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D-Cycloserine, a Positive Modulator of the N-Methyl-D-Aspartate Receptor, Enhances Performance of Learning Tasks in Rats

JOSEPH B. MONAHAN,[1] GAIL E. HANDELMANN, WILLIAM F. HOOD AND ALEXIS A. CORDI

CNS Diseases Research, G.D. Searle Company
700 Chesterfield Village Parkway, St. Louis, MO 63198

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MONAHAN, J. B., G. E. HANDELMANN, W. F. HOOD AND A. A. CORDI. *D-Cycloserine, a positive modulator of the N-methyl-D-aspartate receptor, enhances performance of learning tasks in rats.* PHARMACOL BIOCHEM BEHAV **34**(3) 649–653, 1989.—Glycine has recently been shown to positively modulate the N-methyl-D-aspartate (NMDA) subclass of acidic amino acid receptors which are important in neural pathways involved in learning and memory. We report that d-cycloserine (DCS), an antimycobacterial agent known to cross the blood-brain barrier, binds with high affinity to this glycine modulatory site, functions as a positive modulator, and facilitates performance of learning tasks in rats. In addition, DCS appears to be a potent cognitive enhancer at doses lower than those required for antibacterial activity. Based on these data, we propose that modulation of NMDA receptors via glycinergic mechanisms may be a means of influencing cognitive processes.

NMDA receptor Glycine receptor Cognitive enhancement Learning and memory

THE NMDA receptor is a subclass of acidic amino acid receptors which have been shown to be prominently involved in excitatory transmission in the mammalian central nervous system. Glycine and other select neutral amino acids (e.g., serine and alanine) have been shown in electrophysiological studies to potentiate the responses to NMDA in dissociated cultured cortical neurons (10). Biochemical evidence confirming this interaction of glycine with the NMDA receptor complex has been reported in studies where glycine enhanced the binding of the phencyclidine analogs [^{3}H]1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) and [^{3}H](+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine ([^{3}H]MK-801) to the NMDA receptor coupled ionophore (2, 22, 24, 26). Additionally, a recognition site for [^{3}H]glycine has been identified in the rat brain which has the pharmacological properties (11,18), and anatomical distribution (3,20) expected of the NMDA receptor coupled modulatory site.

Several studies have provided evidence for a role of the NMDA receptor complex in processes of learning and memory. Long-term potentiation (LTP), a synaptic model of memory (5, 12, 13), has been demonstrated in certain synaptic pathways in the hippocampus which are glutamatergic in nature and which contain high densities of NMDA receptors (6,16). NMDA receptor activation has been shown to be a necessary component for LTP induction in the visual cortex and blockade of this receptor with either competitive or noncompetitive antagonists blocks induction of LTP in both the hippocampus and cortex (1, 8, 23).

Finally, a competitive NMDA antagonist, 2-amino-5-phosphonopentanoate, has been shown to impair learning of several types of tasks in rats, including spatial mazes and a passive avoidance paradigm (7,19). It would, therefore, be predicted that compounds which enhance glutamatergic transmission, such as those interacting with this glycine site, may facilitate learning and memory.

DCS is an antimycobacterial agent used primarily for retreatment of nonresponsive or noncompliant patients. The observation that DCS freely crosses the blood-brain barrier (15), coupled with the demonstration in the present study that it is a potent and selective positive modulator of the NMDA receptor, makes this compound a logical choice to analyze behavioral characteristics induced by positive modulators interacting at this glycine site. In this study, the effects of DCS were studied in two types of learning tasks: a shock-motivated passive avoidance test and a food-motivated spatial learning task.

[1]Requests for reprints should be addressed to Joseph B. Monahan, Searle R & D, c/o Monsanto Company, Mail Zone AA5C, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

ABBREVIATIONS

DCS, d-cycloserine; NMDA, N-methyl-D-aspartate; TCP, 1-[1-(2-thienyl)cyclohexyl]piperidine; MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine; LTP, long-term potentiation; SPM, synaptic plasma membranes.

METHOD

Materials

Radioactive ligands (l-[^{3}H]glutamate, 40–50 Ci/mmol; [^{3}H]glycine, 40–50 Ci/mmol; [^{3}H]TCP, 47.1 Ci/mmol; [^{3}H]kainate, 60 Ci/mmol; [^{3}H]strychnine, 24.4 Ci/mmol and [^{3}H]-α-amino-3-hydroxy-5-methylisoxazole-4-propionate, 27.1 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Glycine and l-glutamate were from Pierce Chemical Co. (Rockford, IL). D- and l-cycloserine were from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were reagent grade.

Membrane Preparation

Synaptic plasma membranes (SPM) were prepared as previously described (17). The SPM were stored at a concentration of 10–15 mg/ml in 0.32 M sucrose, 0.5 mM EDTA, 1 mM $MgSO_4$, 5 mM Tris/acetate, pH 7.4, under liquid nitrogen. The identity and purity of the subcellular fractions were confirmed by both electron microscopy and marker enzymes. Protein concentrations were determined by using a modification of the method of Lowry (21).

Receptor Binding Assays

The strychnine-insensitive [^{3}H]glycine (18,25), NMDA recognition site selective l-[^{3}H]glutamate (17) and [^{3}H]TCP binding assays (24,25) were performed as previously described. Briefly, the [^{3}H]glycine and l-[^{3}H]glutamate binding assays were each performed using a similar procedure, where 10 nM of the radiolabeled ligand ([^{3}H]glycine or l-[^{3}H]glutamate) was added to the appropriate concentration of the test compound in 50 mM Tris/acetate, pH 7.4 and the assay initiated by the addition of 0.2–0.4 mg of SPM. Following a 10 min incubation at 2°C, the bound radioactivity was separated from the free by centrifugation and quantitated by liquid scintillation spectrometry. The K_i values were determined by logit-log analysis.

In the [^{3}H]TCP binding assay, SPM (0.16–0.25 mg) were incubated with [^{3}H]TCP (2.0 nM) and the appropriate test compounds for 60 min at 25°C in 5 mM Tris/HCl, pH 7.4 followed by vacuum filtration. The radioactivity retained on the filter disc was quantitated by liquid scintillation spectrometry. The EC_{50} values for the stimulation of [^{3}H]TCP binding were determined using a four parameter logistic regression analysis.

Learning Tests

Male Long-Evans rats weighing about 200 g were used for the behavioral experiments. They were housed two per cage with ad lib food and water. The experiments were performed during the dark portion of the rats' light-dark cycle.

Passive Avoidance Test

DCS was tested for its ability to enhance learning and memory in a one-trial passive avoidance test modified from that described by Carew (4). The apparatus consisted of a lidded Plexiglas box (32 × 26 × 20 cm) with a floor of metal rods spaced 1.8 cm apart. The box was divided into two chambers, one painted black and the other gray. Two doors (12 cm high) in the front of the box allowed access to the chambers. A Y-shaped runway was attached to the front of the box. The stem of the Y was 16 cm long and unpainted, and extended over the edge of the table on which the apparatus was placed so that it was approximately 75 cm above the floor. The arms of the Y (each 14 cm long) led to the two doors and each was painted the color of the chamber to which it led. The metal floor of the box was wired to a Lafayette shock generator to deliver a 0.5 mA shock of 2 sec duration. The apparatus was housed in a sound-attenuated room with dim lights. The test was performed on three consecutive days. On Day 1, each rat was placed on the runway and allowed to enter one of the chambers. The door to that chamber was then closed and the rat again placed on the runway and allowed to enter the other chamber. On Day 2, each rat was placed on the runway and allowed to enter one chamber where it received a footshock for 2 sec. On Day 3, the rat was placed on the runway and allowed to enter a chamber. The rat's latency to enter a chamber, and the chamber it chose, were recorded on both Day 2 and 3. Separate groups of rats were injected IP with DCS dissolved in 0.9% saline or saline alone either: 1) 60 min before the footshock, 2) 10 sec after the footshock, or 3) 60 min before the trial on Day 3.

Place Learning in a T-Maze

The T-maze was constructed of opaque Plexiglas, with the stem and each arm 45 cm in length. The entire maze was enclosed by walls 10 cm high. The food reward was pieces of breakfast cereal (Cheerios), weighing about 50 mg each. To prevent the rats from locating the reward by scent, a food cup, consisting of a plastic bottle cap filled with pieces of cereal and covered with wire mesh, was placed in each arm and used to hold the reward. The maze was placed beneath a 75-W lamp in a darkened room.

The rats were gradually food-deprived to 85% of their initial body weight before beginning the test procedure. On three successive days before the start of training, each rat was allowed to explore the T-maze for 10 min with food available in the arms of the maze. On the first test day, each rat was trained to choose the right arm of the maze to obtain a single food reward placed in the food cup (acquisition). The rats were trained to a criterion of nine correct out of ten consecutive choices, with an interval of about 5 min between choices. On the second test day, the rats were trained to choose the left arm of the maze to obtain a food reward (reversal), again to a criterion of nine correct out of ten consecutive trials. On both test days, each rat's latency to choose was recorded. The latency was measured from when the rat's head entered the cross-point of the T to when its hind legs entered an arm.

The rats were injected with either DCS (3 mg/kg IP) dissolved in 0.9% saline (N = 11) or saline alone (n = 11) 30 min before the beginning of training on both test days.

RESULTS

Receptor Binding Assays

Cycloserine was found to displace [^{3}H]glycine binding in a stereoselective manner with the d-isomer (K_i = 2.33 μM) significantly more potent than the l-isomer (K_i > 100 μM) and approximately an order of magnitude less potent than glycine itself (K_i = 0.23 μM) (Fig. 1; Table 1). The functional assessment of DCS indicates that it enhances [^{3}H]TCP binding with an EC_{50} = 18.1 μM (Table 1). The DCS-induced enhancement of [^{3}H]TCP binding can be blocked by kynurenate (200 μM) (data not shown), an NMDA receptor antagonist shown to act predominantly through the associated glycine modulatory site (25). This kynurenate blockade of DCS enhancement of [^{3}H]TCP binding is consistent with DCS being a positive modulator at the glycine recognition site on the NMDA receptor complex. The specificity of DCS for

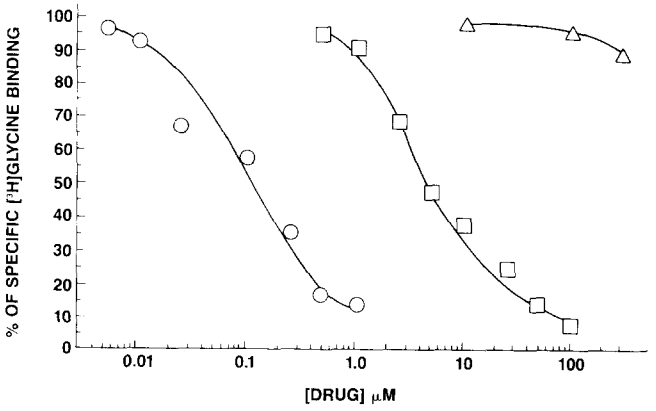

FIG. 1. Displacement analysis of [^{3}H]glycine binding. [^{3}H]Glycine (10 nM) was incubated with SPM (0.2–0.4 mg/ml) and various concentrations of test compounds for 10 minutes at 2°C. [^{3}H]Glycine binding is shown as a percent of maximal binding (measured in the absence of test compound) in the presence of unlabeled glycine (○), DCS (□) or 1-cycloserine (△). Nonspecific binding is defined in the presence of 100 μM glycine. The results are from a single representative experiment (from a total of 3–5 experiments) performed in triplicate.

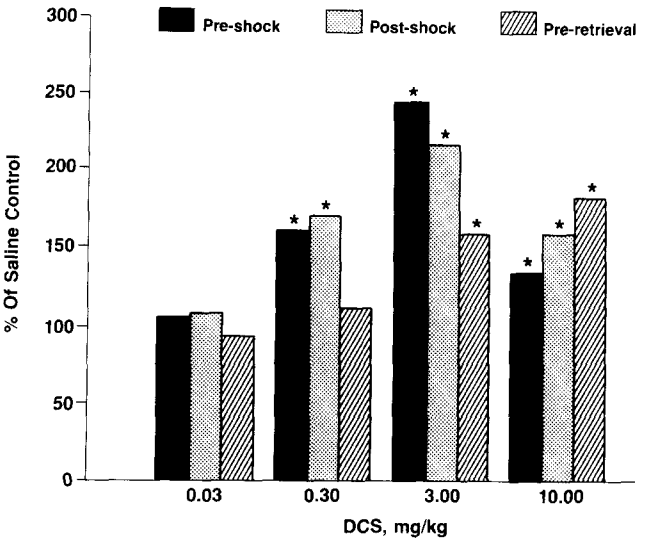

FIG. 2. Latency of rats receiving DCS to enter the apparatus. Rats were administered various concentrations of DCS at different stages of the behavioral test and their latency to enter the apparatus on Day 3 is presented as a percentage of the matched saline-tested group. Latency on Day 3 is taken to indicate how well the rat remembers receiving shock in the apparatus on Day 2. The data were analyzed by one-way analysis of variance [for preshock administration, $F(3,20)=11.29$, $p<0.001$; for postshock administration, $F(3,20)=6.88$, $p<0.001$]. Individual t-tests were then carried out. The control group consisted of 6 rats while the DCS-treated group had 6 rats. *Significantly different from saline-treated group ($p<0.05$, t-test for differences among several means).

this site is further documented by its lack of activity in displacing either l-[^{3}H]glutamate binding to the NMDA recognition site (Table 1), [^{3}H]kainate binding to the kainate subclass of acidic amino acid receptors, [^{3}H]α-amino-3-hydroxy-5-methylisoxazole-4-propionate binding to the quisqualate subclass of acidic amino acid receptors, or [^{3}H]strychnine binding to the inhibitory glycine receptor (data not shown).

Learning Tests

Passive avoidance test. On Day 2, DCS injected before the test had no effect at any dose on the rats' latencies to enter the apparatus, suggesting that DCS did not alter the rats' motivation to enter the apparatus or their locomotor ability. All rats entered the apparatus on Day 2 within 6 sec. Learning in this test is defined as having a longer latency to enter the apparatus on Day 3 than on Day 2. All of the rats but one saline control met this criterion. Latency on Day 3 is taken to indicate how well the rat remembered the shock received on Day 2. The mean latency for all of the saline-treated rats was 15.3 ± 0.9 sec. Rats treated with DCS either before or after the shock on Day 2 had significantly longer latencies on Day 3 than their matched saline controls (Fig. 2), at doses of 0.3 mg/kg or greater. Another strategy rats might use in this test is to avoid, on Day 3, the chamber where shock was received on Day 2. About 50% of each treatment group did so, indicating that choice of chamber was probably random on Day 3 and that DCS did not alter the rats' choice.

Place learning. At the dose which produced the greatest increase in latency in the passive avoidance paradigm, 3.0 mg/kg, DCS did not influence the rate of acquisition of the spatial task, with both DCS- and saline-treated rats showing similar trials to criterion. It is clear that DCS had no deleterious effect on the learning of the initial acquisition since on the first trial of the reversal learning 9 out of 11 of the saline-treated rats and 10 out of 11 of the DCS-treated rats chose the previously rewarded arm of the maze indicating that the majority of both groups remembered the training from the previous day to the same degree. The reversal task was more difficult than the initial acquisition for the saline-treated controls, as indicated by the increased number of trials to reach criterion. DCS significantly increased the rate of learning of the reversal compared to the saline-treated controls (Fig. 3).

TABLE 1

INTERACTION OF DCS WITH THE NMDA RECEPTOR COMPLEX

	[^{3}H]Glycine K_i (μM)	[^{3}H]TCP EC_{50} (μM)	L-[^{3}H]Glutamate K_i (μM)
Glycine	0.23 ± 0.05	1.15 ± 0.2	>100
DCS	2.33 ± 0.29	18.1 ± 2.8	>100
L-Cycloserine	>100	>100	>100

Receptor binding assays were performed using SPM and either [^{3}H]glycine (10 nM, 10 min, 2°C), [^{3}H]TCP (2 nM, 60 min, 25°C) or l-[^{3}H]glutamate (10 nM, 10 min, 2°C, under conditions which specifically label the NMDA recognition site) and various concentrations of the test compounds. K_i values were determined using logit-log analysis while EC_{50} values were determined using a four parameter logistic regression analysis. Results are expressed as the mean ± S.E.M. from at least three separate experiments each performed in triplicate.

DISCUSSION

The biochemical studies show that DCS is a ligand for the NMDA receptor-associated glycine recognition site. That it also enhances [^{3}H]TCP binding indicates agonist activity at the site, and suggests that through this interaction, DCS enhances the opening of the associated ion channel mediated by NMDA receptor agonists.

It was predicted that positive modulation of the NMDA receptor via the glycine recognition site would facilitate processes of learning and memory. Consistent with this hypothesis, DCS

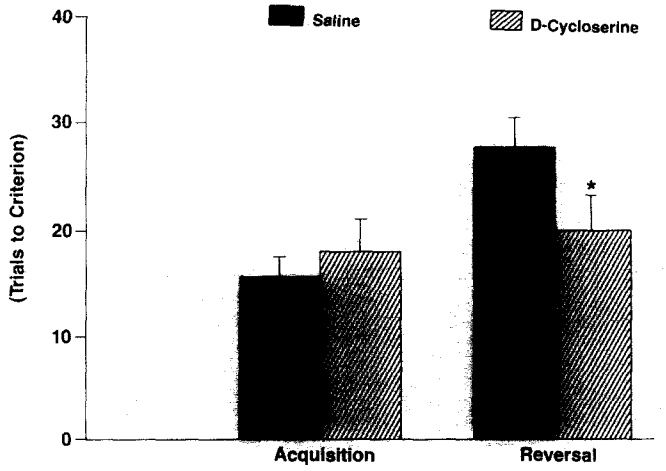

FIG. 3. Rate of place-learning and reversal in a T-maze following administration of DCS (3 mg/kg IP). *Significantly different from saline-treated group ($p<0.05$, t-test).

was found to improve performance of two types of learning tasks. In the passive avoidance task, DCS was effective in increasing the latency to reenter the apparatus where shock was received when it was administered before the shock. This is consistent with the enhancement of the induction of LTP and learning. In addition, DCS was effective in enhancing performance when given after the shock or before the retrieval trial. The fact that it was effective when administered both before and after the shock indicates that DCS did not increase latency by altering the rats' perception of the shock, i.e., making the stimulus more aversive, or by altering their predisposition to move. A common interpretation of such results is that the drug has a positive influence on memory consolidation (14). In addition, DCS increased latency when administered before the retrieval trial. Whether this pattern of results is most consistent with DCS enhancement of both memory consolidation and retrieval, or with enhancement of other aspects of the learning process, such as stimulation, needs to be explored. These results suggest that in addition to the obligatory role the NMDA receptor plays in learning (the induction of LTP), NMDA receptor agonists can modulate additional processes involved in learning.

In the spatial learning task, DCS had no effect on acquisition, but increased the rate of learning of the reversal. These results may be consistent with a site of action in the hippocampus, as hippocampal lesions do not interfere with place learning, but impair learning of reversals (9). While improvement of reversal learning may represent a positive influence on cognitive processes such as spatial mapping or attention, an alternative interpretation of the results would be that DCS removes the interference from prior training by impairing consolidation or longer term storage of information. This latter interpretation is difficult to reconcile, however, with the effects of DSC on passive avoidance learning. In addition, the fact that DCS-treated rats remembered, 24 hours later, the arm that was rewarded during acquisition argues against a deleterious effect of DCS. Clearly, additional behavioral analyses will be required to determine the precise aspects of memory processing influenced by DCS. The data at present, however, indicate a facilitating influence of DCS on performance of two types of learning tasks, distinguished by the type of motivator employed (aversive vs. appetitive) and the type of response required of the rat (passive vs. active).

These data indicate the potential therapeutic effectiveness of compounds acting at the glycine modulatory site. An attractive feature of such compounds is that positive modulators may potentiate the excitatory response to NMDA, but may not alone result in excitation, which could alleviate the possibility of excitotoxic damage as seen with direct NMDA agonists.

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