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Anxiolytic Properties of 1-Aminocyclopropanecarboxylic Acid, a Ligand at Strychnine-Insensitive Glycine Receptors

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TRULLAS, R., B. JACKSON AND P. SKOLNICK. *Anxiolytic properties of 1-aminocyclopropanecarboxylic acid, a ligand at strychnine-insensitive glycine receptors.* PHARMACOL BIOCHEM BEHAV **34**(2) 313–316, 1989.—The effects of 1-aminocyclopropanecarboxylic acid were investigated on performance in an elevated plus-maze. This compound is a high-affinity, partial agonist ligand at strychnine-insensitive glycine receptors of the N-methyl-D-aspartate receptor complex. Like chlordiazepoxide, 1-aminocyclopropanecarboxylic acid increased in a dose-dependent manner both the percent entries into and the percent time spent in the open arms of the plus-maze. However, 1-aminocyclopropanecarboxylic acid was significantly less efficacious than chlordiazepoxide in these measures and increased, while chlordiazepoxide decreased, the time spent in the middle platform of the plus-maze. These findings indicate that ligands acting through strychnine-insensitive glycine receptors on the N-methyl-D-aspartate receptor complex may represent a new class of anxiolytic agents with a profile which differs from the benzodiazepines.

1-Aminocyclopropanecarboxylic acid 7-Chlorokynurenic acid Chlordiazepoxide Glycine Plus-maze Anxiolytics N-methyl-D-aspartate receptors

BOTH competitive and noncompetitive antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor possess anticonvulsant (5, 7, 31), muscle relaxant (31), and anxiolytic properties (3, 6, 28) [see (4) for review]. This spectrum of action is reminiscent of benzodiazepines and barbiturates, and suggests that reducing excitatory output mediated by this "NMDA receptor complex" (8,15) can mimic the effects of facilitating synaptic inhibition through the benzodiazepine/$GABA_A$ receptor complex (26).

Both neurochemical and electrophysiological studies indicate that in addition to its well-described inhibitory actions in brainstem and spinal cord (1), glycine also modulates excitatory neurotransmission in the central nervous system. Thus, glycine augments NMDA receptor-mediated membrane depolarization in cultured neurons (11) and is required for activation of NMDA-gated cation channels expressed in *Xenopus* oocytes (14). These actions are mediated through strychnine-insensitive glycine receptors with markedly different structural requirements for ligand binding (9, 13, 27) and regional distribution (2) than those strychnine-sensitive sites associated with the role of glycine as an inhibitory transmitter (1, 32, 33).

Recently, we reported that 1-aminocyclopropanecarboxylic acid (ACPC) is a high affinity ligand ($K_i \approx 32$ nM) with partial agonist properties at strychnine-insensitive glycine receptors associated with the NMDA receptor complex (20). It was hypothesized that if the requirement for glycine to activate NMDA-gated cation channels in *Xenopus* oocytes (14) is also present in vivo, a high affinity, partial agonist ligand at strychnine-insensitive glycine sites would function as an NMDA antagonist (25). While ACPC was found to protect mice against the convulsant and lethal actions of parenterally administered NMDA (25), it did not possess the broad anticonvulsant spectrum, muscle relaxant, or sedative properties common to both competitive and noncompetitive NMDA receptor antagonists (4). These findings prompted an examination of the effects of ACPC on performance in an elevated plus-maze, a conflict test capable of detecting clinically effective anxiolytics (10, 17, 24).

METHOD

Male (20–25 g), NIH-Swiss mice (Harlan Sprague-Dawley, Frederick, MD) were housed (10–15/cage) in $32 \times 25 \times 15$ cm plastic cages for at least one week before testing with food and water freely available. Mice were injected intraperitoneally (0.1

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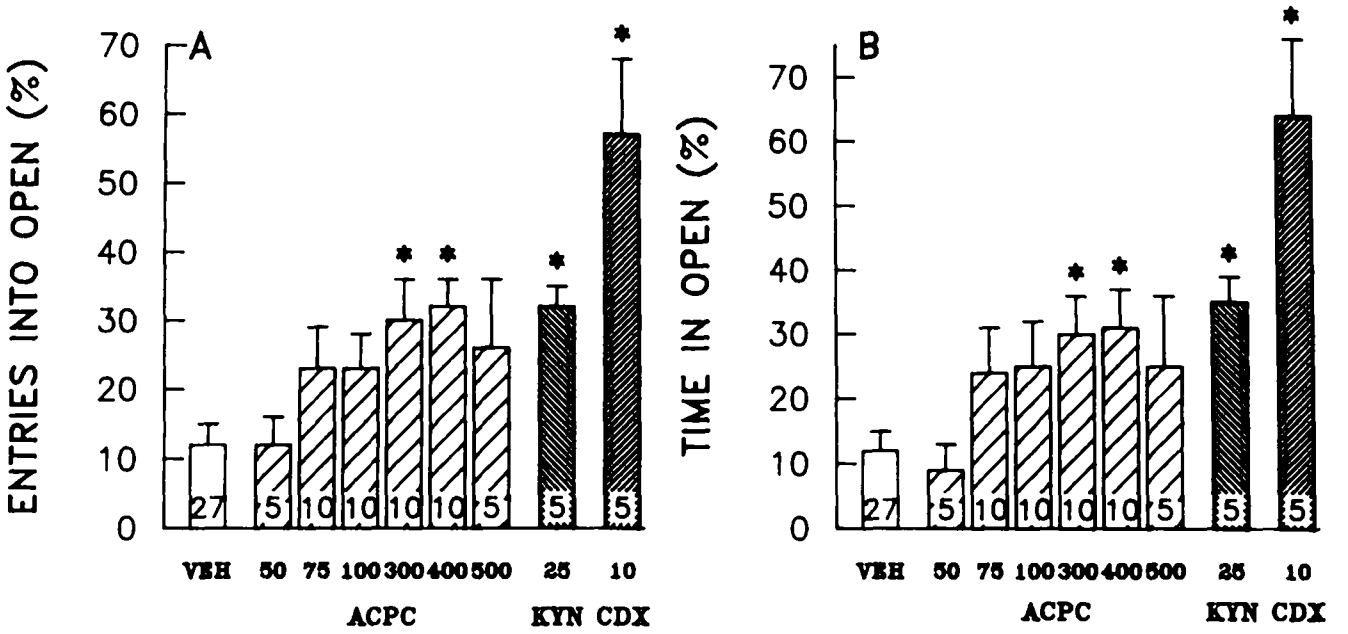

FIG. 1. Effects of 1-aminocyclopropanecarboxylic acid, 7-chlorokynurenic acid, and chlordiazepoxide on plus-maze performance. Animals were evaluated for five minutes under bright light as described in the Method section. (A) Percent entries into the open arms was expressed as the number of entries into the open arms/total entries × 100. (B) Percent time in open arms was expressed as time in the open arms/time in the open arms + time in the enclosed arms × 100. Values represent mean ± SEM of the number of animals shown within the bars per group. *Significantly different from vehicle group, $p<0.05$ (Duncan's test).

ml) with either saline or drugs 15 minutes prior to testing. ACPC was dissolved in distilled water, 7-chlorokynurenic acid (KYN) in 0.1 N NaOH, and chlordiazepoxide (CDX) in saline, respectively.

The elevated plus-maze was a modification (30) of the apparatus described by Lister (17). The plus-maze was constructed entirely of black Plexiglas, consisting of 2 open arms (30 × 5 cm) and 2 enclosed arms (30 × 5 × 15 cm) extending from a center platform (5 × 5 cm). A 1-cm high Plexiglas strip was attached to the sides of the open arms of the maze to prevent animals from falling. The maze was elevated 31 cm above the floor. Testing was performed in a windowless room (held at approximately 21°C) under "bright light," consisting of two 34-watt, 125-cm fluorescent lamps located 134 cm over the center platform. Animals were moved from the colony room to the testing area in their own cages and allowed to adapt to the new environment for one hour before treatment. After injection, the animals were returned to their cages until tested. Performance in the plus-maze was evaluated for 5 min with testing initiated by placing a mouse at the intersection of the maze arms so that its head was in the center of the platform. The apparatus was thoroughly washed with 50% ethanol/water after each test period. Mice were scored as in the open or enclosed arms only when all four limbs were within that area. Time spent in the open arms, enclosed arms, middle platform and number of entries into the arms was recorded using an Apple IIe computer.

ACPC and KYN were obtained from Fluka (Ronkonkoma, NY) and Tocris Neuramin (Essex, England), respectively. CDX (as the HCl salt) was donated by Hoffmann-La Roche, Inc. (Nutley, NJ).

RESULTS

One-way analysis of variance revealed that ACPC produced a dose-dependent increase in the percentage of entries into the open arms, $F(6,70)=3$, $p<0.01$ (Fig. 1A), the percentage of time spent in the open arms, $F(6,70)=2.2$, $p=0.05$ (Fig. 1B), and the total number of arm entries, $F(6,70)=5.8$, $p<0.01$ (Table 1). ACPC increased the percentage of time spent in the open arms by significantly decreasing the time spent in the enclosed arms and increasing the time spent in the middle platform without significantly affecting the time spent in the open arms (Table 1). The effects of ACPC on these parameters were maximum at 300–400 mg/kg, while at the highest dose (500 mg/kg) employed in these studies the increase in both percentage entries into the open arms and percentage time in the open arms was no longer statistically significantly different from the vehicle-treated group (Fig. 1). Like ACPC, KYN (25 mg/kg) significantly increased the percentage of entries into the open arms ($p<0.05$) and percentage of time in the open arms ($p<0.05$) by reducing the time mice spent in the enclosed arms ($p<0.05$) of the maze, without modifying the time spent in the open arms of the maze (Table 1, Fig. 1).

CDX (10 mg/kg) elicited a significant increase in percentage of entries into the open arms ($p<0.05$), percent time in the open arms ($p<0.05$), and total number of entries ($p<0.05$). CDX also increased time spent in the open arms ($p<0.05$), and significantly decreased both time spent in the middle platform and time spent in the enclosed arms ($p<0.05$) (Table 1).

An analysis of covariance using the total number of arm entries as the covariate showed that the significant effects of ACPC, KYN, and CDX to increase the percent entries into the open arms, $F(8,77)=2.0$, $p=0.05$, and percent time in the open, $F(8,77)=2.2$, $p<0.05$, were independent of increases in motor activity.

DISCUSSION

Previous studies have shown that both competitive [2-amino-7-phosphonoheptanoic acid (AP 7), 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP), CGS 19755] and noncompetitive (MK-801, phencyclidine) NMDA receptor antagonists possess

TABLE 1

EFFECTS OF 1-AMINOCYCLOPROPANECARBOXYLIC ACID (ACPC), 7-CHLOROKYNURENIC ACID (KYN) AND CHLORDIAZEPOXIDE (CDX) ON PERFORMANCE IN AN ELEVATED PLUS-MAZE

Drug (dose)	n	TIMEO	CROSO	TIMEC	CROSC	TIMEM	TE
VEHICLE	27	24 ± 7	2 ± 1	162 ± 8	13 ± 1	113 ± 5	15 ± 1
ACPC							
(50)	5	17 ± 6	2 ± 1	170 ± 16	16 ± 1	113 ± 13	18 ± 1
(75)	10	43 ± 14	4 ± 1	133 ± 14*	12 ± 1	123 ± 11	16 ± 1
(100)	10	45 ± 13	5 ± 1	122 ± 12*	15 ± 1	133 ± 10	20 ± 2
(300)	10	52 ± 14	9 ± 3*	110 ± 8*	15 ± 1	139 ± 10*	24 ± 3
(400)	10	49 ± 10	9 ± 1*	107 ± 11*	17 ± 1	144 ± 7*	26 ± 2*
(500)	5	51 ± 23	7 ± 3	122 ± 8*	16 ± 1	127 ± 20	23 ± 2*
KYN							
(25)	5	63 ± 8	7 ± 2	115 ± 7*	15 ± 2	122 ± 7	22 ± 2
CDX							
(10)	5	152 ± 29*	15 ± 2*	88 ± 29*	15 ± 6	60 ± 11*	30 ± 6*

Doses (in parentheses) are in mg/kg. Animals were evaluated in the plus-maze for five minutes as described in the Method section. Values represent the mean ± SEM of n animals per group. TIMEO: time spent in open arms; CROSO: crosses into the open arms; TIMEC: time spent in enclosed arms; CROSC: crosses into enclosed arms; TIMEM: time spent in the middle platform; TE: total entries.

*Significantly different from the vehicle group ($p<0.05$, Duncan's test).

anticonvulsant, muscle relaxant, and anxiolytic properties in animal models (3–5, 7, 29, 31). The present study, examining the effects of ACPC in a behavioral paradigm capable of detecting anxiolytics (10, 17, 24, 30), was prompted by several recent findings. Thus, we initially observed that ACPC was a potent and selective ligand at strychnine-insensitive [^{3}H]glycine receptors, but was less efficacious than glycine in enhancing [^{3}H]MK-801 binding to sites associated with NMDA-gated cation channels (20). It was hypothesized that a high-affinity ligand with partial agonist actions at strychnine-insensitive glycine sites would function as an antagonist in situ (25) if glycine was required for the gating of NMDA-operated cation channels as has been observed in *Xenopus* oocytes (14). While ACPC blocked the convulsant and lethal actions of parenterally administered NMDA in mice, it differed from other NMDA antagonists in that it was ineffective against other convulsants, and did not produce muscle relaxation or ataxia (25). These findings, together with the lack of consensus (6, 16, 29) surrounding the efficacy of noncompetitive NMDA antagonists (MK-801, phencyclidine) in animal models of anxiety, stimulated the present investigation.

Like CDX, ACPC produced a significant increase in both the percent time and entries into the open arms of the plus-maze (Fig. 1). Nonetheless, there were significant differences between these compounds in their effects on plus-maze performance. Thus, the efficacy of ACPC to increase these measures was significantly lower than a standard test dose of CDX (Fig. 1). While this lower efficacy may be directly related to the qualitative differences in plus-maze performance between these agents (see below), competitive NMDA receptor antagonists exhibit efficacies comparable to benzodiazepine receptor ligands in several conflict paradigms including the Cook/Davidson test (16), 4-plate test (29), and plus-maze (29). Since several lines of evidence (20, 21, 23) indicate the presence of multiple classes of NMDA receptors that may be differentially regulated by glycine (21), the lower efficacy of ACPC (compared to NMDA antagonists) may be related to a heterogeneity of organization of the NMDA receptor complex in that not all NMDA receptor-operated cation channels may be coupled to glycine receptors.

ACPC significantly increased, while CDX decreased the amount of time spent in the middle platform of the plus-maze (Table 1). While the mechanisms responsible for this qualitative difference in plus-maze performance are unknown, it has been suggested that the anticonflict action of benzodiazepines in animal models of anxiety is related to an alteration of a decision making mechanism rather than to a specific effect on behavioral disinhibition (18). If time spent in the middle platform of the plus-maze is considered as the period in which "no decision" is made to enter the maze arms, this phenomenon could ultimately be related to a distinct anxiolytic profile of substances that act through the NMDA receptor complex. This hypothesis is currently under investigation.

The potency of ACPC to affect plus-maze behavior (Fig. 1, Table 1) was comparable to its potency in blocking NMDA-induced convulsions and lethality (25), but was low compared to CDX (Fig. 1, Table 1). This low potency [relative to its affinity for strychnine-insensitive [^{3}H]glycine binding sites in vitro (20)] is consistent with the zwitterionic nature of ACPC at physiological pH which would impede penetration into the central nervous system. A similar explanation has also been invoked to explain the low potencies of competitive NMDA antagonists such as AP-7 in anticonvulsant and anxiolytic paradigms following parenteral administration (16,19).

Despite the neurochemical evidence indicating that ACPC is a high-affinity, partial agonist ligand at strychnine-insensitive glycine receptors (20,22), the mechanism(s) by which ACPC exerts an anticonflict action should be considered speculative. Nonetheless, several lines of evidence are consistent with an action mediated through the NMDA receptor complex. Thus, if the effects of ACPC are due to a functional antagonism of the NMDA receptor complex through a partial agonist action at glycine receptors (25), it would be predicted that a glycine antagonist would exert a similar effect. The finding that 7-chlorokynurenic acid [which has electrophysiological and biochemical properties of a glycine antagonist at the NMDA receptor complex (12)] elicits an anticonflict action with a strikingly similar pattern of behavior to ACPC (Table 1) supports this hypothesis. Moreover, the observation that a supramaximal dose of ACPC (500 mg/kg), which results in a reduced efficacy in some measures of plus-maze

performance (Fig. 1, Table 1), also results in a lower anticonvulsant efficacy against NMDA-induced convulsions (25) provides further support for this hypothesis.

In summary, the present findings indicate that the strychnine-insensitive glycine receptor is a potentially important locus for drug design. Since ACPC appears to lack the muscle relaxant and ataxic properties (25) common to both competitive and noncompetitive NMDA antagonists (3,4), it could represent the prototype of a novel class of anxiolytics with a profile that differs from benzodiazepines.

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