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Effects of D-Cycloserine and Cycloleucine, Ligands for the NMDA-Associated Strychnine-Insensitive Glycine Site, on Brain-Stimulation Reward and Spontaneous Locomotion

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HERBERG, L. J. AND I. C. ROSE. *Effects of D-cycloserine and cycloleucine, ligands for the NMDA-associated strychnine-insensitive glycine site, on brain-stimulation reward and spontaneous locomotion.* PHARMACOL BIOCHEM BEHAV **36**(4) 735–738, 1990.—D-Cycloserine (DCS) binds with high affinity to the glycine site associated with the NMDA receptor in rat brain. Systemic injections of DCS have been reported to facilitate performance on learning tasks, possibly by promoting long-term changes at the NMDA receptor complex. In the present study, DCS failed to affect spontaneous locomotor activity or variable-interval self-stimulation response rate. Cycloleucine, a competitive antagonist of glycine at the glycine site, produced a brief depression of self-stimulation, but only after relatively large doses which were not antagonised by injection of DCS in the dose reported to be optimal for the facilitation of learning. Improvements in learning and retention reported after administration of DCS are therefore unlikely to be accounted for by nonassociative motivational, or performance, factors.

Cycloleucine D-Cycloserine Glutamate Glycine Learning Locomotor activity NMDA Self-stimulation

ACTIVITY at central NMDA glutamate receptors has been shown to play a critical role in the acquisition and retention of learnt behaviour (3,18). Compounds regulating activity at this receptor might thus offer important benefits in the management of learning or memory impairments. Unfortunately, compounds affecting NMDA transmission commonly have unwanted side-effects: direct agonists, in particular, may induce hyperactivity (4,7), motivational changes (9) and seizures (19), and are strongly neurotoxic (24). Recently, a strychnine-insensitive glycine-recognition site has been identified that positively modulates the NMDA-receptor/ion-channel complex, to which it is allosterically coupled (12). Compounds active at the glycine site would be limited in their effects by the availability of endogenous glutamate (12), and might thus enjoy a wider margin of safety in possible clinical applications than compounds acting directly on the NMDA receptor.

Agonists at the glycine site have recently been reported to produce significantly enhanced performance in a number of learning tasks (8,17). In a passive avoidance study, low doses of D-cycloserine (DCS, 3.0 mg/kg IP), selectively increased latency to enter the shocked arm of a Y-maze (though it failed to affect the choice of arm); the same compound also facilitated the reversal of a food-rewarded side-preference (though it did not facilitate the initial acquisition). Similar results were obtained, in another study (8), with a glycine precursor, 2-N-pentylaminoacetamide. This compound also prevented the disruption of delayed spontaneous alternation by amnesia-inducing doses of scopolamine or diazepam (8).

A remarkable feature of these studies was that glycine agonists appeared to be effective not only in promoting new learning, but also in facilitating retrieval of previous learning, if injected just before testing took place (8,17). This feature, and the somewhat erratic spectrum of positive effects on acquisition, suggest the possibility that nonassociational 'performance' factors may have contributed to the apparent enhancement of acquisition and/or recall. For example, since glycine antagonists are reported to be anxiolytic (27), the corresponding agonists might be expected to be anxiogenic and to enhance passive avoidance in learning tests (8,17) without actually facilitating learning or recall in the strict sense. The possibility of nonspecific arousal was considered by the respective investigators (8,17), who point out that effective doses of the respective compounds did not alter the unshocked rat's readiness to explore the test apparatus; and in any case simple motor hyperactivity could not have accounted for lengthened latencies on passive avoidance tasks (17). In the present study, we have looked for evidence of other, more complex nonassociational changes that might account for the reported effects. Manipulation of the NMDA receptor may have widespread behavioural effects,

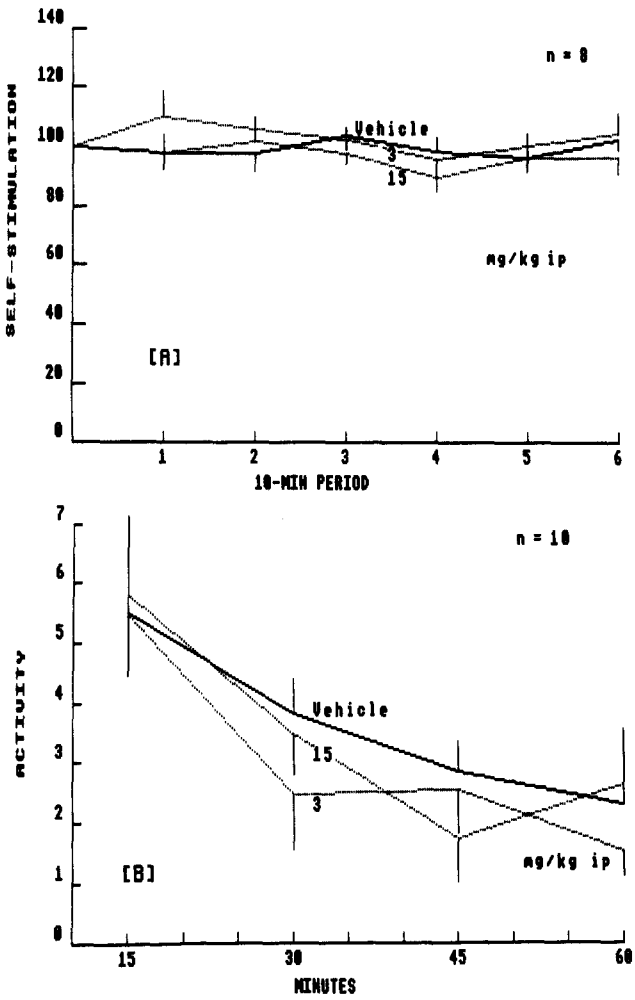

FIG. 1. (A) Time course of variable-interval self-stimulation at threshold current in the 60 min after IP injection of D-cycloserine. Response rates are expressed as percentages of the respective preinjection rates. Vertical bars indicate standard errors. (B) Time course of the effect of DCS on spontaneous locomotor activity, shown as the square root of the number of counts recorded at 15-min intervals in the 60 min after injection.

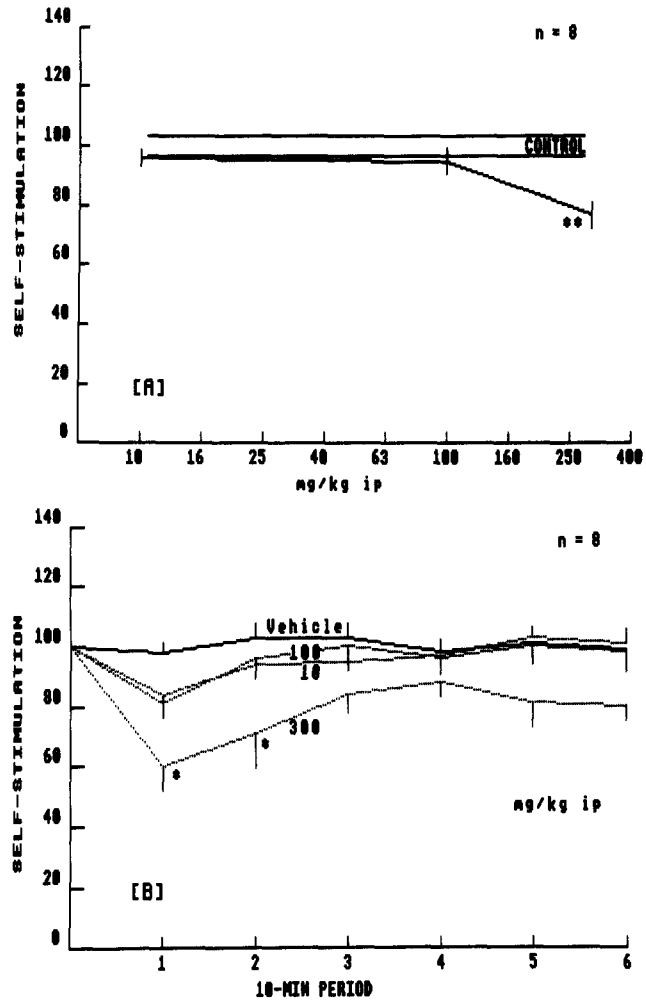

FIG. 2. (A) Dose-response curve for the effect of cycloleucine on self-stimulation. Paired horizontal lines indicate the mean response rate (± SE) during the corresponding period after injection of vehicle. *Significantly different from vehicle ($p<0.05$); **significantly different from vehicle ($p<0.01$). (B) Time course of responding recorded at 10-min intervals after injection of cycloleucine or vehicle. Details as above.

including anticonflict effects (1), enhancement of brain-stimulation reward (10) and spontaneous motor activity (2), while some antagonists have been found to affect behaviour in a manner similar to phencyclidine-like psychotomimetics (15).

Variable-interval response rates for near-threshold currents in hypothalamic self-stimulation have been shown to be sensitive to anxiolytic (11) and anxiogenic drugs (21,22), and to the stimulant effects of phencyclidine-like indirect NMDA antagonists (10). These effects occur in fully trained rats, and are thus unlikely to depend on modulation of learning processes. The present study examined whether doses of DCS reported to facilitate learning, had similar, nonassociative, effects on self-stimulation, or on spontaneous locomotor activity. Self-stimulation tests were also carried out with cycloleucine (cLEU), a compound recently shown to act as a competitive antagonist at the glycine receptor (25).

METHOD

Subjects

Male hooded rats (type PVG, Banting and Kingman Ltd, Hull, England) weighing 250–300 g were anaesthetised with pentobarbitone and implanted with twisted bipolar stainless steel electrodes (Plastic Products Co., Roanoke, VA) aimed at the midlateral hypothalamus, with coordinates A 5.0, ± 1.4, 8.6 (20). Electrode placements were verified on 50-μm unstained frozen sections at the end of the experiment.

Self-Stimulation

On recovery from surgery, the rats were trained to obtain a 0.5-sec 50-Hz sinewave constant-current reinforcing stimulus available at randomly varied intervals of 12 sec mean duration (VI 12 sec). Stimulus intensities were fixed at the lowest value that would just maintain responding, as determined in preliminary trials with intensities decremented in 1-decilog steps. Variable-interval responding at this threshold intensity occurs at approximately half the maximal response rate (23), and is maximally sensitive to small changes in motivational state (23). The relatively slow rate of responding is also well within the rat's motor capacity, and performance is thus relatively unaffected by physical constraints (16).

Locomotor Activity

Locomotor activity was measured in enclosed circular bowls 35 cm in diameter resting on a central pivot and six microswitches spaced about the perimeter. Counts made by movement of the rat from one part of the bowl to another were cumulated and recorded automatically at 5-min intervals for 1 hr after injection of drug or vehicle. Grooming or postural movements without locomotion had no appreciable effect on the count.

Drugs

Fresh solutions of DCS (D-cyloserine, Sigma, Poole, England) or cLEU (cycloleucine, Sigma) were prepared daily in sterile isotonic saline, titrated to approximately pH 7.6 by addition of 0.1 N NaOH. Injections were made intraperitoneally in volumes of 1.0 ml/kg. Control injections contained the corresponding vehicle.

Procedure

Dose-response studies on self-stimulation were carried out on separate groups of rats with DCS (0, 3.0 and 15 mg/kg IP), and cLEU (0, 10, 100 and 300 mg/kg IP), given in random order of dose at intervals of not less than 48 hr, each rat receiving each dose once. At each test, the rat was allowed to self-stimulate for approximately 45 min, the last 30 min before injection providing a predrug baseline. The response rate after each injection was scored as a percentage of the preceding predrug rate, and group scores were submitted to analysis of variance. If significant dose-related effects were obtained, individual doses were compared to vehicle by means of a Wilcoxon matched-pairs test of significance. The interaction between DCS and cLEU was examined by the same procedure in a separate group of rats injected with 3.0 mg/kg DCS (the dose reported optimal for enhancement of learning), or vehicle, followed 5 min later by 300 mg/kg cLEU (the smallest dose producing significant depression of self-stimulation in the present study). Self-stimulation scores after treatment with DCS plus cLEU were compared to scores after cLEU alone.

The effect of DCS on spontaneous locomotor activity was tested in a further group which had been familiarised with the test chambers in seven daily 1-hr sessions. Drug doses were given in counterbalanced order at 48-hr intervals. Vehicle controls were derived from the mean of tests at the beginning and end of the series. Locomotor counts were analysed by a Friedman ANOVA, and normalised, for plotting, by a square-root transform.

RESULTS

D-Cycloserine, in doses that included the dose reported optimal for enhanced learning (3.0 mg/kg) (8,17), had no discernible effect on self-stimulation in the 60 min after injection [Fig. 1A; Friedman $\chi_r^2(2)=0.75$, n.s.]

Locomotor scores, under all treatment conditions, showed a steep within-test decrement [Fig. 1B; $\chi_r^2(3)=15.48$, $p<0.01$], but there was no significant effect of dose, $\chi_r^2(2)=2.45$, n.s.

Cycloleucine produced a significant depression of self-stimulation [Fig. 2A; Friedman $\chi_r^2(3)=11.5$, $p<0.01$], but Fig. 2B shows this result was largely due to a fall in responding in the first 20 min after injection after the highest dose (300 mg/kg). Lower doses had no significant effect.

Depression of self-stimulation by cLEU (300 mg/kg) in the 10-min period after injection was replicated in a further group of eight rats [Wilcoxon $T(8)=1$, $p<0.01$ relative to vehicle; results not shown]. Depression of responding by cLEU was also seen even after pretreatment with DCS (3.0 mg/kg) [Wilcoxon $T(8)=1$, $p<0.01$ relative to vehicle], and response rates after DCS plus cLEU ($69\pm5.8\%$ SEM) were not appreciably higher than after cLEU alone [$65\pm8.0\%$; Wilcoxon $T(8)=20$, n.s.].

DISCUSSION

DCS did not affect self-stimulation or locomotor activity, despite the sensitivity of these measures to NMDA agonists and antagonists (2, 4, 7, 10). This result is not altogether surprising. Endogenous glycine occurs throughout the brain in relatively high concentrations such that the glycine receptor may ordinarily be saturated, and unaffected by any further application (6,13); responses to NMDA measured in vitro are generally not enhanced by exogenous glycine, presumably for this reason (5). Ambient glycine concentrations might be lower in the immediate vicinity of the synapse (26), but precise focal application by an intracranial route would seem necessary to exploit this possibility (6,26).

How, then, does DCS facilitate learning? The mechanism underlying the apparent effect of glycine agonists on learning tasks (8,17) remains unresolved, but the present findings appear to rule out any substantial contribution by motivational, anxiogenic, or 'performance' factors; if systemic injections of DCS could influence these factors, such injections would doubtless have affected self-stimulation and/or spontaneous locomotion.

There are stronger grounds for expecting behavioural effects from *antagonists* of the glycine site. For example, the competitive antagonist, 7-chlorokynurenic acid, inhibits the NMDA-receptor complex in rat cortical slices even in the absence of added glycine (14), indicating a tonic influence by endogenous glycine, susceptible to interruption by antagonists (5, 6, 13). The competitive glycine antagonist, cLEU, might thus be expected to elicit behavioural stimulant effects similar to those elicited by NMDA antagonists (2,25). In the present study, however, cLEU depressed self-stimulation; this outcome is the opposite to that obtained in self-stimulation studies with other indirect NMDA antagonists, e.g., MK-801 (10), or with anxiolytic agents (11). However, the present result should be treated with caution: depression of responding was brief, and was limited to high doses (100 and 300 mg/kg), suggesting the possibility of a systemic or nonspecific mode of action. Furthermore, the failure of DCS to prevent depression of responding by cLEU strengthens the possibility that cLEU acted through some route other than, or in addition to the glycine receptor.

Our failure to demonstrate a clear role for glycine in established self-stimulation does not conflict with its reported facilitation of learning (8,17), since the possibility remains that glycine agonists might facilitate the *acquisition* (''shaping'') of self-stimulation. Other learning paradigms would be more suitable for examination of this alternative.

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