

**Chronic treatment with the uncompetitive NMDA receptor antagonist memantine influences the polyamine and glycine binding sites of the NMDA receptor complex in aged rats**

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**Summary.** Receptor binding studies on rat cortical membranes were used to characterize the NMDA receptor in aged rats (22 months) treated for 20 months with a memantine containing diet delivering 30mg/kg/day in comparison to aged and young/adult rats treated with control-diet. Spatial memory impairing effects of (+)-MK-801 (0.16mg/kg) in the radial maze was not altered within the course of memantine-treatment (up to 16 months). However, chronic memantine-treatment significantly increased the number of [<sup>3</sup>H]MK-801 binding sites and the affinity of [<sup>3</sup>H]glycine. A non-significant trend to such changes was also seen in aged-control rats. Glycine-dependent [<sup>3</sup>H]MK-801 binding (functional binding under non-equilibrium conditions at a fixed L-glutamate concentration) revealed that a decreased ability of glycine to stimulate channel opening in aged rats was partially attenuated by the long-term memantine treatment. Furthermore, an increased ability of spermidine to enhance [<sup>3</sup>H]MK-801 binding in aged-control rats was even more pronounced in the aged memantine-treated group. Together these findings may indicate that changes in functional receptor-channel properties during the process of aging occur prior to a detectable loss of binding sites and that memantine enhances an endogenous compensatory mechanism triggered by glutamatergic hypofunction which is suggested to take place in aging.

**Keywords:** Memantine, N-methyl-D-aspartate (NMDA), aging, [<sup>3</sup>H]MK-801, [<sup>3</sup>H]glycine, spermidine, radial maze.

**Introduction**

The amino-adamantane derivative memantine (3,5-dimethyl-1-amino-adamantane) has been used clinically for many years for different indications.

Initially memantine was used in Parkinson's disease (Schneider et al., 1984; Rabey et al., 1992), subsequently the application was extended by the findings that this compound was also effective in the treatment of neuroleptic-malignant syndrome, spasticity and dementia (Meldrum et al., 1986; Ditzler, 1991; Görtelmeyer and Erbler, 1992; Kornhuber et al., 1993; Pantev et al., 1993). However, the major mechanism of action of memantine has only recently been clarified.

Kornhuber and co-workers (1989) demonstrated that memantine displaces [ $^3\text{H}$ ]MK-801 ([ $^3\text{H}$ ]-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) binding at low micromolar concentrations in postmortem human frontal cortex and Bormann (1989) showed that memantine blocks N-methyl-D-aspartate-(NMDA-) mediated current responses of cultured mouse spinal neurons in the same concentration range. Since then a number of subsequent studies have confirmed that many of the *in vitro* and *in vivo* effects of memantine can be attributed to NMDA antagonism. The reported effects of memantine in electrophysiological and receptor binding studies were found at concentrations that are in the range or even below the brain concentrations obtained with a treatment of 2 X 10 mg memantine/day in humans (Wesemann et al., 1980). Moreover, several groups showed that neuroprotective properties of memantine are likely to be related to NMDA antagonism. Seif el Nasr and co-workers (1990) demonstrated a neuroprotective effect of memantine comparable to MK-801 in a rat model of transient forebrain ischemia and on cultured neurons from chick embryo retina. Subsequent findings by others have also demonstrated that memantine is able to protect cultured neurons from different brain areas against NMDA- or L-glutamate-mediated toxicity (Erdö and Schafer, 1991; Pellegrini and Lipton, 1993; Weller et al., 1993). However, unlike the classical uncompetitive NMDA receptor antagonist MK-801, memantine is clinically well-tolerated. Investigations by Chen and co-workers (1992) using patch clamp experiments to elucidate further the difference between the action of memantine and MK-801 have led them to the conclusion that the better clinical tolerability of memantine may be due to its faster kinetics of channel blocking and unblocking, respectively. In turn, they also suggested that physiological NMDA receptor activity is preserved in the presence of low micromolar concentrations of memantine but not of MK-801.

In demented patients memantine is given chronically, however there are no preclinical data on the consequences of chronic memantine-treatment in laboratory animals. To address these questions, changes of NMDA receptor properties in rats treated with either control or memantine-containing diet for 20 months were studied. Additionally the sensitivity of these animals to a learning impairment induced by a challenging dose of another NMDA antagonist, MK-801, was tested repeatedly.

## Materials and methods

### Animals

For long-term memantine treatment male Sprague-Dawley rats at the age of 3 months were used. The animals (5 per cage) were housed in an illumination-controlled room

(light period 6 A.M. to 6 P.M.). The room temperature was kept at 21°C. The rats had free access to water but food was limited to 15 g/animal/day to limit their body weight gain and to obtain sufficient appetitive motivation. Animals were divided into two groups with one group receiving control-diet (Altromin) and the other group receiving a diet delivering 30 mg/kg/day memantine (Altromin). At the age of 22 months, rats were sacrificed by decapitation. 48 hours before decapitation the memantine-treated group was switched to a control-diet.

For binding experiments using young adult (3 months) male Sprague-Dawley rats, animals (obtained from Interfauna) weighing 200-250 g were used. Until the day of sacrifice the animals were fed a complete commercial pelleted diet (Altromin) and had free access to food and tap water. Two days after delivery they were killed by decapitation.

### ***Radial maze testing***

The behavioral testing was conducted in an 8-arm-radial maze, made of plastic coated wood. It consisted of an octagonal central platform (50 cm diameter) and 8 adjoining arms (length 70 cm, height 35 cm). Small black glass cups were mounted at the end of each arm and were used for food pellet placement. Rats were injected i.p. with (+)-MK-801 at a dose of 0.16 mg/kg 30 min before radial maze testing. One randomly chosen arm that changed every day was baited. Each rat was given ten trials a day. Each trial was terminated as soon as the rat found the baited arm. Successive trials started from different starting arms. This procedure was performed once a day for five days at the beginning and then after 3, 6, 8, 12 and 16 months of treatment. The number of visited arms (except baited arm) was scored as a measure of spatial learning.

### ***Membrane preparation***

For receptor binding studies on homogenized membranes, tissue preparation was performed according to Foster and Wong (1987). In brief, rats were decapitated and their brains were removed rapidly. Cortices were dissected and homogenized in 20 volumes of ice-cold 0.32 M sucrose using a glass-Teflon homogenizer. The homogenate was centrifuged at 1,000  $\times$  g for 10 min. The pellet was discarded and the supernatant centrifuged at 20,000  $\times$  g for 20 min. The resulting pellet was resuspended in 20 volumes of distilled water and centrifuged for 20 min at 8,000  $\times$  g. Then the supernatant and the buffy coat were centrifuged three times (48,000  $\times$  g for 20 min) in the presence of 5 mM Tris-HCl, pH 7.4. All centrifugation steps were carried out at 4°C. After resuspension in 5 volumes of 5 mM Tris-HCl, pH 7.4, the membrane suspension was frozen rapidly at -80°C until the day of assay. On the day of assay the membranes were thawed and washed five times by resuspension in 5 mM Tris-HCl, pH 7.4, and centrifugation at 48,000  $\times$  g for 20 min. The final pellet was suspended in assay buffer.

The amount of protein in the final membrane preparation was determined according to the method of Lowry (1951) with some modifications (Hartfree, 1972).

### ***[<sup>3</sup>H]MK-801 binding assay***

[<sup>3</sup>H]MK-801 binding studies were performed using an assay modified from Ransom and Stec (1988). Before use membrane preparations were suspended in assay buffer containing 5 mM Tris-HCl (pH 7.4, 4°C). Non-specific binding (approximately 20% of total binding) was determined by the addition of 10  $\mu$ M (+)-MK-801. To determine incubation times for equilibrium or non-equilibrium conditions membranes from young/adult animals were incubated at increasing incubation times with 5 nM [<sup>3</sup>H]MK-801 in the absence of any modulator or in the presence of 100  $\mu$ M spermidine, 10  $\mu$ M glycine, 10  $\mu$ M L-glutamate or combinations, respectively (glycine/L-glutamate 10/10  $\mu$ M, glycine/L-glutamate/spermidine 10/10/100  $\mu$ M).

Rat cortical membranes of aged memantine-treated, aged-control and young/adult rats were used to investigate the influence of age and long-term memantine treatment on

NMDA receptor properties: For saturation studies [<sup>3</sup>H]MK-801 binding was stimulated by the addition of 10  $\mu$ M glycine and 10  $\mu$ M L-glutamate to the incubation media. Specific binding was determined at increasing concentrations of radiolabeled MK-801 (0.5/1/2.5/5/10/20/30/50 nM). The samples were incubated for 120 min at 23°C. To determine L-glutamate-dependent binding of [<sup>3</sup>H]MK-801 increasing concentrations of L-glutamate (0.1  $\mu$ M–1 mM) were added in the presence of 10  $\mu$ M glycine. Control experiments were performed in the absence of L-glutamate. The same experiment was conducted vice versa to test glycine-dependent binding. When spermidine-dependent binding was investigated the experiments were performed in the presence of glycine (10  $\mu$ M) and L-glutamate (10  $\mu$ M). The incubation time for these “functional” binding studies was 30 min at 23°C (non-equilibrium).

Incubation was terminated using a Millipore filter system. The samples were rinsed three times with 2.5 ml ice cold assay buffer over glass fiber filters obtained from Schleicher & Schuell. Following separation and rinse, the filters were placed into scintillation liquid (5 ml; Ultima Gold) and radioactivity retained on the filters was determined by using a conventional liquid scintillation counter (Hewlett Packard, Liquid Scintillation Analyzer; efficacy about 50%).

### ***[<sup>3</sup>H]glycine binding assay***

[<sup>3</sup>H]glycine binding assays were performed according to the method described by Kessler and co-workers (1989). Rat cortical membranes were prepared as previously described and the final pellet was suspended in 50 mM Tris-acetate, pH 7.4. Saturation experiments were performed by incubating the membranes with various concentration of [<sup>3</sup>H]glycine (1, 5, 10, 20 nM) or with unlabeled glycine (10, 30, 100, 300, 1000, 3000) added to constant [<sup>3</sup>H]glycine concentrations (20 nM). Samples were incubated for 30 min at 4°C. All experiments were conducted in the presence of 100  $\mu$ M strychnine. Non-specific binding (approximately 30% of total binding) was determined by including 0.1 mM glycine in the incubation mixture. The incubation was terminated by diluting the samples with 2 ml of stop solution (50 mM Tris-HCl including 10 mM magnesium sulfate, pH 7.4, cooled to <2°C) and filtration, followed by a further rinse with 2.5 ml buffer. Filtration was performed as rapidly as possible. Radioactivity retained on the filters was determined as detailed above.

### ***Data analysis***

Saturation parameters were determined with the computer program GRAFIT (Leatherbarrow, 1990). All values are given as mean  $\pm$  S.E.M. of at least 3-4 experiments each performed in triplicates. Statistical analysis was performed with the computer program SIGMASTAT (Jandel Scientific).

### ***Drugs***

Memantine was synthesized and tested for purity at Merz + Co, GmbH & Co, Frankfurt, Germany. (+)-MK-801 hydrogen maleate, spermidine trihydrochloride and L-glutamic acid hydrochloride were obtained from RBI and glycine was obtained from Aldrich Chemie. [<sup>3</sup>H]Glycine (Glycine, [<sup>2-3</sup>H]-; specific activity 48.4 Ci/mmol) and [<sup>3</sup>H]MK-801 (MK-801, (+)-[<sup>3-3</sup>H]-; specific activity 22.0 Ci/mmol) were from Du Pont de Nemours GmbH, Germany, NEN-Research Products.

## **Results**

### ***Radial maze testing***

Both control and memantine-treated animals showed identical increase in the body weight during the whole study (not shown). Hence a similar diet restric-

tion (15g/rat/day) was used for all animals. Treatment with (+)-MK-801 (0.16mg/kg) impaired memory to the same extent in control and memantine-treated animals at all test periods after 3, 6, 8, 12 and 16 months of treatment (Fig. 1).

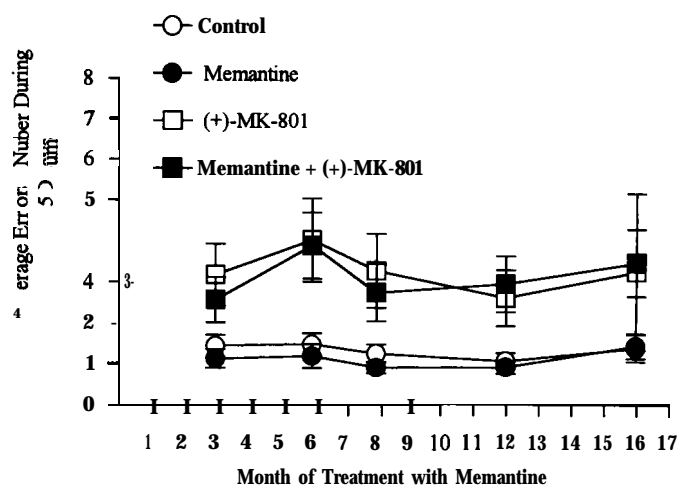
### **Binding experiments**

No influence of age or chronic treatment with memantine on the protein content in cortical membrane preparations was detected by one way ANOVA ( $38 \pm 1.5$ ,  $42 \pm 5.3$  and  $41 \pm 5.8 \mu\text{g protein/mg wet weight}$  for young, aged-control and aged memantine-treated rats respectively). In all groups the amount of protein in the final membrane preparation was about 4% of the wet weight used.

The rate of association of [ $^3\text{H}$ ]MK-801 was found to be strongly dependent on the presence of glutamate, glycine and spermidine (Fig. 2). [ $^3\text{H}$ ]MK-801 binding reached equilibrium within 120min in the presence of L-glutamate and glycine (both  $10 \mu\text{M}$ ).

Binding parameters for [ $^3\text{H}$ ]MK-801 binding to rat cortical membranes were determined in young, aged-control and aged memantine-treated rats. Both groups of aged rats revealed an increase in the amount of [ $^3\text{H}$ ]MK-801 binding sites which reached significance in memantine-treated animals as compared to young rats (Table 1). In addition the affinity of [ $^3\text{H}$ ]MK-801 tended to decrease in both groups of aged rats without reaching statistical significance.

Furthermore, chronic treatment with memantine significantly increased the affinity of [ $^3\text{H}$ ]glycine to the strychnine-insensitive glycine binding site of the NMDA receptor complex as compared to young animals (Table 2). This



**Fig. 1.** Effect of (+)-MK-801 (0.16mg/kg) on allocentric orientation in the radial maze in control and memantine-treated rats. Values are mean number of errors made during 5 days of experiment and S.E. is used as a measure of variation. Two way ANOVA with repetitive measures showed significant effect of (+)-MK-801 treatment but not that of memantine

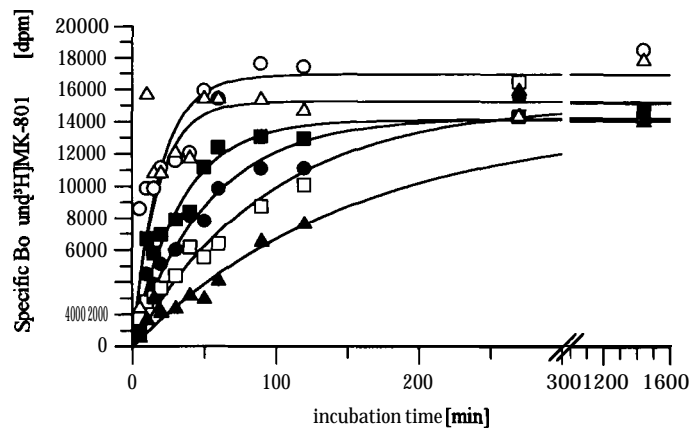


Fig. 2. Representative association curves of specific [<sup>3</sup>H]MK-801 binding determined in rat cortical membranes at different conditions: ○ spermidine (100 μM); ● glycine (10 μM); □ L-glutamate (10 μM); ■ L-glutamate/glycine (10/10 μM); △ L-glutamate/glycine/spermidine (10/10/100 μM); A control

Table 1. Binding parameters for [<sup>3</sup>H]MK-801 binding on rat cortical membranes in the presence of 10 μM glycine and 10 μM L-glutamate. Data represent mean ± S.E.M. of three animals tested

Treatment	K <sub>D</sub> [nM]	B <sub>max</sub> [pmol/mg protein]	Hill coefficient
Young	3.29 ± 0.33	1.98 ± 0.02	1.096 ± 0.053
Aged-control	8.47 ± 2.59	2.58 ± 0.27	0.953 ± 0.019
Aged memantine-treated	6.98 ± 1.79	3.25 ± 0.35 <sup>a</sup>	0.938 ± 0.027

<sup>a</sup>significantly different from young rats,  $p < 0.05$  (Student-Newman-Keuls test)

Table 2. Binding parameters for [<sup>3</sup>H]glycine binding on rat cortical membranes. Data represent mean ± S.E.M. of five to six animals tested

Treatment	K <sub>D</sub> [nM]	B <sub>max</sub> [pmol/mg protein]	Hill coefficient
Young	169.47 ± 19.94	0.987 ± 0.200	1.011 ± 0.076
Aged-control	116.24 ± 21.08	0.951 ± 0.241	0.914 ± 0.049
Aged memantine-treated	48.16 ± 13.85 <sup>a</sup>	0.505 ± 0.119	1.088 ± 0.063

<sup>a</sup>significantly different from young rats,  $p < 0.05$  (Dunn's test)

trend was seen to a non-significant extent in the aged-control group. In both groups of aged rats no significant changes in receptor density for [<sup>3</sup>H]glycine was found.

Hill coefficients for [<sup>3</sup>H]MK-801 and [<sup>3</sup>H]glycine binding were close to unity; thus receptor heterogeneity was not indicated (Tables 1, 2).

In addition, [<sup>3</sup>H]MK-801 binding was determined under non-equilibrium conditions in the presence of different combinations and concentrations of

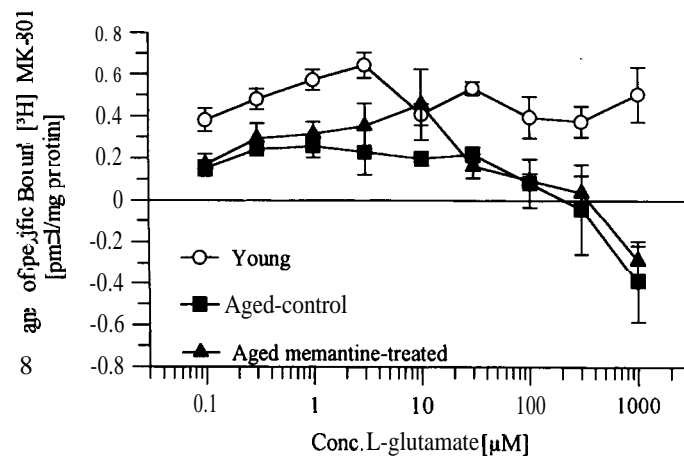


Fig. 3. L-glutamate-dependent binding of [ $^3\text{H}$ ]MK-801 at non-equilibrium conditions (incubation time: 30min) in the presence of glycine ( $10\mu\text{M}$ ). Control values were specific bound [ $^3\text{H}$ ]MK-801 in the presence of glycine ( $10\mu\text{M}$ ). Two way ANOVA with repetitive measures showed significant effect of treatment (age, memantine-treatment), concentration and interaction revealing significant differences between aged (control and memantine-treated) and young animals,  $p < 0.05$  (Student-Newman-Keuls test)

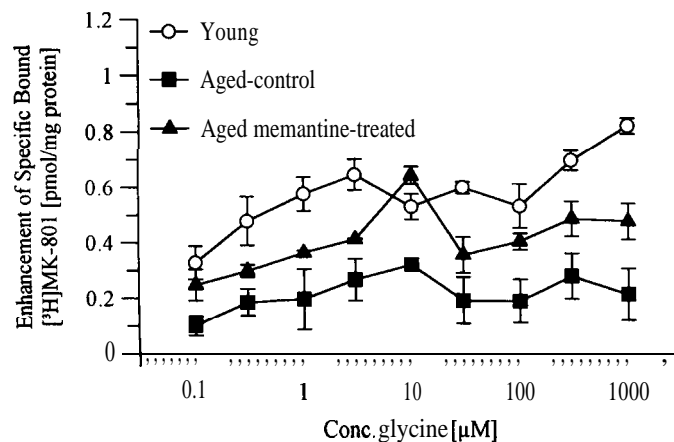
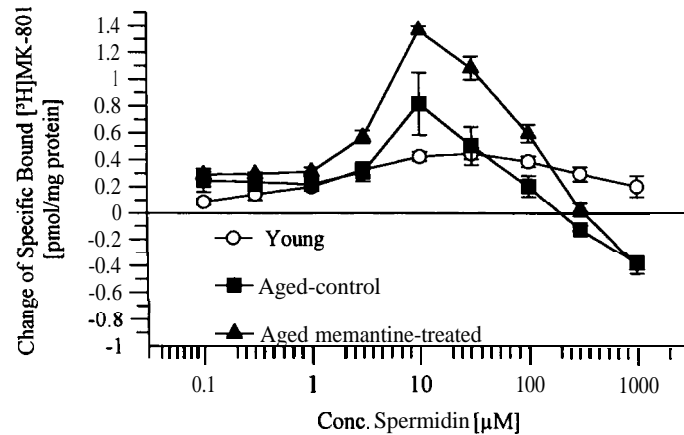


Fig. 4. Glycine-dependent binding of [ $^3\text{H}$ ]MK-801 at non-equilibrium conditions (incubation time: 30min) in the presence of L-glutamate ( $10\mu\text{M}$ ). Control value was specific bound [ $^3\text{H}$ ]MK-801 in the presence of L-glutamate ( $10\mu\text{M}$ ). Two way ANOVA with repetitive measures showed significant effect of treatment (age, memantine-treatment), concentration and interaction revealing significant differences between each pairwise comparison,  $p < 0.05$  (Student-Newman-Keuls test)

modulators. L-Glutamate-dependent stimulation of specific [ $^3\text{H}$ ]MK-801 binding in the presence of a fixed glycine ( $10\mu\text{M}$ ) concentration revealed similar binding curves for both groups of aged rats (Fig. 3). In contrast, [ $^3\text{H}$ ]MK-801 binding determined in cortical membranes from young rats as compared to aged rats resulted in a binding curve with a significant higher increase of specific bound [ $^3\text{H}$ ]MK-801 at all L-glutamate concentrations tested (Fig. 3). The same was found to be true for glycine-dependent [ $^3\text{H}$ ]MK-801 binding at a fixed glutamate concentration (Fig. 4). However, the glycine-



**Fig. 5.** Spermidine-dependent binding of [ $^3\text{H}$ ]MK-801 at non-equilibrium conditions (incubation time: 30min) in the presence of L-glutamate and glycine (both  $10\ \mu\text{M}$ ). Control value was specific bound [ $^3\text{H}$ ]MK-801 in the presence of L-glutamate and glycine (both  $10\ \mu\text{M}$ ). Two way ANOVA with repetitive measures showed significant effect of treatment (age, memantine-treatment), concentration and interaction revealing significant differences between memantine-treated animals in comparison with the two other groups,  $p < 0.05$  (Student-Newman-Keuls test)

dependent stimulation of specific bound [ $^3\text{H}$ ]MK-801 was significantly higher in memantine-treated rats when compared to aged-control animals (Fig. 4). When the same experiments were performed at increasing concentrations of spermidine at fixed concentrations of glycine and L-glutamate (both  $10\ \mu\text{M}$ ) the increase of specific binding at maximal effective spermidine concentrations ( $10\ \mu\text{M}$ ) was significantly higher in the memantine-treated group than in young and aged-control animals. The course of concentration-dependent stimulation in the aged group was similar showing a rapid and significant decline in spermidine-dependent [ $^3\text{H}$ ]MK-801 with spermidine concentrations over  $10\ \mu\text{M}$ . Spermidine above  $100\ \mu\text{M}$  even lowered specific binding in these two groups when compared to basal values (in the absence of spermidine). In contrast, for young animals there was no reduction of specific binding by spermidine in the tested concentration range (Fig. 5).

## Discussion

### *Influence of age on NMDA receptor properties*

Results from saturation experiments on rat cortical membranes from young adult rats (3 months) characterizing the PCP/MK-801 and glycine binding sites of the NMDA receptor ion-channel complex were in accordance to those previously described (e.g. Kessler et al., 1989; Ebert et al., 1991). In the present study no significant changes in the density of [ $^3\text{H}$ ]MK-801 and [ $^3\text{H}$ ]glycine binding sites were observed in cortical membranes of rats at the age of 22 months as compared to young animals. These findings are not consistent with some other findings demonstrating a decreased density of [ $^3\text{H}$ ]MK-801 and [ $^3\text{H}$ ]glycine binding sites in the cortex of aged rats (Miyoshi et al., 1990; Tamaru et al., 1991). However, a recent investigation of

Saransaari and Oja (1993a) on mouse cerebral cortex using [<sup>3</sup>H]TCP to characterize the channel domain also failed to detect a change in the density of binding sites during aging. Moreover, the same authors detected an increase in the number but not affinity of [<sup>3</sup>H]glycine binding sites up to two weeks and then again in aged animals (Saransaari and Oja, 1993b). The assay conditions applied by this group were more consistent with those used for our study, and in accordance with their finding, we detected no significant change in the affinity of [<sup>3</sup>H]glycine during aging. The extreme differences in the assay conditions used by different authors e.g. concerning the separation technique applied or incubation time may be one reason for the inconsistency of the findings. Furthermore, it has been reported that age-related changes in the number of binding sites may even underlie strain-specific alterations in NMDA receptor properties (Peterson and Cotman, 1989). Another explanation for these discrepancies could be that most previously reported investigations showing a decrease in  $B_{\max}$  values were performed on rats at the age of 24 months or even older animals. The aged rats used for this study were sacrificed at the age of 22 months. At this stage of aging other mechanisms than simple loss of binding sites may predominate such as compensatory mechanisms (see below).

Enhancement of [<sup>3</sup>H]MK-801 binding is known to be use-dependent which implies that MK-801 preferentially binds to the activated state of the channel. As such, [<sup>3</sup>H]MK-801 binding can provide an index of receptor function. There are only a few investigations on functional changes of the NMDA receptor ion-channel complex underlying age-dependent processes. Recently, three studies on rat and mouse brain have been published demonstrating age-dependent changes of glycine and L-glutamate-dependent channel activation (Cohen and Müller, 1992, 1993; Serra et al., 1994). In addition to an age-dependent loss of [<sup>3</sup>H]MK-801 binding sites, Cohen and Müller (1992) have reported an enhanced efficacy of L-glutamate and glycine to stimulate [<sup>3</sup>H]MK-801 binding in the mouse forebrain of aged (20 months) as compared to young animals (3 months). A more recent study by Serra and co-workers (1994) has indicated that the age-dependent increase of glycine- and L-glutamate-dependent stimulation of [<sup>3</sup>H]MK-801 binding may only occur during a certain period of aging and may also depend on the brain region. They detected an enhanced sensitivity of [<sup>3</sup>H]MK-801 binding to glycine and L-glutamate stimulation in the hippocampus of rats at the age of 18 months. However, this enhancement was clearly absent in cortex and striatum and was found to be normalized in the hippocampus of rats at the age of 24 months. They therefore suggested that these findings may reflect an increase of NMDA receptor activity to compensate for the decrease in receptor density (Serra et al., 1994).

To investigate the influence of age on receptor function, glycine-dependent stimulation of [<sup>3</sup>H]MK-801 binding was performed in the presence of 10  $\mu$ M L-glutamate and vice versa. Binding experiments with increasing concentrations of spermidine were performed in the presence of 10  $\mu$ M glycine and 10  $\mu$ M L-glutamate. A small and variable amount of endogenous L-glutamate and glycine is likely to be present despite extensive washing of the

membranes. Therefore the addition of a fixed agonist concentration which is high enough to saturate the binding sites should insure that conditions are the same for each sample. This approach was taken to mimic more physiological conditions of receptor activation where glycine and L-glutamate are expected to be present. Another important difference of the incubation conditions applied in this study compared to those previously described is that this functional assay was deliberately performed under non-equilibrium conditions (incubation time: 30min). This is reasonable considering that [ $^3\text{H}$ ]MK-801 may also slowly gain access to the channel domain by diffusion through the membrane in the absence of agonists (Javitt and Zukin, 1989).

These variations in assay conditions are likely to underlie the differences between our results and those reported by the above mentioned groups. In the presence of a fixed glycine concentration (10  $\mu\text{M}$ ) L-glutamate-dependent stimulation over a concentration range from 0.1  $\mu\text{M}$  to 1 mM was found to be decreased in aged animals. This also held true for glycine-dependent stimulation when transmitter recognition sites for L-glutamate were saturated.

Maximal spermidine-dependent stimulation of [ $^3\text{H}$ ]MK-801 binding in the presence of glycine and L-glutamate was enhanced in aged rats compared to young rats. Binding curves in the presence of spermidine showed the typical bell-shaped characteristic which has previously been reported on rat brain as well as human cortical membranes (Williams et al., 1989; Steele et al., 1990): at spermidine concentrations higher than 10–30  $\mu\text{M}$  specific [ $^3\text{H}$ ]MK-801 binding decreased. Interestingly, in rats aged 22 months this blocking activity of spermidine was even more evident. The physiological concentrations of spermine and spermidine in rat and human brain have been reported to be about 100  $\mu\text{M}$  in cortical membrane preparations (Shaw and Pateman, 1973). Considering this, the inhibitory action of polyamines may be of particular relevance. However, from these findings on homogenates it is difficult to assess the actual polyamine concentration relevant for receptor activation and it is important to realize that polyamine concentrations significantly differ between cellular and subcellular elements. Furthermore, one can not exclude that these polyamine-dependent effects are partially abolished in vivo by the presence of endogenous inhibitors of the polyamine binding site like putrescine. Although the occupancy of the polyamine binding site, in contrast to the transmitter binding site and the glycine regulatory binding site, does not seem to be required for channel activation, this binding site may be of importance in developmental changes of the NMDA receptor ion-channel complex. Williams and co-workers (1991) for instance have observed an increase in the potency of spermine and in the magnitude of the enhancement of binding of [ $^{125}\text{I}$ ]MK-801 by spermine in rat forebrain between days 3 and 10 of postnatal life. They suggested that these changes in the effects of polyamines during development may be directly related to alterations in the properties of the polyamine binding site during development. Furthermore, a change in polyamine sensitivity of the NMDA receptor during aging may also reflect changes in subunit composition. Molecular cloning studies by Durand and co-workers (1992), demonstrating different polyamine-dependent potentiation of NMDA responses in distinct subunits, may support this suggestion.

In addition, a more recent study by Williams and co-workers (1994) has demonstrated that the polyamine sensitivity of NMDA receptors is mainly controlled by NMDAR2 subunits. Since subunit composition of NMDA receptors also undergoes age-dependent changes, our findings may reflect a shift in the subunit composition to receptors with increased polyamine sensitivity.

The findings reported here are in good correlation to those recently observed in human cortical membrane preparations. Piggott and co-workers (1992) have shown that the effect of glycine (30  $\mu\text{M}$ ) and L-glutamate (50  $\mu\text{M}$ ) on [ $^3\text{H}$ ]MK-801 binding is decreased in aged humans. Furthermore their findings indicate that spermidine-dependent binding of [ $^3\text{H}$ ]MK-801 is especially altered by age. They observed a reduction in binding in fetal cases of up to 80% and an increase in binding in young adults of up to 50%, which was gradually diminished and was abolished in extreme old age irrespective if spermidine was added alone or in combination with glycine and L-glutamate (Piggott et al., 1992). Thus, changes in the functional properties of the NMDA receptor-channel complex may occur prior to a detectable loss of binding sites. However, the present study did not assess the status of aging or whether compensatory or degenerative processes were predominating.

#### ***Influence of chronic memantine treatment of NMDA receptor properties***

Investigations on rat cortical membranes of aged animals treated chronically with memantine (30 mg/kg/day) revealed no changes concerning parameters of [ $^3\text{H}$ ]MK-801 saturation experiments as compared to control aged rats. However, the tendency for increased  $B_{\text{max}}$  of [ $^3\text{H}$ ]MK-801 binding seen in aged animals was further enhanced by memantine-treatment so that a significant difference to young animals was seen. Moreover, the affinity of [ $^3\text{H}$ ]glycine was significantly increased after this long-term treatment. This was unexpected as memantine does not interact directly with this binding site (unpublished data from strychnine-insensitive [ $^3\text{H}$ ]glycine displacement studies). In addition, in the present study we found that L-glutamate-dependent channel opening measured as a function of specific [ $^3\text{H}$ ]MK-801 binding remained unchanged in aged rats after a long-term memantine-treatment. A decreased ability of glycine to stimulate channel opening in aged as compared to young animals was partially attenuated by long-term treatment with memantine although failing to reach the level of the glycine effect observed in young animals.

To our knowledge the present study is the first investigation on changes in NMDA receptor binding properties occurring after a chronic treatment with an NMDA receptor channel blocker over such a long period of time (more than one year). However, some previous studies reported the influence of a subchronic treatment with uncompetitive NMDA receptor antagonists on binding parameters of the NMDA receptor: Investigation on subchronic administration of MK-801 (0.5 mg/kg twice daily for 7 days) in rats suggested a down-regulation of cortical NMDA receptors when determined by [ $^3\text{H}$ ]D-2-amino-5-phosphonopentanoic acid binding (Mannalack et al., 1989).

This reduction was not paralleled by an alteration in [ $^3\text{H}$ ]N-(1-[2-thienyl]cyclohexyl)piperidine ([ $^3\text{H}$ ]TCP) binding which was in agreement with findings from brain tissue from a human PCP-addicted population (Mannalack et al., 1989; Weissmann et al., 1991). In contrast, a more recent study by Saransaari and co-workers (1993) detected a significant increase in binding capacity ( $B_{\text{max}}$ ) of [ $^3\text{H}$ ]TCP in cortical synaptosomal preparations after PCP-treatment in mice (1 mg/d/mouse for 3 days). Moreover, they reported that the effect of L-glutamate and glycine on [ $^3\text{H}$ ]TCP binding was increased in PCP-treated animals which suggested an increase in the number of these binding sites or a modification in their function (Saransaari et al., 1993). Although some of the previous results by others are in line with our findings it is difficult to compare these data to the present study where animals have been treated for a long period of time.

Our finding concerning the increased effect of glycine to stimulate [ $^3\text{H}$ ]MK-801 binding after a long-term memantine treatment is clearly supported by the higher affinity of [ $^3\text{H}$ ]glycine observed. However, it is difficult to draw any conclusions from these findings concerning the therapeutic meaning since there are only a few investigations which focused on disease-dependent changes in the coupling of the glycine binding site and the MK-801/PCP binding site of the NMDA receptor complex: Data from previous studies on cortical grey matter of subjects with Alzheimer's disease by Procter and co-workers (1989a,b, 1991) suggested that the strychnine-insensitive glycine recognition site is selectively altered in Alzheimer's disease. They showed that, in addition to a significant loss of [ $^3\text{H}$ ]glycine binding sites occurring in connection with this disease, glycine-dependent [ $^3\text{H}$ ]MK-801 binding is decreased in these subjects (Procter et al., 1991). Considering this, one may speculate that compounds regulating these Alzheimer's-dependent alterations in the glycine binding site may be of therapeutic benefit.

Recent findings from [ $^3\text{H}$ ]MK-801 binding studies on rat hippocampal membranes, showing that the inhibitory effect of memantine was reduced in the presence of 100  $\mu\text{M}$  spermine, have for the first time led to the supposition that the action of memantine may involve the polyamine binding site (Berger et al., 1992). We here report that chronic memantine-treatment may also influence the polyamine binding site: The enhanced ability of spermidine to enhance [ $^3\text{H}$ ]MK-801 binding in aged rats as compared to young animals was even more pronounced in the aged memantine-treated group of animals. As was the case in aged-control animals the spermidine-dependent inhibition (above 10  $\mu\text{M}$  spermidine) of [ $^3\text{H}$ ]MK-801 binding was also more evident in the aged memantine-treated group. However, further investigations especially from electrophysiological studies, will be necessary to further evaluate the precise mechanism underlying the action of memantine at the polyamine binding site. The reported observations may also support the hypothesis by Reynolds (1994) that polyamines may act at more than one binding site distinct from that originally described by Ransom and Stec (1988).

In general the effects of long-term memantine-treatment on NMDA receptor properties are for several reasons unlikely to be due to the fact that

memantine is still present in the membrane preparations: first the memantine-treated animals received a control diet 48 hours before decapitation to avoid an enrichment of memantine in the brain which could disturb binding experiments; second, control values of specific [ $^3\text{H}$ ]MK-801 binding of both groups of aged rats when 10  $\mu\text{M}$  of glycine and 10  $\mu\text{M}$  of L-glutamate were added and  $K_D$  values from saturation experiments did not reveal significantly changed data in the memantine-treated group which would be expected if memantine were still present.

However, behavioral experiments revealed that long-term memantine-treatment is not able to prevent the animals from a memory impairment caused by MK-801 application.

### **General Conclusions**

In conclusion, there is clear evidence for the suggestion that, at the time of decapitation, the aged rats may have reached a state of aging at which compensatory mechanisms predominate rather than a loss of binding sites. Long-term memantine treatment was found to exhibit a significant influence on the strychnine-insensitive glycine binding site coupled to the NMDA receptor ion-channel complex by increasing the affinity of [ $^3\text{H}$ ]glycine as well as the ability of glycine to stimulate channel opening. Since the glycine co-agonistic site of the NMDA receptor complex may be of relevance for learning and memory it may be suggested that the decreased glycine-dependent [ $^3\text{H}$ ]MK-801 binding and loss of [ $^3\text{H}$ ]glycine binding sites in patients suffering from Alzheimer's disease may contribute to the impairment of learning and memory in connection with this disease (Procter et al., 1991; Thompson et al., 1992). One could therefore speculate that treatment with memantine may produce its cognitive enhancing effects in dementia patients by compensating this deficit in the glycine site (Ditzler, 1991; Gdrtelmeyer and Erbler, 1992; Pantev et al., 1993). Recent investigations on the effect of a chronic treatment with memantine (infusion via osmotic pumps for two weeks) has revealed a reversal of entorhinal lesion-induced deficits in a spatial reference memory task (W. Zajaczkowski, personal communication). This provides the first animal model supporting the observed symptomatic improvement in demented patients. This is unexpected considering that NMDA receptor antagonists, although acting as neuroprotectants, are known to produce impairment of learning and memory (e.g. Danysz and Archer, 1994).

Recently, Parsons and co-workers (1993) have suggested that the faster kinetics of memantine to block and unblock the NMDA receptor-linked ion-channel and strong voltage-dependency as compared to high-affinity antagonists like MK-801 may account for the ability of this compound to prevent pathological NMDA receptor activation whilst leaving physiological processes intact (Rogawski, 1993). However, data from the present study clearly suggest that some of the reported *in vivo* and *in vitro* properties of memantine after chronic treatment may be attributable to its indirect modification of the NMDA receptor ion-channel complex through sites distinct from the PCP/MK-801 recognition site.

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[<sup>3</sup>H]MK-801

Bowen

Stec

[<sup>3</sup>H]MK-801

N-

[<sup>3</sup>H]CGP

Mg<sup>2+</sup>

N-methyl-D-aspar-  
-arcaine-

2,3-benzodiazepines.

Mech

125-

Mech

Rossberg

