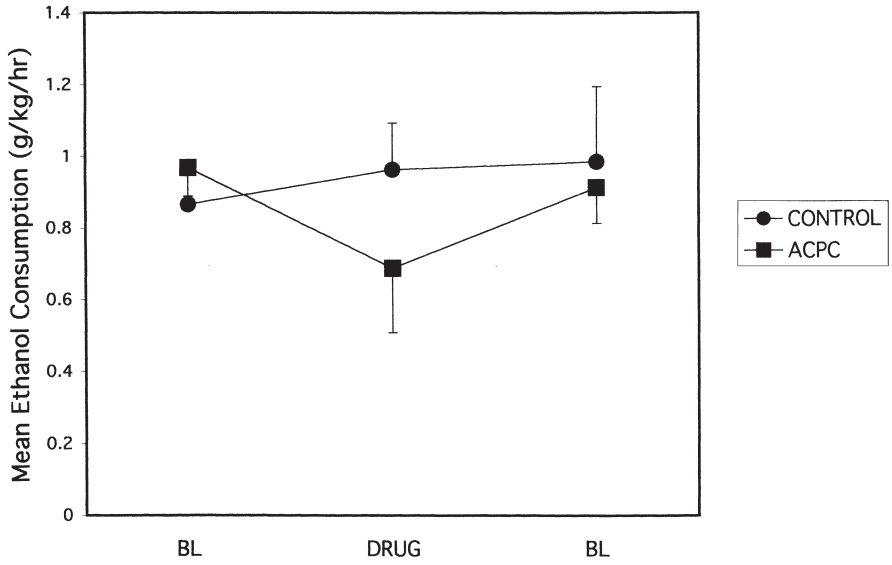

FIG. 3. The effect of bilateral microinjection of ACPC, 25 μg, or vehicle on ethanol consumption (g/kg/h) in Experiment 2 (±SEM).

the 24-h period. The ACPC rats did not differ in their gross behavior from the control rats following infusions. Histologic analysis following completion of the experiment confirmed that all cannulae were placed in the central region of the nucleus accumbens.

GENERAL DISCUSSION

The present experiments provide initial evidence that ACPC, a partial agonist selective for the strychnine-insensitive glycine subunit on the NMDA receptor complex, can reduce consumption of ethanol in rats. The results further suggest that ACPC's effect on ethanol consumption is, in part, due its interaction with NMDA receptors located in the nucleus accumbens, an area hypothesized to underlie the reinforcing effects of many drugs with abuse potential.

The precise mechanism of ACPC's effect on ethanol drinking is unclear. One possibility is that ACPC alters the neuronal hyperexcitability of NMDA receptors that occurs after chronic ethanol use (9). The rats in the present study were chronically exposed to ethanol, both continuously and in limited access, for over 1 month, producing the potential for NMDA receptor upregulation and the development of neuronal hyperexcitability in the absence of ethanol (9,13,21,43). Although the limited access paradigm does not result in a detectable withdrawal syndrome, it remains to be determined if there are changes in expression of NMDA receptor subunits. In previous behavioral studies, evidence from drug discrimination studies demonstrated that noncompetitive NMDA antagonists working at the ion channel (MK-801, phencyclidine, and memantidine) could substitute for ethanol (10,14,31,33). NMDA antagonists have also been shown to attenuate the seizures induced by withdrawal from chronic exposure to ethanol (4,23). Although these drugs are effective in both substituting for the ethanol stimulus and reducing withdrawal symptoms related to hyperexcitability, they also have been shown to produce toxicity at the cellular level and psychotomimetic effects at the behavioral level. These adverse effects have limited their clinical usefulness.

ACPC, a partial agonist at the strychnine-insensitive glycine receptor on the NMDA receptor complex (27), has been demonstrated to have neuroprotective effects without adverse behavioral effects in rats. In addition to the glutamate and phencyclidine regions, sites are also present for binding of polyamines and glycine. The importance of the glycine binding site is suggested by data showing that the addition of glycine can reverse ethanol's inhibitory effect on NMDA activity in some brain regions (8,28). However, there are other data suggesting that glycine does not reverse ethanol's effects on NMDA activity (25). Similarly, there are conflicting data on the ability of glycine antagonists (1,3,14) or partial agonists (2) to substitute for ethanol's stimulus properties in a discrimination procedure. In addition, the glycine selective antagonists, L-701,324, has been shown to produce a dose-dependent reduction in ethanol-induced withdrawal symptoms (19).

How glycine modulates ethanol's effects via the NMDA receptor remains to be determined. However, these preliminary findings are encouraging because drugs with selective activity at the glycine site appear to be able to modify ethanol's effects without the toxic and behavioral problems inherent in NMDA antagonists that block the ion channel. Interestingly, acamprosate, which is also thought to act on the NMDA receptor (20), has been shown to decrease ethanol reinstatement, but not basal ethanol consumption in animal models (12,37) and also cravings for alcohol in human subjects (32). Although the experiments presented here did not evaluate the effect of ACPC on reinstatement following a period of deprivation, ACPC shows a difference from acamprosate in its ability to attenuate basal ethanol consumption in the limited access model used here (Stromberg, unpublished data). Despite the fact that both ACPC and acamprosate are hypothesized to work at the NMDA receptor complex, there may be critical differences related to a specific target site that determine the effects of these two drugs on ethanol consumption.

Part of the motivation underlying continuing ethanol consumption may be due to ethanol's ability to inhibit NMDA activity, thereby reducing neuronal hyperexcitability. Following this, one potential explanation for ethanol consumption, un-

der the parameters of the limited access procedure used in these experiments, is that it was maintained, at least in part, by negative reinforcement. An unproven hypothesis is that ACPC may function as an antagonist at the glycine site on the NMDA receptor complex, reducing the level of hyperexcitability prior to ethanol reexposure. The present results (Table 1, Figs. 1 and 2), demonstrating a reduction in ethanol consumption of approximately 20 to 30%, is consistent with the data showing that ACPC reduced the maximal glycine-induced increase in NMDA activity by approximately 20% (41). A second possible mechanism by which ACPC reduces ethanol drinking is by disrupting its positive reinforcing properties. Similar findings were observed after injection of 2-amino-5-phosphopentanoic acid (AP-5) into the nucleus accumbens (29).

Glutamate is the most widely spread excitatory neurotransmitter in the brain. Glutamatergic projections from the hippocampus, amygdala, and prefrontal cortex terminate in the nucleus accumbens (40). Recent attention has focused on glutamatergic projections from the PFC to the nucleus accumbens and VTA. Lesions of the medial PFC, but not the hippocampus and amygdala, have been shown to prevent behavioral sensitization to cocaine (26). In addition, glutamate antagonists administered to the VTA attenuated DA release in the nucleus accumbens following electrical stimulation of the PFC (34,38). This PFC stimulation has been demonstrated to increase extracellular glutamate in the VTA (30). The systemic administration of ethanol has also been shown to increase extracellular levels of glutamate in the nucleus accumbens of Lewis, but not F344 rats (35). However, ethanol in this same experiment failed to increase glutamate levels in the PFC of either strain. Whereas the importance of glutamate in modulating those neurobiological substrates underlying the motivation to consume many drugs with abuse potential is becoming apparent, much work remains to be done to elucidate the specific mechanisms involved.

In summary, the results of these experiments suggest that ACPC, a partial agonist at the strychnine-insensitive glycine receptor, significantly reduced ethanol consumption in a dose-dependent manner without disrupting other appetitive behavior. In addition, the results suggest that some of this effect is due to the action of ACPC at NMDA receptors in the nucleus accumbens. These results may be clinically significant in the treatment of recovering alcoholics because ACPC has been shown to have a neuroprotective effect and have none of the psychotomimetic properties of other drugs with effects at the NMDA receptor.

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