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Effects of D-cycloserine and Aniracetam on Spatial Learning in Rats with Entorhinal Cortex Lesions

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ZAJACZKOWSKI, W., W. DANYSZ. *Effects of D-cycloserine and aniracetam on spatial learning in rats with entorhinal cortex lesions.* PHARMACOL BIOCHEM BEHAV **56**(1) 21–29, 1997.—A great body of behavioural and neurophysiological evidence suggests that excitatory amino acids are involved in mechanisms of learning and memory. Moreover, degeneration of glutamatergic pathways may underlie the cognitive deficits seen in various disorders such as Alzheimer's dementia. As direct stimulation of glutamatergic receptors with agonists may increase the risk of toxicity and accelerate neuropathological changes, a more valid approach seems to be positive modulation of glutamatergic receptors that may reverse the symptoms with a lower risk of excitotoxic effects. Such a possibility offered by partial agonists of the strychnine-insensitive glycine site of the NMDA receptor (Gly-B site) or positive modulators of AMPA receptors, such as aniracetam. In the present study, the effects of d-cycloserine and aniracetam were tested in two animal models of cognitive deficits (entorhinal cortex lesion-induced deficits evaluated in the radial maze and scopolamine-induced amnesia evaluated in passive avoidance test). D-cycloserine (6 mg/kg, for 10 days) had no effect on spatial working memory deficit induced by entorhinal cortex lesions. It did, however, reverse scopolamine-induced deficits in the passive avoidance test when given acutely at the same dose. In contrast, aniracetam (50 mg/kg, for 10 days) produced beneficial effects in the radial maze test in rats with entorhinal cortex lesions, but given at the same dose acutely did not influence scopolamine-induced amnesia. The positive effect of d-cycloserine against scopolamine-induced amnesia may be probably related to the cholinergic-glutamatergic interaction in the hippocampus. The negative data obtained with d-cycloserine in the model of entorhinal cortex lesions-induced cognitive deficits could be taken as a hint that it is probably not suitable for the symptomatological therapy of Alzheimer's disease. The mechanism of positive action of aniracetam cannot be explained on the basis of AMPA receptor modulation, as the dose used (50 mg/kg) is well below that required for the effect at AMPA receptors. Other actions such as peripheral effects or modulation of metabotropic receptors seem more likely.

Aniracetam D-cycloserine Glutamate receptors Radial maze Passive avoidance
Entorhinal cortex lesions Scopolamine Rat

AMONG various forms of dementia, the most common is that of the Alzheimer's type. For populations above the age of 85, the number of people suffering from this disease exceeds 40% (17). Alzheimer's disease is characterised by specific histopathological signs of neuronal degeneration that also involve glutamatergic neurones (27,37). Progressive neurodegeneration occurring in Alzheimer's disease leads to deterioration of higher functions of the central nervous system, with characteristic disturbances in cognitive processes (44). Short-term memory loss is a distinct feature of the early stages of this disease (21).

The first theory concerning the neuroanatomical basis of the symptomatological changes occurring in Alzheimer's disease was the cholinergic deficiency hypothesis (12). At present, however, there is an accumulating body of evidence that disturbances of glutamatergic systems may play a key role in both pathologic processes and symptomatological disturbances related to Alzheimer's disease (22). There is also considerable neurophysiological and behavioural data confirming that excitatory amino acids play an important role in cognitive functions, such as learning and memory under physiological conditions (10,11,43).

Glutamatergic receptors are divided in two main classes: ionotropic and metabotropic receptors. The ionotropic class

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of glutamatergic receptors include three major subtypes: NMDA, AMPA, and kainate receptors. The NMDA receptor gates an ion channel permeable for Ca^{2+}, Na^{+}, and K^{+} and is blocked in a voltage-dependent manner by Mg^{2+} (33,39), whereas most of AMPA receptors are normally only permeable for Na^{+} and K^{+}. It has been suggested that tonic low-level activation may even be the cause of at least some kinds of excitotoxicity (8) and, therefore, the direct stimulation of main binding sites of glutamatergic receptors to improve glutamatergic transmission may a priori be excluded. One of the modulatory sites of the NMDA receptor is the strychnine-insensitive glycine site (Gly-B site) (32), where glycine is a co-agonist (32,34). Modulation of NMDA receptors at this site, could theoretically allow the preservation of the physiological pattern of stimulation by phasic release of transmitter glutamate with only slight or no changes in the basal level of tonic activation. Modulation of this type could be obtained by full agonists such as glycine, or partial agonists such as d-cycloserine or ACPC (25). Indeed, positive effects of d-cycloserine on cognitive functions have been shown both in animals (41) and in humans (67), but in the acute models only.

Positive modulation of AMPA receptors has been demonstrated for several classes of drugs such as cyclothiazide, aniracetam, and AMPAkines (2,30,69), which all inhibit desensitisation of AMPA receptors (29,69). Beneficial effects of aniracetam on cognitive functions have been shown in rats tested in the radial maze (38) and some improvement of psychobehavioural parameters has also been observed in Alzheimer's patients (47,63). Thus, positive modulation of both NMDA and AMPA receptors may be considered as a potential therapeutic approach for the symptomatological treatment of Alzheimer's disease.

At early stages of the disease, the neuropathological changes are restricted to structures of the limbic cortical areas, such as the entorhinal cortex (4). During progression of the disease, neurodegeneration spreads to other areas such as the hippocampus + parietal cortex and influences mainly neurones bearing glutamate receptors. In fact, in Alzheimer's disease some functional changes of the Gly-B site have been observed (7,55). This provides further evidence that modulation of this site might have a positive symptomatological effects in Alzheimer's disease.

The aim of present study was to test the potential procognitive properties of positive modulators of the glutamatergic receptors, d-cycloserine, and aniracetam, in two animal models of memory deficits: entorhinal cortex lesion-induced memory deficits tested in the radial maze and, for comparison, scopolamine-induced long-term memory deficits evaluated in the passive avoidance test. The doses were selected on the basis of other behavioural data showing positive effects of both drugs on cognitive functions. For d-cycloserine the dose selected was 6 mg/kg, as at similar range of doses d-cycloserine has been shown to reverse both scopolamine-induced amnesia (19,64) and the negative effects of hippocampal (62) or entorhinal cortex lesions (48). For aniracetam we selected the dose of 50 mg/kg, as similar range of doses has been previously shown to produce positive effects against scopolamine- or ECS-induced deficits (9), as well as hypoxia-induced deficit in the passive avoidance test (9,13). Moreover, d-cycloserine was active in the passive avoidance test but not in the radial maze test, while the oposite was true for aniracetam. Thus, for both drugs, the doses used seem to be behaviourally active.

METHODS

Animals

Male Sprague–Dawley rats, weighing 250–280 g (radial maze test) or 200–220 g (passive avoidance test) at the beginning of experiments were housed 3–5 per cage, under a 12-h light-dark cycle (light on at 6 a.m.), and controlled temperature (21°C). In experiments with the passive avoidance test both water and food were available ad lib. while during experiments with the radial maze test, food was restricted to 15 g/day and given once daily, directly after testing. Experiments were performed between 12.00 and 18.00.

Surgery

Rats were anaesthetised with Nembutal (60 mg/kg, ip) and placed into a stereotaxic apparatus (Stoelting) with the incisor bar 3.1 mm below the interaural line. Two small holes were drilled on each side of the skull with the following coordinates: AP: -7.8 and -6.8 mm; L: -3.5 mm measured from the bregma. A microsyringe was then rotated 10° outwards in the coronal plane. The needle was inserted 5 mm (measured from the dura) at AP = -7.8, and 5.5 and 7.0 mm from the dura at the AP = -6.8. The coordinates for the injection sites were selected according to the stereotaxic atlas (51). At each of three points (bilaterally), 60 nmol of quinolinic acid (dissolved in saline with addiction of NaOH and adjusted to pH 7.5) in a volume of 0.5 μl was administered over 30 s. The control rats were injected with 0.5 μl saline. After completion of each injection the cannula was left in place for 30 s.

Histology

One week after the end of each experiment the rats were killed and the brains were removed and kept for 36 h in a 10% formalin solution. Afterwards the brains were washed for 4 h in water and transferred to a 30% saccharose solution for 2 days. The brains were then cut on a freezing microtome -21°C into 20 μm slices. The slices were stained with Cresyl violet (5).

Behavioural Procedures

Radial maze. The effects of drugs on entorhinal cortex lesions-induced deficit were tested in the radial maze according to the paradigm described by Olton et al. (49) with some modifications introduced. The radial maze consisted of eight arms with walls and a central region (52 cm in diameter). The arms (68 cm long, 35 cm high, 17 cm wide) were made of wood covered with dark brown painted plastic. A small cup with opaque walls was placed at the end of each arm. Noyes precision food pellets (45 mg) were used as a reinforcement. A plastic tube measuring 32 cm in diameter and 35 cm height was placed in the centre of the maze. The experiments were performed in a lighted room with several external cues in a fixed configuration and a video camera for observation of the rats' behaviour.

One week before surgery, the animals were handled (2 days) and allowed to adapt to the apparatus (3 days). On the third day, the rats were placed for 5 min in groups of 4–5 into the maze and food pellets were scattered randomly throughout the maze (4–5 pellets per rat). On the fourth day the food cups were placed at the end of each arm. The four randomly selected arms were baited with one pellet of food each. Rats were placed individually into the centre of the maze and re-

mained inside the maze until they had collected all food pellets but not for longer than 5 min. Rats were given 3–5 trials, depending on their performance (collection of all pellets within 5 min). On the fifth day, the same procedure was repeated, except that the rats were placed in the centre of the maze in the plastic tube which was then removed after 15 s. In the experiment with d-cycloserine, two additional days of pretraining were given (day 6 and 7). However, since in this experiment no lesion-induced deficit in reference memory was seen, in the next experiment (with aniracetam) this additional pretraining was omitted.

Surgery was performed 4–6 days after adaptation as described above. Training began 10–12 days after surgery. Rats were placed into the plastic tube that was removed after 15 s. In each trial four of the arms were baited with one pellet of food. The position of the baited arms was left unchanged during the whole experiment. Each rat was given four trials daily for 10 consecutive days. The rats were observed with the video camera and each entry with all four paws into each arm was scored. The rats remained inside the maze until they had collected all pellets of food or 10 min had elapsed, whichever came first. Two types of errors were scored: working memory errors (i.e., entry into an arm already visited during the same trial) and reference memory errors (i.e., each entry into an arm that was never baited). Additionally, the time spent by the rat in the maze during each trial was measured for evaluation of general locomotor performance.

Passive Avoidance

The two-choice dark avoidance system (40) consisted of three identical-size compartments (25W $\times$ 22D $\times$ 20H) connected so that a vertically sliding door (6H $\times$ 9W cm) controlled access from the start compartment to both choice compartments (dark and light). Each compartment was equipped with a light source and a grid floor connected to a shock generator (Geltz Labortechnik, Niefern-Oeschelbronn, Germany).

The experiments were performed during 4 days. On day 1 each animal received 5 min of handling. On day 2, rats were handled again for 5 min and then habituated to the apparatus (5 min) by placing them in the start box. During habituation, only one (light) choice compartment was available. To avoid directional preference, the left compartment was lit (and therefore available) for 50% of each group, and the right compartment for the other 50%.

For this experiment, a single-trial acquisition procedure was used. According to this procedure, on day 3 (training), after injection of tested agents, the rat was placed into the starting compartment with access to both choice compartments. The time to leave the starting compartment and to enter the dark compartment was recorded. As the rat entered the dark compartment, the sliding door was closed and two electroshocks (1 mA, 1 s, interval 3–4 s) were delivered. After a 15 s delay the rat was returned to the home cage. On day 4, retention was tested using a cut-off time of 300 s. For statistical analysis the same parameters were used as during the training.

Drugs and Treatment

D-cycloserine (Sigma, St. Louis, MO, USA) and scopolamine hydrobromide (RBI, Boston, MA, USA) were dissolved in 0.9% sodium chloride solution and given intraperitoneally in a volume of 1 ml/kg b.w., while aniracetam (Hoffman-LaRoche, Basel, Switzerland) was suspended in 0.5% carboxymethylcellulose and given orally in a volume of 2 ml/kg. In the radial maze test, d-cycloserine (6 mg/kg) was given once daily for 10 days, 30 min before the trial. In the passive avoidance test, d-cycloserine was given at the same dose, 35 min before the training. Aniracetam (50 mg/kg) was given either repeatedly for 10 days, 60 min before the radial maze test or acutely, 60 min before training in the passive avoidance test. Scopolamine (0.5 mg/kg) was given 30 min before training in the passive avoidance test.

Data Analysis

In the radial maze test, values for the following parameters were analysed: frequency of working memory errors, and frequency of reference memory errors. The frequency was calculated as the total number of working memory errors or reference memory errors made over 2 days (2 $\times$ 4 trials) divided by the total number of entries into the arms. The average exploration time was calculated as the time elapsing from the beginning until the end of the trial divided by the total number of arms visited. This average exploration time of one arm was used as a measure of general locomotor performance.

All parameters were calculated as 2-day blocks and are presented as means $\pm$ SEM. The data were analysed by two-way analysis of variance (ANOVA; treatment and blocks as factors) followed, if significant, by the Newman–Keuls test for pairwise comparisons (SigmaStat software, Jandel Scientific, Erkath, Germany). The number of rats in each group was eight.

For the passive avoidance test, nonparametric ANOVA (Kruskal–Wallis test) was used which, if significant, was followed by Dunn's test for pairwise comparisons. Results are expressed as medians with percentiles (10th, 25th, 75th, and 90th) as a measure of variation. The number of rats in each group was eight. In all calculations a difference at $p < 0.05$ was regarded as significant.

RESULTS

Histological Evaluation

Examination of the horizontal sections around the area of the quinolinic acid lesions revealed that lesions of all the rats used were well contained within the entorhinal cortices (Fig. 1). In several cases, the subiculum and dentate gyrus were also partially affected, however these animals were not excluded from the analysis, because a similar distribution of changes was seen in all groups.

Effects of D-cycloserine and Aniracetam in Entorhinal Cortex Lesioned Rats

In both experiments, four groups were tested: sham-operated vehicle treated rats, sham-operated rats given a drug, entorhinal cortex lesioned rats given vehicle, and lesioned rats treated with either d-cycloserine or aniracetam. Because preliminary analysis did not show any difference between both sham-operated groups, regardless if they were given vehicle or drug, we have included to the further statistical analysis the sham-operated vehicle treated groups only. As revealed by two-way ANOVA (treatment and 2-day blocks as factors), in the experiment with d-cycloserine (Fig. 2) there was a significant main effect of treatment ($F(2,119) = 8.69, p < 0.0003$),

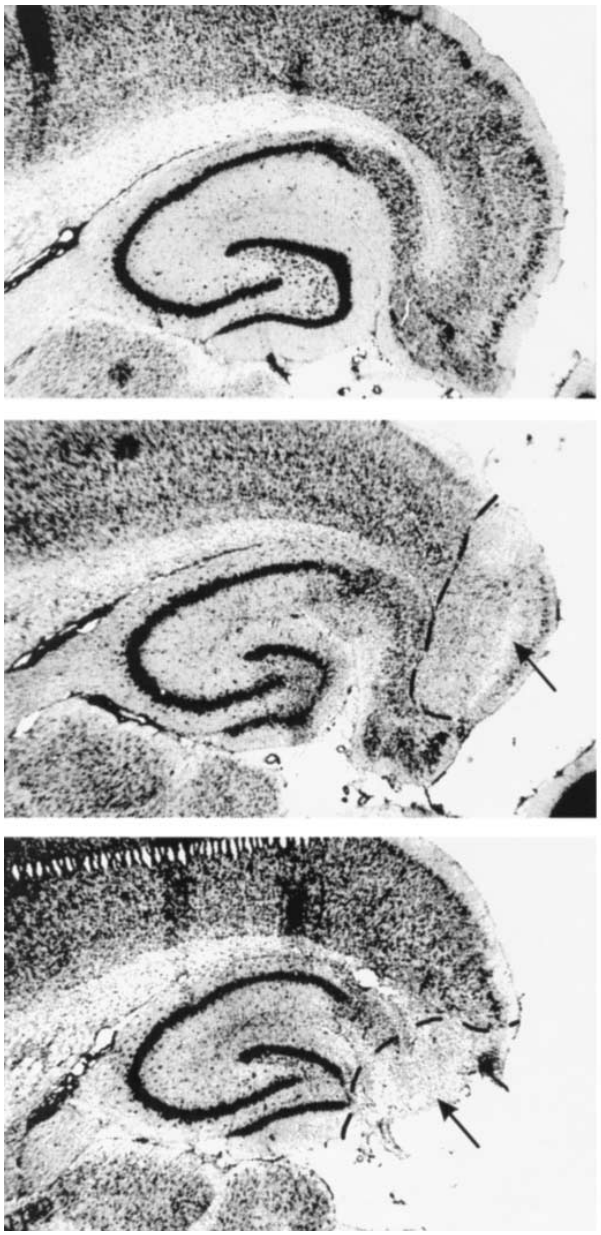

FIG. 1. Average entorhinal cortex lesions shown in horizontal sections. (A) control animal; (B) and (C) typical extent of entorhinal cortex lesion.

but neither block ($F(4,119) = 1.49$, $p > 0.2$) nor treatment × block interaction ($F(8,119) = 0.45$), $p > 0.88$) on the frequency of working memory errors. Posthoc multiple pairwise comparisons revealed that the rats with entorhinal cortex lesions had a significantly higher frequency of working memory errors than control rats ($p < 0.05$; Fig. 2A), but repeated injections of d-cycloserine for 10 days did not reverse the deficit in entorhinal cortex lesioned rats. For the second parameter measured —reference memory errors— two-way ANOVA revealed only a significant main effect of block ($F(4,119) = 18.31$, $p < 0.0001$), but neither treatment ($F(2,119) = 0.6$, $p > 0.55$) nor treatment × block interaction ($F(8,119) = 0.86$), $p > 0.55$) (Fig. 2B).

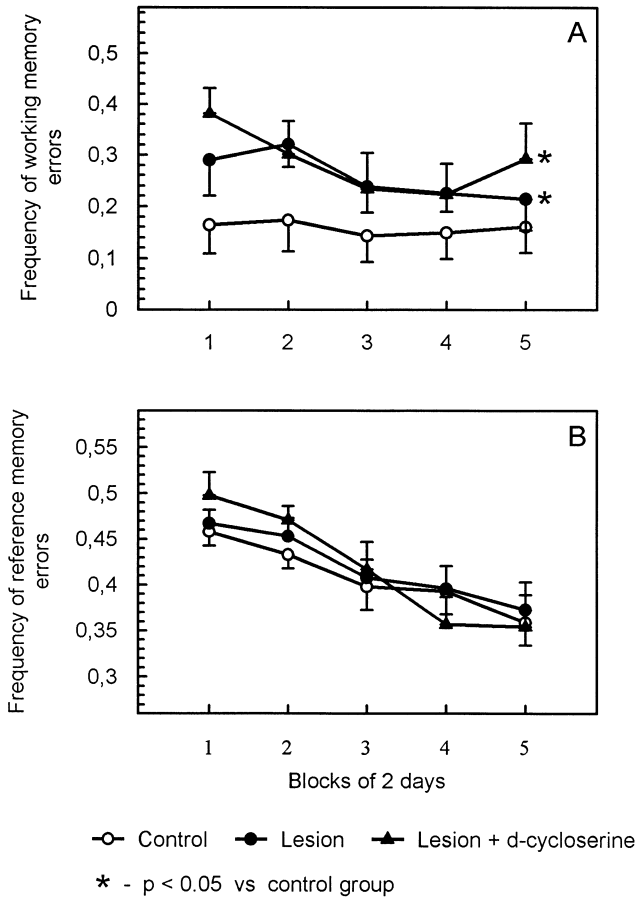

FIG. 2. The effects of chronic administration of d-cycloserine (6 mg/kg, for 10 days) on learning deficits in entorhinal cortex-lesioned rats (lesion) in the radial maze. The effects on the frequency of working memory errors (A) and on the frequency of reference memory errors (B) are shown. Each point represents the mean ± SEM in 2-day blocks of training (2 × 4 trials). *$p < 0.05$ significant difference as compared to sham-operated group for the whole learning period (Newman–Keul's test). $N = 8$.

Two-way ANOVA (treatment and 2-day blocks as factors) performed for the experiment with aniracetam, showed a significant main effect of treatment ($F(2,119) = 31.44$, $p < 0.0001$) and block ($F(4,119) = 3.57$, $p > 0.009$), but no treatment × block interaction ($F(8,119) = 0.35$), $p > 0.94$) on frequency of working memory errors. Posthoc multiple pairwise comparisons revealed a significant increase in the frequency of working memory errors in the group with entorhinal cortex lesions ($p < 0.05$, Fig. 3A). The entorhinal cortex lesioned rats injected with aniracetam showed a lower frequency of working memory errors than lesioned untreated rats ($p < 0.05$, Fig. 3A) but higher frequency than control rats ($p < 0.05$, Fig. 3A). Similar effects were also observed in the frequency of reference memory errors. A two-way ANOVA showed significant effects of treatment ($F(2,119) = 10.27$, $p < 0.0001$) and block ($F(4,119) = 8.94$, $p < 0.0001$), but not treatment × block interaction ($F(8,119) = 1.22$, $p > 0.29$). The entorhinal cortex lesioned rats injected with aniracetam showed a lower frequency of reference memory errors than lesioned vehicle treated rats ($p < 0.05$, Fig. 3A) but a higher frequency than control rats ($p < 0.05$, Fig. 3A). The average exploration time

TABLE 1

EFFECT OF REPEATED ADMINISTRATION OF D-CYCLOSERINE (6 MG/KG) OR ANIRACETAM (50 MG/KG) ON AVERAGE EXPLORATION TIME IN RADIAL MAZE IN SHAM-OPERATED AND ENTORHINAL CORTEX LESIONED RATS

Treatment	Exploration time (s/arm)
Sham/Vehicle	4.5 ± 0.3
Sham/d-cycloserine	4.7 ± 0.2
Lesion/Vehicle	4.3 ± 0.1
Lesion/d-cycloserine	5.0 ± 0.3
Sham/Vehicle	6.0 ± 0.7
Sham/Aniracetam	6.2 ± 0.3
Lesion/Vehicle	5.8 ± 0.9
Lesion/Aniracetam	5.1 ± 0.5

Results were averaged for the whole training period (10 days) and are presented as means ± S.E.M. One-way ANOVA showed no significant effect. N = 8.

(average time spent by each rat for exploration of an arm) was not influenced by any treatment (Table 1). Under applied schedule of food deprivation the body weight loss of animals was about 5%.

Effects of D-cycloserine and Aniracetam on Scopolamine-induced Amnesia in the Passive Avoidance Test

During training, neither of the parameters measured was affected by the drugs tested (Fig. 4), as revealed by Kruskal–Wallis ANOVA. During the retention test, scopolamine given alone did not influence the latency to leave the start compartment, but decreased the latency to enter the dark compartment ($p < 0.05$, Dunn's test). This amnesic effect of scopolamine was reversed by d-cycloserine ($p < 0.05$, Dunn's test), but not by aniracetam (Fig. 4). The latency to leave start box was significantly lower in rats injected with both scopolamine and aniracetam, as compared to the control group ($p < 0.05$, Dunn's test). The results of preliminary experiments where all drugs were tested in the continuous multiple-trial dark avoidance paradigm (see ref. 40, for method description), suggest that none of the drugs influenced the pain sensitivity of rats at the range of doses used (not shown).

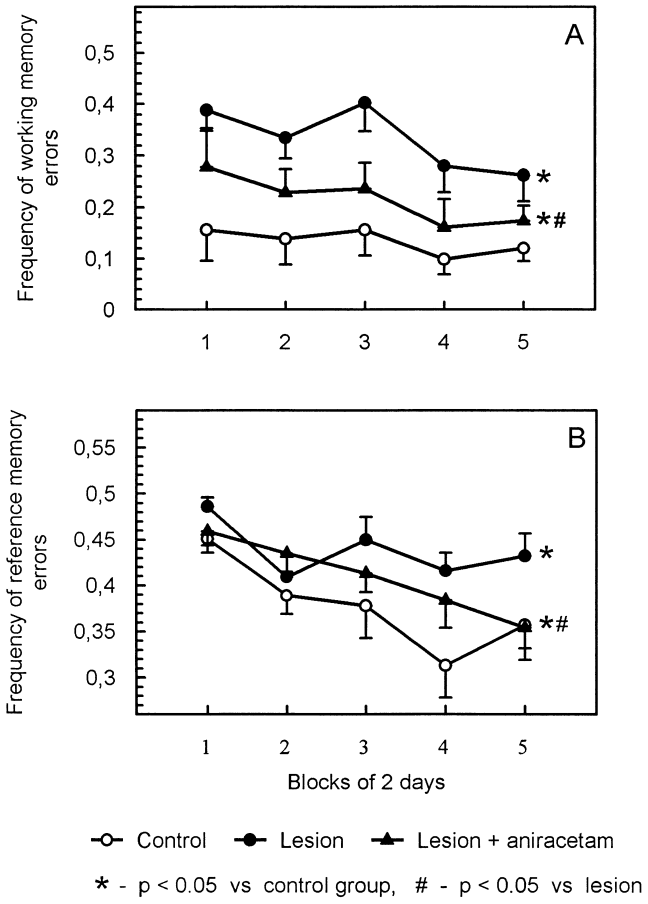

FIG. 3. The effects of chronic administration of aniracetam (50 mg/kg, for 10 days) on learning deficit in entorhinal cortex-lesioned rats (lesion) in the radial maze. The effects on the frequency of working memory errors (A) and on the frequency of reference memory errors (B) are shown. Each point represents the mean ± SEM in 2-day blocks of training (2 × 4 trials). *$p < 0.05$ significant difference as compared to sham-operated group for the whole learning period (Newman–Keul's test). #$p < 0.05$ significant difference as compared to entorhinal cortex-lesioned rats for the whole learning period (Newman–Keul's test). $N = 8$.

DISCUSSION

Lesions of discrete brain areas by injection of neurotoxins are commonly used to study the physiological role of different brain structures and as models of neuropathological changes occurring in neurodegenerative diseases of the central nervous system (14). Lesions of structures belonging to the hippocampal formation are regarded as suitable models of the pathology and symptomatology of Alzheimer's disease (46).

In several earlier studies, it has been shown that entorhinal cortex lesions produce both working and reference spatial memory deficits in rats (24,26). The lesions performed in the present study affected not only the entorhinal cortex, but also partially the para-, presubiculum, and subiculum. The extent of these lesions is similar to those obtained by Hunt et al. (26) and Johnson and Kesner (31), however slightly bigger than shown by Hölscher and Schmidt (24). In the experiment with d-cycloserine a negative effect of lesions was observed on the frequency of working memory errors but not of reference memory errors. In the experiment with aniracetam and in other experiments described in a previous article (70), we observed reference memory deficits as well. The lack of the effect of lesions on reference memory in the experiment with d-cycloserine may have been partially due to a slightly different experimental schedule, i.e., excessive pretraining. The observed negative effects of lesions on working memory-dependent learning may be either due to a decrease of the capacity of systems involved in working memory or it may result from the use of a false strategy by animals. For instance, according to Morris et al. (45), the entorhinal cortex may be involved in the selection of the appropriate spatial strategy which is then processed by the hippocampus.

Positive modulation of the Gly-B site has been proposed to be a possible therapeutic approach for the symptomatological

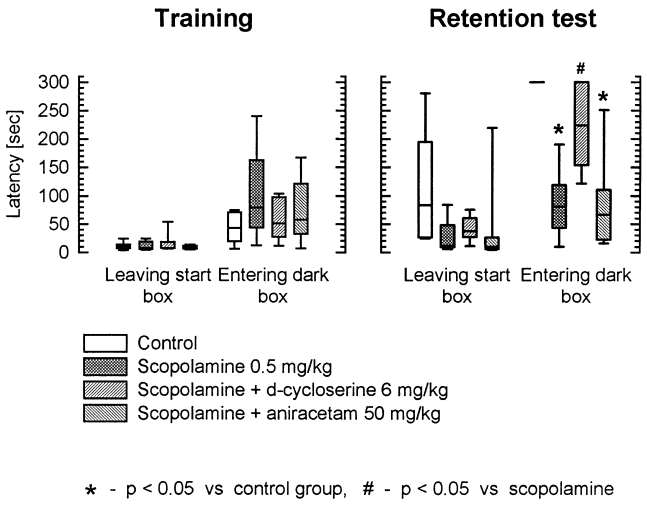

FIG. 4. The effects of d-cycloserine and aniracetam on scopolamine-induced amnesia in the dark avoidance test. Left and right parts show respectively the results of training (acquisition) and retention tests (performed 24 h later). Drugs were administered 30 (scopolamine), 35 (d-cycloserine), and 60 min (aniracetam) before the training. Results are expressed as medians and percentiles (10, 25, 75, and 90). $p < 0.05$ as compared to the control group, $p < 0.05$ as compared to scopolamine-injected group (Dunn's test). $N = 8$.

treatment of Alzheimer's disease (3). This suggestion was based on the assumption that stimulation of the Gly-B site might have positive effects on cognitive functions, especially under conditions of deficit in glutamatergic system function resulting, for example, from decreased levels of endogenous agonists. In the first model, d-cycloserine failed to influence cognitive functions both in naive rats and in the rats with entorhinal cortex lesions. In this experiment, deficits were only observed in working memory but not in reference memory. Therefore, it is not possible to exclude definitely a positive effect of d-cycloserine on reference memory components in this test.

Previously, several studies addressed the role of the Gly-B site in modulation of cognitive functions. In these experiments, agents such as glycine (47), milacemide (glycine prodrug) (23), d-cycloserine (partial agonist) (20,41), and Gly-B site antagonists (10,18) were studied. It was concluded that stimulation of the Gly-B site may improve cognitive functions both in naive animals (20,23,41) and under conditions of memory deficits, induced by scopolamine injection (19,64), hippocampal lesions (62), or entorhinal cortex lesions (46,47). However, either studies in primates (60) or clinical studies failed to confirm these positive effects, for either milacemide (6,15) or d-cycloserine (57). In the above mentioned studies, d-cycloserine was used at low doses (1–30 mg/kg). At the high doses (80–320 mg/kg) or at brain concentrations of around 1 μM (59), d-cycloserine usually shows anticonvulsive activity (36,52,68). This is consistent with in vitro data indicative that d-cycloserine interacts with the Gly-B site with an EC_{50} of 3 μM (25). Although d-cycloserine easily crosses the blood–brain-barrier, after systemic injection at a dose of 5 mg/kg only trace amounts have been shown in brain (68). However, d-cycloserine given systemically to mice in a range of doses 1.25–50 mg/kg shows distinct biphasic effect on the accumulation of cGMP in the cerebellum (16) with stimulatory effect at lower doses (1.25–10 mg/kg) and inhibitory effect at higher doses (50 mg/kg). Within a similar range of doses (3 and 9 mg/kg), d-cycloserine also has stimulatory effect on the excitability of the granular dentate gyrus cells (53). As such, no clear relationship between the in vitro potency of d-cycloserine at NMDA receptors and in vivo brain concentration at doses enhancing learning can be made.

In the entorhinal cortex lesion model, d-cycloserine given repeatedly had no effect at a dose influencing cognitive function in several other studies (41) and in the presented article in the passive avoidance test. Hence, the dose used in the present study was high enough to reverse scopolamine-induced amnesia, but has been too low to obtain positive effects under conditions of both functional and anatomical deficits where higher level of stimulation might be necessary. However, other possible reason for the lack of effect of d-cycloserine in the entorhinal cortex lesion model could be adaptive receptor changes, resulting from chronic repeated treatment and leading to the development of tolerance. For instance, positive cognitive effects of d-cycloserine in mice have been shown after acute injection but this effect was no longer seen if d-cycloserine was given repeatedly (3 mg/kg), for 15 days before acute injection (56). Also Wlaz et al. (68) have shown that the anticonvulsive effects of d-cycloserine decrease during repeated injections at a dose of 80 mg/kg for 8 days.

Aniracetam is a nootropic drug having beneficial effects on the function of the central nervous system via multiple mechanisms of action. Positive cognitive effects of aniracetam has also been shown in clinical studies in Alzheimer's patients (35,50,63). In the present study, aniracetam reversed spatial working and reference memory deficits induced by entorhinal cortex lesions but had no effect in naive animals and no effect on scopolamine-induced amnesia. An enhancement of cholinergic transmission has been suggested for a long time as the main mechanism of action of aniracetam (65). A number of studies, have shown positive effects of aniracetam on cognitive functions both in naive animals (38) and under conditions of cognitive deficits induced by impairment of the cholinergic system function, electroshock application or ischaemia (9,13, 65). Also enhancement of long-term potentiation (LTP) has been shown, at low concentrations (0.01 and 0.1 μM), but not higher (61). These results are consistent with behavioural data showing bell-shaped dose–response curves for the effects of aniracetam on learning (65). In later studies, an additional effect of aniracetam has been demonstrated, namely inhibition of AMPA receptor desensitisation at very high concentrations (c.a. 2 mM) (30). Moreover, at concentrations as low as 5 μM, aniracetam can potentiate phospholipase C activity stimulated by quisqualate (54) and enhances quisqualate- and tACPD-mediated neuroprotective effects. Therefore, the changes in phosphoinositide turnover which seem to play an important role in both LTP and learning (1,58) may also underlie the neuroprotective effects of aniracetam and are seen at c.a. 500–1000 times lower concentrations than the inhibition of AMPA receptor desensitisation.

In the present article, aniracetam positively influenced disturbances of cognitive functions observed in the radial maze test in rats with entorhinal cortex lesions when injected repeatedly (50 mg/kg, for 10 days). However, pharmacokinetic data suggest that within this dose range aniracetam only crosses the blood–brain-barrier to a minor extent (28) and after systemic injection only trace amounts are observed in brain. Therefore, if central mechanism of action of aniracetam is assumed, it is more likely related to the activation of metabotropic glutamate receptors since in vitro this effect is seen at 1000 fold lower

concentrations (low μM range) (30) than those necessary for modulation of AMPA receptors (low mM range) (54). Another possibility to explain the cognitive effects of aniracetam arises from the observation that these effects are no longer seen in mice after adrenalectomy (42). A further hypothesis (66) suggests that the positive effects of nootropic drugs could be related to an increase in glucose production in liver and in turn, to an enhancement of glucose availability and ACh synthesis in the brain.

In the present study, for comparison with the first model, scopolamine-induced amnesia evaluated in the passive avoidance test was selected, because this model is commonly used for testing procognitive effects of drugs in humans (67). Scopolamine decreased the latency to enter the dark box without affecting other parameters indicating specific effect on memory at the dose used. The scopolamine-induced amnesia observed in the passive avoidance test was reversed by d-cycloserine but not aniracetam. Positive effects of d-cycloserine may result from interactions between the glutamatergic and cholinergic systems. For example, such an interaction could take place in the hippocampus, which receives dense glutamatergic innervation from entorhinal cortex and cholinergic innervation from septal nuclei.

In contrast to the present study, in several (but not all) earlier studies, an inhibition of scopolamine-induced amnesia by aniracetam has also been shown (13,65). However, in the present study aniracetam given repeatedly at the same dose did reverse the cognitive deficit induced by entorhinal cortex lesions indicating that this dose is sufficient for cognitive effects.

In conclusion, entorhinal cortex lesions seem to be more useful for the evaluation of procognitive effects of potential drugs for the treatment of dementia of the Alzheimer's type, in contrast to scopolamine-induced amnesia evaluated in passive avoidance test. The results obtained with d-cycloserine suggest that d-cycloserine may be not suitable the for therapy of Alzheimer's disease where of neuronal loss is observed and chronic treatment is necessary. The positive effect of acutely given d-cycloserine on scopolamine-induced amnesia in the passive avoidance test may be limited to this model, which however lacks construct validity. Positive effects of aniracetam on entorhinal cortex lesions induced deficits are probably not related to the known effects of high concentrations on AMPA receptor function. The results obtained are consistent with clinical data and confirm that entorhinal cortex lesions induced cognitive deficits under conditions of chronic drug application may be a valid symptomatological model of Alzheimer's disease.

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