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Infusion of (+)-MK-801 and memantine – contrasting effects on radial maze learning in rats with entorhinal cortex lesion

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Abstract

(+)-5-Methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate ((+)-MK-801) and 1-amino-3,5-dimethyladamantane (memantine), two uncompetitive antagonists of the NMDA receptor were tested in an allocentric version of the radial maze test (with four out of eight arms reinforced) both in normal rats and after quinolinic acid-induced entorhinal cortex lesions. Both agents were infused s.c. using Alzet osmotic minipumps in order to assure steady state drug levels in the serum and brain during the experiment. In non-lesioned rats, (+)-MK-801 (0.312 mg/kg per day) produced disturbances in learning of spatial information dependent on reference memory but not that involving working memory. In contrast, memantine (20 mg/kg per day) had no effect in normal rats. In rats with entorhinal cortex lesions, (+)-MK-801 enhanced the lesion-induced deficit in reference memory. In contrast, memantine reversed the lesion-induced increase in reference memory errors. The divergent effects of those two uncompetitive NMDA receptor antagonists could, at least partially, be due to the differences reported in their channel blocking kinetics and voltage dependence. The results indicate that under conditions of pathological impairment of brain structures such as entorhinal cortex lesion, memantine might produce beneficial effects on cognitive functions.

Keywords: Radial maze learning; Entorhinal cortex lesion; NMDA receptor antagonist; Chronic infusion; Memantine; (+)-MK-801; (Rat)

1. Introduction

Glutamate is the main fast excitatory neurotransmitter in the central nervous system and mediates, besides normal neural transmission, learning and memory processes and also developmental plasticity (Collingridge and Singer, 1990; Watkins and Evans, 1981; Wroblewski and Danysz, 1989). On the other hand, it also most probably plays a role in pathological processes such as epileptogenesis and acute or chronic neuropathology (Greenamyre, 1986; Meldrum and Garthwaite, 1990). The ionotropic glutamate receptors are divided into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate subfamilies (McBain and Mayer, 1994). The NMDA subtype of glutamate receptors is coupled to ion channel permeable for the physiological cations Ca^{2+}, Na^{+} and K^{+} (McBain and Mayer, 1994). NMDA receptor blockade can be achieved by e.g. competitive antagonism with respect to glutamate, at the NMDA binding site e.g. APV (2-amino-5-phosphonovalerate) or uncompetitive blockade of the NMDA receptor ion channel by PCP (phencyclidine), (+)-5-methyl-10,11-dihydro-5*H*-dibenzocyclohepten-5,10-imine maleate ((+)-MK-801) or 1-amino-3,5-dimethyladamantane (memantine) (Kornhuber et al., 1989: Parsons et al., 1993; Watkins and Evans, 1981; Wong et al., 1986; Wroblewski and Danysz, 1989). The NMDA receptor has attracted considerable interest due to its suggested involvement in the pathophysiology of neurodegenerative diseases such as Huntington's, Parkinson's and Alzheimer's (Greenamyre, 1986; Meldrum and Garthwaite, 1990). According to the hypothesis of Greenamyre et al. (1988), in Alzheimer's disease, over-activation of glutamate receptors results in a progressive neurotoxicity i.e. degeneration of neurones bearing glutamate receptors, including glutamatergic

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neurones. This in turn might lead to secondary hypofunction of the glutamatergic system (Francis et al., 1993; Greenamyre et al., 1988) and symptomatological effects such as cognitive impairment. Therefore, it has been suggested that the use of glutamatergic receptor antagonists, in particular NMDA receptor antagonists, in early stages of this disorder might prevent excitotoxicity and slow the progression of these neurodegenerative processes (Lawlor and Davis, 1992).

The neuroprotective potential of both competitive and uncompetitve NMDA receptor antagonists has been confirmed in numerous studies (Meldrum, 1992; Meldrum and Garthwaite, 1990). Unfortunately, but not unexpectedly, it has been shown that inhibition of the NMDA receptors usually impairs learning and memory processes (Danysz et al., 1988, 1995; Morris et al., 1986). Thus, when NMDA receptor antagonists are considered as potential neuroprotective therapy of dementia, their likely negative effects on cognitive functions should be taken into account. However, memantine, an NMDA receptor channel blocker (Chen et al., 1992; Kornhuber et al., 1989; Parsons et al., 1993) has been used for several years for the treatment of dementia and positive effects on symptoms have been reported (Ditzler, 1991; Görtelmeyer and Erbler, 1992). Therefore, there is an apparent contradiction between findings in preclinical studies and clinical observations.

Hence, the rationale for the present study was to test whether memantine infused chronically to insure steady state serum levels within the therapeutic range, produces negative effects on cognitive processes in rats. Memantine and (+)-MK-801, a more potent NMDA channel blocker, were compared in both normal and entorhinal cortex-lesioned rats since this brain region is known to be affected early in Alzheimer's disease (Braak and Braak, 1991). In previous studies, such entorhinal cortex lesions have been shown to produce learning impairment in tasks involving spatial memory (Hölscher and Schmidt, 1994). Therefore, allocentric orientation in the radial maze with modifications allowing the evaluation of learning dependent on both working and reference memory was used.

2. Materials and methods

2.1. Subjects

Male Sprague-Dawley rats, weighing 250–280 g at the beginning of the experiment were housed three to five per cage, under a 12-h light-dark cycle (light on at 6 a.m.), and controlled temperature (21°C). Water was available ad libitum but food was restricted to 15 g per day. The rats received food once daily, directly after testing. Experiments were performed between 2:00 and 6:00 p.m.

2.2. Surgery

Rats were anaesthetised with Nembutal (60 mg/kg, i.p.) 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 dura) at AP = −7.8, and 5.5 and 7.0 mm from the dura at the AP = −6.8. At each point, 60 nmol of quinolinic acid (dissolved in saline 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.

Alzet osmotic minipumps for delivering drugs s.c., were implanted on the back of the animals, under Hypnorm anaesthesia (0.04 ml/100 g i.m.), 7–13 days after lesioning (1 or 4 days before the training). Animals not receiving drugs underwent the complete surgical procedure, but pumps were not actually implanted.

2.3. Histology

1 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 (Burck, 1982).

2.4. Apparatus

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 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.

2.5. Procedure

1 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 four to five into the maze and food pellets were scattered randomly throughout the maze (four to five 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 center of the maze and remained inside the maze until they had collected all food pellets but not for longer than 5 min. Rats were given three to five 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 center of the maze in the plastic tube which was then removed after 15 s.

Surgery was performed 4–6 days after adaptation as described above. Training began 9–13 days after surgery. Rats were placed into the 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 12 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. Rats requiring more than 10 min to finish the trial were excluded from further experiment. 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.

2.6. Data analysis

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 (two × four trials) divided by total number of entries into the arms. The average exploration time (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 ± S.E.M. 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). The number of rats in each group was seven to ten.

2.7. Drugs

(+)-MK-801 hydrogen maleate (RBI, USA) and memantine (Merz + Co., Germany) were dissolved in 0.9% sodium chloride solution. Memantine (20 mg/kg per day) or (+)-MK-801 (0.312 mg/kg per day) were delivered with Alzet osmotic pumps (2ML2 and 2002 type, with an infusion rate of 5.0 and 0.5 μl/h, respectively). The pumps were implanted s.c., 4 days before the start of training (except preliminary study, see Results). Considering the reservoir volume and the pumping rate it can be assumed that the infusion continued throughout the learning experiment (12 days).

3. Results

3.1. Histological evaluation

Examination of the horizontal sections around the area of the quinolinic acid lesion revealed that lesions of all the rats used were well contained within the entorhinal cortices (Fig. 1). In a few cases, the subiculum and dentate gyrus were also partially affected.

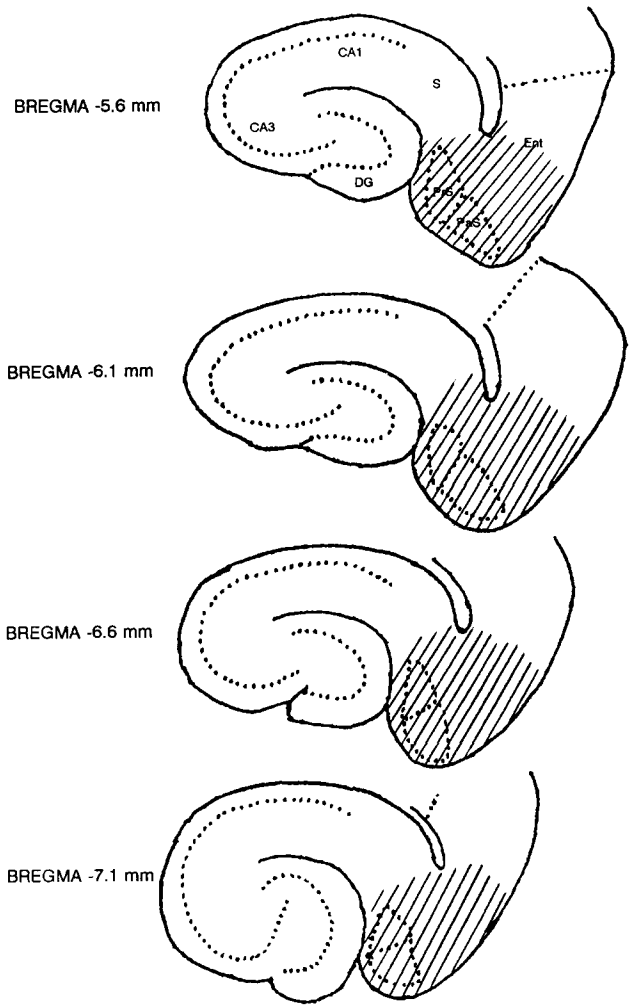

Fig. 1. Average extent of lesion shown in horizontal sections. EC – entorhinal cortex; PrS – presubiculum; PaS – parasubiculum; S – subiculum; DG – dentate gyrus.

3.2. Effects of (+)-MK-801 and memantine in normal rats

Three groups were tested: the control group, the group infused with (+)-MK-801 (0.312 mg/kg per day) and the group infused with memantine (20 mg/kg per day). As revealed by two-way ANOVA (treatment and 2-day blocks as factors), learning dependent on working memory was not affected by either drug (Fig. 2A). There was no significant main effect of either treatment ($F(2,162) = 2.29$, $P > 0.1$) or treatment × block interaction ($F(10,162) = 0.68$), $P > 0.74$) on frequency of working memory errors. However, for the second parameter – reference memory-dependent learning – ANOVA revealed a significant main effect of treatment ($F(2,162) = 34.55$, $P < 0.0001$). Post-hoc multiple pairwise comparisons performed with the Newman-Keuls test revealed that the rats infused with (+)-MK-801 had a significantly higher frequency of reference memory errors than control rats ($P < 0.05$; Fig. 1B). The treatment × block interaction effect was not significant ($F(10,162) = 1.07$, $P < 0.39$). The memantine-infused group did not differ from the control group (Fig. 1B). The average exploration time (average time spent by each rat for exploration of an arm) was not influenced by either drug (Table 1). For all parameters measured there was a significant main effect of block ($F(5,162) = 9.92$, 19.46, and 10.77, for working memory errors, reference memory errors, and exploration time $P < 0.0001$).

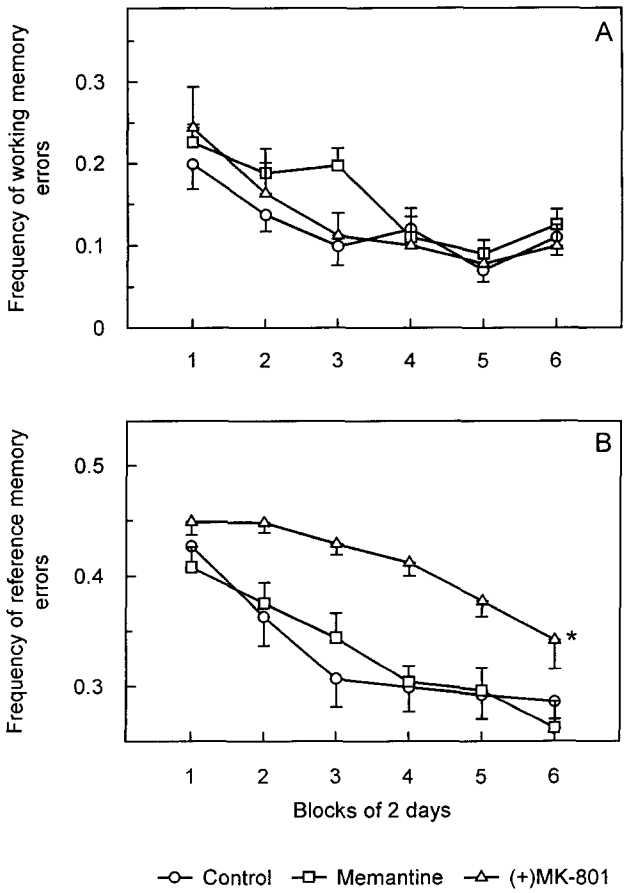

Fig. 2. The effect of chronic infusion of (+)-MK-801 (0.312 mg/kg per day) and memantine (20 mg/kg per day) on learning by rats in the radial maze. Alzet osmotic minipumps were implanted 4 days before the start of training. The effects on the frequency of working memory errors (A) and on the frequency of reference memory errors (B) are shown. Each point represents the mean ± S.E.M. in 2 day blocks of training (2×4 trials). $^*P < 0.05$ significant difference as compared to control group for the whole learning period (Newman-Keuls test). $n = 10$.

Table 1
The effect of chronic infusion of (+)-MK-801 (0.312 mg/kg per day) and memantine (20 mg/kg per day) on average exploration time in radial maze in sham-operated and entorhinal cortex-lesioned rats

Treatment	Exploration time s/arm
Control	5.04 ± 0.62
(+)-MK-801	4.04 ± 0.40
Memantine	4.72 ± 0.27
Sham	4.34 ± 0.19
Lesion	4.59 ± 0.17
Lesion/(+)-MK-801	4.10 ± 0.17
Lesion/memantine	5.13 ± 0.31

Results were averaged for the whole training period (12 days) and are presented as means ± S.E.M. One-way ANOVA showed no significant effect. $n = 7–10$.

3.3. Effects of (+)-MK-801 and memantine in rats with lesioned entorhinal cortex

In the preliminary experiment, osmotic pumps were implanted 1 day before the start of training and three groups were tested: sham-operated/untreated group, lesioned/untreated group and lesioned/memantine-infused group. Memantine increased the deficit observed in frequency of working memory errors in entorhinal cortex-lesioned rats during the first 2-day block (data not shown). The frequency of reference memory errors in both lesioned groups (memantine-treated and untreated) was increased in comparison to control rats (data not shown). However, during the 5th and 6th block, rats infused with memantine showed improvement, i.e. did not differ from the control rats and showed a significantly lower frequency of reference memory errors than did lesioned rats (data not shown). Therefore, in the next experiments we decided to implant minipumps earlier (4 days before the start of training) in order to lengthen the time of adaptation of the rats to increased drug levels.

In the next experiment, the effects of (+)-MK-801 and memantine were compared in rats after entorhinal cortex lesion. Four groups were tested: sham-operated/untreated rats, lesioned/untreated rats, le-

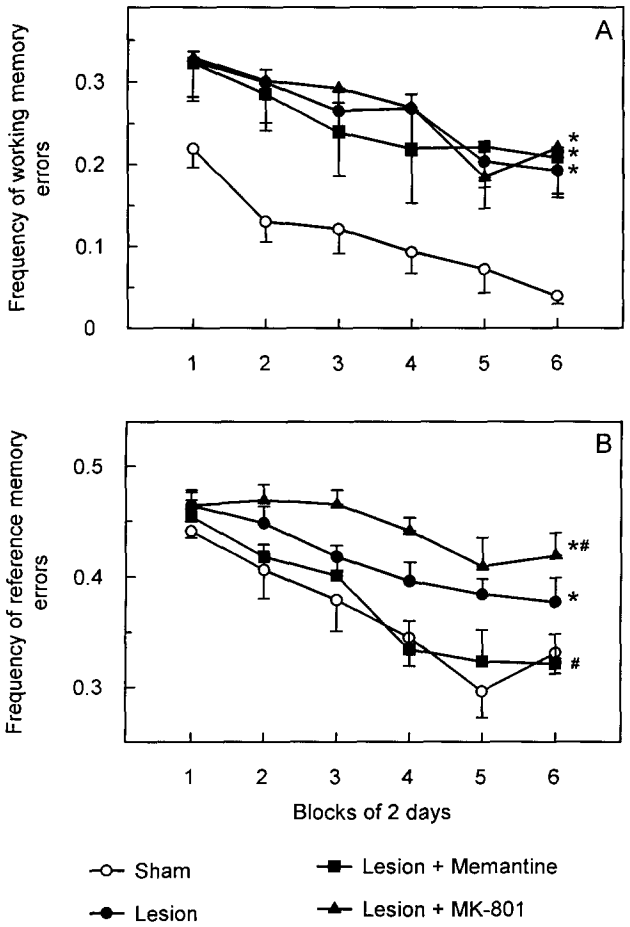

Fig. 3. The effect of chronic infusion of (+)-MK-801 (0.312 mg/kg per day) and memantine (20 mg/kg per day) on learning by entorhinal cortex-lesioned rats (lesion) in the radial maze. The effects on the frequency of working memory errors (A) and on the frequency of reference memory errors (B) are shown. Each point represents the mean ± S.E.M. 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-Keuls test). $^{\#}P < 0.05$ significant difference as compared to entorhinal cortex-lesioned rats for the whole learning period (Newman-Keuls test). $n = 7–10$.

sioned/(+)-MK-801-infused rats and lesioned/memantine-infused rats. Two-way ANOVA showed significant main effect of treatment ($F(3,180) = 14.82$, $P < 0.0001$) but no treatment × block interaction ($F(15,180) = 0.27$, $P > 0.99$) when frequency of working memory errors was analysed. Post-hoc multiple comparisons showed a significant increase in the frequency of working memory errors in all lesioned groups either untreated or infused with (+)-MK-801 or memantine ($P < 0.05$) (Fig. 3A). The frequency of reference memory errors was also changed as revealed by a significant main effect of treatment ($F(3,180) = 20.37$, $P < 0.0001$, ANOVA) but no treatment × block interaction ($F(15,180) = 0.87$, $P > 0.6$). Post-hoc multiple comparisons showed that lesioned rats infused with (+)-MK-801 had a significantly higher frequency of reference memory errors than did control rats and lesioned/untreated rats ($P < 0.05$) (Fig. 3B). A lower frequency of the reference memory errors was observed in rats with entorhinal cortex lesions infused with memantine as compared to the lesioned/untreated group ($P < 0.05$). Hence, reversal of the negative effect of lesioning was seen (Fig. 3B). For all parameters measured there was a significant main effect of block ($F(5,180) = 6.11$, 18.31, and 29.17, for working memory errors, reference memory errors, and exploration time, respectively, $P < 0.0001$). Exploration time was not affected by the treatment (Table 1).

4. Discussion

(+)-MK-801 – an uncompetitive NMDA receptor antagonist – infused chronically (using Alzet osmotic minipumps) in naive animals impaired reference memory but had no effect on the working memory-dependent parameter of the radial maze task. Previous studies have shown that (+)-MK-801 disrupts working memory-dependent radial maze learning in an unfamiliar but not in a familiar environment (Shapiro and O'Connor, 1992), indicating the importance of the reference memory component in this task (see also Danysz et al., 1995). In the present experiments, the rats were allowed to adapt to the experimental room and to the procedure before the start of training. The lack of effect on working memory could be also explained, at least to some extent, by the use of strategies, i.e. selection of adjacent arms, by rats infused with (+)-MK-801. On the other hand, the strategy that was beneficial in terms of working memory errors, was wrong in terms of reference memory errors i.e. where the locations of never baited arms should be learned, and consequently omitted. Hence, it cannot be excluded that the selection of a wrong strategy was responsible for the reference memory deficit. Of course a strategy may be an adaptive reaction to an inability to learn the spatial position of baited arms (reference memory deficit) as observed previously after repetitive injections of (+)-MK-801 (Shapiro and O'Connor, 1992).

Memantine, another uncompetitive NMDA receptor antagonist (Chen et al., 1992; Kornhuber et al., 1989; Parsons et al., 1993; Kornhuber et al., 1994), is used in the treatment of dementia (Ditzler, 1991; Görtelmeyer and Erbler, 1992). The dose of memantine (20 mg/kg per day) used in the present study was selected on the basis of results of preliminary studies since this treatment leads to pseudo steady state serum levels close to the therapeutic range (1.2 μM) (Kornhuber et al., 1994; Misztal et al., 1994). At this concentration an effective and selective blockade of NMDA receptors might be expected (Parsons et al., 1993;

Kornhuber et al., 1994). (+)-MK-801 was used at a dose 70 times lower than memantine, considering in vitro and in vivo potency differences (Bisaga et al., 1993; Parsons et al., 1993; Kornhuber et al., 1994) and similar half-life after acute i.p injection (Vezzani et al., 1989; Wenk et al., 1994). This treatment (memantine, 20 mg/kg per day) did not affect learning in naive rats, which is an apparent contradiction bearing in mind the crucial role of NMDA receptors in learning processes (Collingridge and Singer, 1990), and the clear disruption produced by (+)-MK-801. However, the two drugs show different NMDA channel kinetics (Parsons et al., 1993) (see below).

The lesions in the present study involved apart from the entorhinal cortex, the para- and pre-subiculum and partially the subiculum. This extent of the lesion was similar to the results of Hunt et al. (1994) and Johnson and Kesner (1994) but somehow bigger than reported by Hölscher and Schmidt (1994). Entorhinal cortex lesions have previously been shown to produce spatial working and reference memory deficits (Hölscher and Schmidt, 1994; Hunt et al., 1994). In the present study, similar impairment was observed. The negative effect of lesions on working memory-dependent learning may result from a decreased capacity of the working memory buffer and/or from a lack of appropriate strategy used by rats. As proposed by Morris et al. (1990), the entorhinal cortex may be involved in selecting spatial strategies utilised by the hippocampus. If this is the case, then entorhinal cortex lesion-induced working memory impairment might involve inability to adapt a strategy (in contrast to rats infused with (+)-MK-801, see above). Nevertheless, it is clear that working memory performance improves with training as the animals learn the position of baited arms (reference memory) and, in consequence, in the later phase of training the rats need to remember fewer arms previously visited.

The lesioned rats infused chronically with (+)-MK-801 showed an increased frequency of reference memory errors in comparison to lesioned/untreated rats but no change was seen in the frequency of working memory errors. The general locomotor performance was also not changed as shown by analysis of the average exploration time. In lesioned rats, memantine, similarly to (+)-MK-801, had no effect on working memory-dependent learning but, in contrast to (+)-MK-801, it surprisingly reversed the lesion-induced impairment of reference memory. This positive effect was seen after several days of infusion in lesioned, but not in sham-operated animals. Similarly, previous studies showed that administration of low doses of NMDA receptor antagonists may improve cognitive functions when the performance of the animals is poor (Mondadori et al., 1989; Walker and Gold, 1991; also see Danysz et al., 1995 for review).

In the present study it was impossible to clearly distinguish whether the effects observed were due to continuous presence of the drug (e.g. possible up-regulation of NMDA receptors) and/or the direct effect of the drug itself (inhibition of NMDA receptors). These two actions would be apparently contradictory. However, the same infusion of MK-801 and memantine prevented both the deterioration of cholinergic system and the behavioral deficit (T-maze learning) induced by parallel i.c.v. infusion of quinolinic acid (Misztal et al., 1994). This indicates that the effect of receptor blockade prevails over possible up-regulation (increase in the receptor number), since the latter change alone should instead increase the sensitivity to quinolinic acid. Nevertheless, the mode of drug delivery used in the present study (chronic application, steady state serum levels) seems to be the most appropriate when clinical relevance is a primary aim.

The crucial question is what is the mechanism of cognition enhancing effects of memantine in rats with learning deficits?

One possible explanation could be based on findings of Jones et al. (1990), showing learning impairment in a passive avoidance task after systemic administration of NMDA. Therefore, one might speculate that administration of NMDA increases the stimulation of the NMDA receptors in a non-temporal way and thereby decreases the signal-to-noise ratio. This could result in a decreased selectivity of systems responsible for the association and in turn, in learning impairment. Similar effects may also occur in Alzheimer's disease, where over-activation of glutamate receptors may be assumed to take place at certain stages of the disease (Greenamyre et al., 1988). In such cases NMDA receptor antagonists might produce beneficial effects by restoring the normal signal-to-noise ratio. However, up to now we have no indication that an over-activation of NMDA receptors takes place after entorhinal cortex lesion.

It cannot be excluded that non-associative, but cognition-supporting effects of memantine, e.g. on vigilance, attention, etc. which are not necessarily directly related to its NMDA receptor blocking activity are responsible for the cognitive effects observed (Bubser et al., 1992; Danysz et al., 1994). However, in the present study, memantine influenced only reference memory, but not working memory, as could have been expected for unspecific effects.

The present study showed a contrasting effect of two uncompetitive NMDA receptor antagonists on learning, namely (+)-MK-801 and memantine. Although both produce uncompetitive blockade of the NMDA receptor channel in a use-dependent manner, meaning that the antagonistic effect can be only seen in the presence of an agonist (Huettner and Bean, 1988; Kemp et al., 1987), other features determine their profile as well. Memantine shows fast open-chan-

nel blocking kinetics (Chen et al., 1992; Parsons et al., 1993) and strong voltage dependence, meaning that it can easily enter into the channel but also unblocks it quickly upon depolarisation. On the contrary, (+)-MK-801 shows weaker voltage dependence, and very slow kinetics (ibid.). In fact, some data confirm a better therapeutic index for memantine than (+)-MK-801. Wenk et al. (1995) studied the effects of NMDA-induced lesions of cholinergic neurones in the NBM on levels of the choline acetyltransferase in the cortex. Both memantine and (+)-MK-801 given i.p. before NMDA microinjection produced clear cut protection, with respective ED_{50}s of 2.8 mg/kg and 0.07 mg/kg (Wenk et al., 1995). However, when neuroprotective potency was compared to side-effects such as ataxia, myorelaxation, and stereotyped behaviour, a much better therapeutic index was obtained for memantine (ca. 7) as compared to (+)-MK-801 (ca. 1). It also cannot be excluded that the behavioural differences between (+)-MK-801 and memantine are not related to the NMDA receptor antagonism per se. For example, phosphoinositide turnover which seem to play an important role in both LTP and learning (Anwyl, 1991; Riedel et al., 1994) is stimulated by memantine but not by (+)-MK-801 (Osborne and Quack, 1992; Mistry et al., 1995).

In summary, the electrophysiological properties of memantine such as relatively strong voltage dependence and fast NMDA receptor channel unblocking kinetics, in comparison to (+)-MK-801, could explain the differences in behavioural effects of the two drugs in the present study. Thus, the properties of memantine allow a reduction in the risk of negative effects on cognitive functions and therefore make it suitable as treatment for Alzheimer's disease and other dementias (Ditzler, 1991; Görtelmeyer and Erbler, 1992). The results obtained support this assumption and indicate that memantine may even have cognition-enhancing properties in deficient subjects. The explanation of this beneficial effect of memantine requires, however, further study.

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