



Different Binding Affinities of NMDA Receptor Channel Blockers in Various Brain Regions—Indication of NMDA Receptor Heterogeneity

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Summary—The *N*-methyl-D-aspartate (NMDA) receptor–channel complex exists in multiple forms which probably have different physiological and pharmacological properties. To further evaluate this concept of different NMDA receptor subtypes, receptor binding and autoradiographic techniques were used to study the phencyclidine (PCP) binding site of the NMDA receptor ion-channel complex. [³H]MK-801 was employed to characterize binding properties of (+)-MK-801, (–)-MK-801, phencyclidine (PCP), (±)-ketamine, amantadine (1-amino-adamantane) and memantine (3,5-dimethyl-1-amino-adamantane) in different brain regions. Saturation experiments on homogenized membranes revealed the existence of single classes of binding sites in cortex and cerebellum but with significant different affinities between these regions ($K_{D,Cortex} = 4.59$ nM, $B_{max,Cortex} = 0.836$ pmol/mg protein; $K_{D,Cereb.} = 25.99$ nM, $B_{max,Cereb.} = 0.573$ pmol/mg protein) suggesting that the lower affinity in cerebellum indicates another population of NMDA receptor channels. In contrast, in striatum there was clear evidence for two binding sites ($K_{D,high} = 1.43$ nM, $B_{max,high} = 0.272$ pmol/mg protein; $K_{D,low} = 12.15$ nM, $B_{max,low} = 1.76$ pmol/mg protein). Displacement studies (autoradiography and binding) revealed a lower affinity for unlabeled (+)-MK-801 in striatum which was clearly not the case for memantine. In cerebellar membranes there was a significant decrease in the affinity for both MK-801 enantiomers and PCP but not for the 1-amino-adamantanes. In contrast, all compounds showed lowered affinity in the dentate gyrus. These findings support NMDA receptor heterogeneity which may be of particular relevance for the development of subtype-selective drugs.

Keywords—Memantine, amantadine, [³H]MK-801, phencyclidine (PCP), (±)-ketamine, *N*-methyl-D-aspartate (NMDA), receptor binding, autoradiography, receptor heterogeneity.

Glutamate has been shown to be a predominant factor for neurotoxicity associated with seizures, ischaemia and several degenerative neurological disorders like Huntington's disease and Parkinson's disease (Choi, 1988; Meldrum and Garthwaite, 1990). A key event in this process seems to be an overstimulation of the NMDA (*N*-methyl-D-aspartate) receptor–channel complex and a subsequent influx of Ca²⁺. Because of the potential involvement of NMDA receptors in chronic and acute neurological disorders, extensive investigations on NMDA receptor antagonists have been undertaken. Amantadine (1-amino-adamantane) and its 3,5-dimethyl derivative memantine have been proposed to be beneficial for the treatment of Parkinson's disease and dementia, respectively, and are also known to possess antispastic properties (Schwab *et al.*, 1969; Schneider

et al., 1984; Ditzler, 1991; Rabey *et al.*, 1992). There is now clear evidence that these compounds bind to the phencyclidine (PCP) binding site of the NMDA receptor complex at therapeutic concentrations (Kornhuber *et al.*, 1989, 1991) and that they block NMDA-evoked currents (Bormann, 1989; Parsons *et al.*, 1994). Moreover, several groups have shown neuroprotective properties of amantadine and memantine against NMDA receptor-mediated toxicity (Erdö and Schäfer, 1991; Chen *et al.*, 1992; Pellegrini and Lipton, 1993; Weller *et al.*, 1993). However, there are still a number of questions remaining concerning the precise mechanism of action of these compounds, e.g. why they exhibit differential pharmacological profiles as compared to high-affinity NMDA receptor antagonist such as (+)-MK-801 [(5*R*, 10*S*)-(+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)-cyclohepten-5,10-imine; dizocilpine].

A number of recent studies have revealed the heterogeneity of the NMDA receptor. So far, two subunits

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designated NMDAR1 and NMDAR2 (the mouse counterparts of the rat are designated as ζ and ϵ subfamilies) have been cloned (Moriyoshi *et al.*, 1991; Meguro *et al.*, 1992). For the NMDAR1 subfamily there exist at least eight isoforms (NMDAR1A–NMDAR1H) which were generated by alternative splicing (Sugihara *et al.*, 1992; Durand *et al.*, 1993; Hollmann *et al.*, 1993). There is now clear evidence that the NMDAR2 subfamily consists of four individual subunits, NMDAR2A–NMDAR2D (Meguro *et al.*, 1992; Monyer *et al.*, 1992; Ikeda *et al.*, 1992). Homo-oligomeric structures of the NMDAR1 possess properties characteristic for the NMDA receptor–channel complex which have been reported from other systems such as electrophysiological studies on cell cultures and receptor binding studies on homogenized membrane preparations. Moriyoshi and co-workers (1991) could demonstrate that ion-channels formed by a single protein encoded by the rat NMDAR1 cDNA clone show a typical profile for agonists and antagonists. In addition, the cloned NMDAR1 receptors are highly permeable to Ca^{2+} , blocked in a voltage-dependent manner by Mg^{2+} , inhibited by Zn^{2+} and their activity is enhanced by glycine and polyamines (Moriyoshi *et al.*, 1991; Durand *et al.*, 1993). In contrast, NMDAR2 subunits have to be expressed in combination with NMDAR1 subunits to yield functional NMDA receptor channels (e.g. Meguro *et al.*, 1992). Thus NMDAR1 seems to be the essential subunit for the expression of functional NMDA receptor channel complexes. Heteromeric NMDA receptor channels formed by combinations of NMDAR1 and NMDAR2 subunits are known to differ in gating properties and magnesium sensitivity and therefore have distinct functional and pharmacological properties (e.g. Monyer *et al.*, 1992). Furthermore Kutsuwada and co-workers (1992) could demonstrate using subunits of the mouse NMDA receptor–channel that the different subunits are distinct concerning their affinities for agonists and competitive antagonists. However, combined expression of NMDAR1 and NMDAR2 subunits reveals receptors with markedly potentiated responses to NMDA and glutamate as compared to homomeric NMDAR1 receptors (Kutsuwada *et al.*, 1991; Meguro *et al.*, 1992). Moreover, distribution of NMDAR2 subunits in the adult brain is not uniform as was shown for the total of NMDAR1 subunits (Kutsuwada *et al.*, 1992; Monyer *et al.*, 1992).

In addition to these findings from molecular biological studies, several autoradiographic and receptor binding studies have also indicated that there are multiple NMDA receptor sites and/or states with different distributions and different affinities for agonists and antagonists (Ebert *et al.*, 1991; Monaghan, 1991; Yoneda and Ogita, 1991; Porter and Greenamyre, 1995). The aim of the present study was to elucidate the pharmacological diversity of NMDA receptors (labeled with [^3H]MK-801) in different brain regions with autoradiographic and receptor binding studies by assessing the potency of

(+)-MK-801, (–)-MK-801, PCP, (\pm)-ketamine, amantadine and memantine.

METHODS

Receptor binding studies on rat brain homogenates

Male Sprague–Dawley rats (200–250 g) were decapitated and their brains were removed rapidly. Cerebral cortex, striatum and cerebellum were dissected and pooled separately. Tissue preparation was performed according to Foster and Wong (1987).

In brief, the brain structures were homogenized in 20 vol of ice-cold 0.32 M sucrose using a glass–Teflon homogenizer. The homogenate was centrifuged at 1000 *g* for 10 min. The pellet was discarded and the supernatant centrifuged at 20,000 *g* for 20 min. The resulting pellet was resuspended in 20 vol of distilled water and centrifuged for 20 min at 8000 *g*. Then the supernatant and the buffy coat were centrifuged three times (48,000 *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 vol of 5 mM Tris–HCl, pH 7.4 the membrane suspension was frozen rapidly at –80°C until use.

On the day of assay the membranes were thawed and washed four times by resuspension in 5 mM Tris–HCl, pH 7.4 and centrifugation at 48,000 *g* for 20 min. The final pellet was suspended in assay buffer (5 mM Tris–HCl, pH 7.4). Displacement and saturation studies were performed using an assay modified from Ransom and Stec (1988). Membranes were incubated at 23°C in an incubation vol of 0.5 ml with approx 100–225 μg of membrane protein per tube (protein was determined according to the method of Lowry *et al.*, 1951). All experiments were carried out in triplicates and were repeated at least 3–4 times.

In displacement and saturation studies [^3H]MK-801 binding was fully stimulated by the addition of 10 μM of L-glutamate and 10 μM of glycine to the incubation media. Non-specific binding (approx 20% of total binding) was determined with 1 μM of unlabeled (+)-MK-801. Incubation was stopped by filtration using a Millipore filter system for vacuum filtration over glass fiber filters (Schleicher and Schuell). The membranes retained on the filters were rinsed three times with 2.5 ml ice cold 5 mM Tris–HCl buffer.

For saturation studies concentrations up to 50 nM were achieved by increasing the concentration of radiolabeled MK-801 from 0.5 to 50 nM, while those above 50 nM (in cerebellar membranes) were produced by adding unlabeled MK-801 to a fixed [^3H]MK-801 concentration (5 nM). To assure equilibrium conditions the samples were incubated for at least 120 min at 23°C. Displacement studies were carried out at a fixed [^3H]MK-801 concentration of 5 nM and increasing concentrations of (+)-MK-801, (–)-MK-801, PCP, (\pm)-ketamine, memantine and amantadine.

Radioactivity retained on the filters was determined by using a conventional liquid scintillation counter (Hewlett Packard, Liquid Scintillation Analyzer).

Autoradiographic studies on rat brain sections

Male Sprague-Dawley rats weighing 200–250 g were decapitated and their brains were removed rapidly and frozen in 2-methylbutane cooled with dry-ice. Serial cryostat sections of 16 μM thickness were thaw-mounted on gelatin-coated slides and stored at -20°C for 24 hr.

Assays were performed according to the method described by Sakurai and coworkers (1991) with some modifications. In brief, on the day of assay the sections were prewashed for 15 min in 5 mM Tris-HCl (pH 7.4) at 4°C and dried under a stream of cold air. Quadruplicates of tissue sections were incubated in 5 mM Tris-HCl buffer (pH 7.4) containing 5 nM [^3H]MK-801. Glycine (10 μM) and L-glutamate (10 μM) were added to stimulate channel opening and shorten the association time of [^3H]MK-801. The sections were incubated at room temperature in Hellendahl glasses (with upper extension, suitable for 16 slides) containing 60 ml of incubation medium for approx 80 min. Non-specific binding was determined in the presence of 10 μM unlabeled (+)-MK-801 (average non-specific binding was 30%).

To study competition of [^3H]MK-801 with (+)-MK-801, memantine and amantadine, these drugs were included in the incubation mixture at increasing concentrations. Following the incubation, sections were rinsed by dipping them quickly two times in 5 mM Tris-HCl buffer (pH 7.4), distilled water and 1.25% glutaraldehyde in acetone. All washing solutions were ice-cold and the washing procedure did not take longer than 30 sec. Then sections were dried under a stream of warm air.

The dried sections and tritium standard (Microscales, Amersham) were apposed to tritium sensitive films ([^3H] Hyperfilm, Amersham) for 2 weeks at 4°C in X-ray cassettes. After exposure the films were developed with D-19 (Kodak) developer, fixed and dried. Autoradiograms were analyzed and quantified by a computer assisted image analyzing system (N.I.H. Image, Version 1.51). The measured densities of gray levels were converted to [^3H] binding values using calibrated tritium standards.

Data analysis

IC_{50} values and saturation parameters were determined with the computer program GRAFIT (Leatherbarrow, 1990). The K_i values were determined by the method of Cheng and Prusoff (1973) using the formula: $K_i = \text{IC}_{50}/(1 + ([L]/K_D))$. K_D values for [^3H]MK-801 binding in autoradiographic studies were calculated from the displacement studies when unlabeled (+)-MK-801 was added. All values are given as mean \pm SEM for at least three determinations. Statistical analysis was made by one way analysis of variance using the SIGMASTAT software (Jandel Scientific)

which, if significant, was followed by the Student-Newman-Keuls test.

Materials

[^3H]MK-801 (22.0 Ci/mmol) was purchased from Dupont NEN. (+)-MK-801, (-)-MK-801, (\pm)-ketamine and PCP were obtained from RBI (Research Biochemicals Incorporated) and the 1-amino-adamantanes were synthesized and analyzed for purity at Merz + Co. All other reagents were obtained from Sigma or Aldrich-Chemie.

RESULTS

Receptor binding studies on rat brain homogenates

With Scatchard analysis a single class of binding sites for cortical membranes was detected with Hill coefficients close to unity and no indication for heterogeneity (Table 1, Fig. 1). In cerebellar membranes a single class of binding sites was observed but with significantly lower affinity and a decreased density as compared to cortex (Table 1). In contrast to cortex and cerebellum in striatal membranes there were clear indications for heterogeneous binding sites. The Hill coefficient for the one-site model was significantly different from unity and the curve derived from a two site equation for Scatchard analysis could describe binding behavior much better (Fig. 2). The high-affinity binding site in striatum had lower K_D and B_{max} values than cortical and cerebellar membranes. On the other hand the low-affinity binding site was characterized by a K_D value 3 times higher than in cortex and an increased density of binding sites (Table 1).

The affinity of unlabeled (+)-MK-801 was significantly lower in cerebellar and striatal membranes as compared to cortical membranes, suggesting that under these conditions the affinity of (+)-MK-801 to striatum represents the low-affinity site of this brain region (Table 2). This tendency for a decrease in affinity to striatal and cerebellar [^3H]MK-801 binding sites was also seen for (-)-MK-801 although failing to reach statistical

Table 1. Binding parameters of [^3H]MK-801 binding (stimulated with 10 μM glycine and 10 μM L-lutamate) in homogenized rat brain membranes, isolated from different brain regions. The values are mean \pm SEM from at least three experiments

Brain region	Binding parameters		
	K_D [nM]	B_{max} [pmol/mg protein]	Hill coefficient
Cortex	4.59 \pm 1.26*	0.836 \pm 0.091†	0.978 \pm 0.022
Cerebellum	25.99 \pm 3.26	0.573 \pm 0.064†	1.023 \pm 0.053
Striatum	1 site:	1 site:	0.789 \pm 0.017‡
	10.08 \pm 1.74	2.157 \pm 0.163	
	2 site:	2 site:	
	1.43 \pm 0.89	0.272 \pm 0.095	
	12.15 \pm 3.96	1.76 \pm 0.315	

* $P < 0.05$ significantly different from cerebellum by ANOVA (Student-Newman-Keuls test); † $P < 0.05$ significantly different from striatum by ANOVA (Student-Newman-Keuls test); ‡ $P < 0.05$ significantly different from unity by Student's t -test.

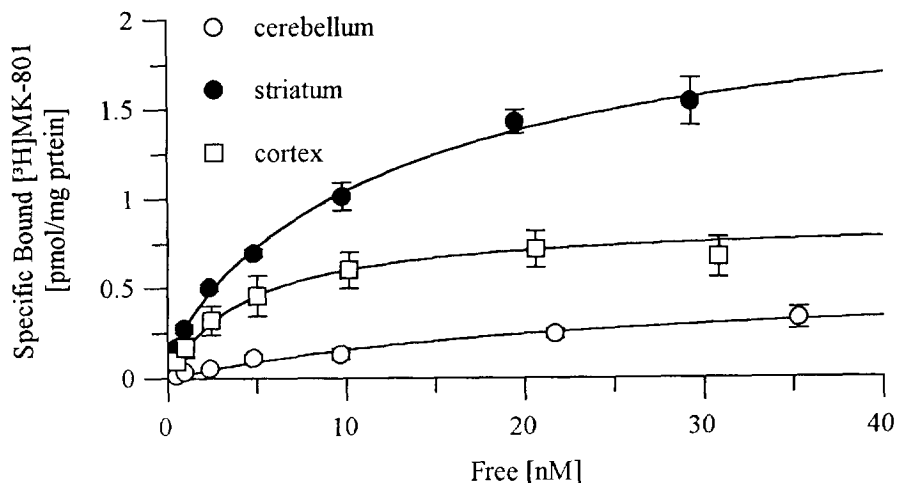


Fig. 1. Saturation curves for the binding of [³H]MK-801 to membrane preparations from rat striatum (●), cortex (□) and cerebellum (○). Values depict mean ± SEM from at least three experiments.

Table 2. Displacement of [³H]MK-801 binding (stimulated with 10 μM glycine and 10 μM L-glutamate) in homogenized rat brain membranes, isolated from different brain regions. K_i values (mean ± SEM) were calculated using the equation $K_i = IC_{50}/(1 + ([L]/K_D))$ (Cheng and Prusoff, 1973)

Competitor	Cortex K_i [μM]	Cerebellum K_i [μM]	Striatum K_i [μM]
(+)-MK-801	0.0025 ± 0.0002*†	0.0094 ± 0.0013	0.0065 ± 0.0013*
(-)-MK-801	0.0137 ± 0.0028	0.0537 ± 0.0218	0.0249 ± 0.0116
PCP	0.0422 ± 0.0056‡	0.1800 ± 0.0100	not tested
Ketamine	1.190 ± 0.237	2.507 ± 1.900	not tested
Memantine	0.69 ± 0.0998	0.700 ± 0.1877	0.433 ± 0.0524
Amantadine	22.8 ± 3.84	23.1 ± 6.94	34.3 ± 13.1

* $P < 0.05$ significantly different from cerebellum by ANOVA (Student–Newman–Keuls test); † $P < 0.05$ significantly different from striatum by ANOVA (Student–Newman–Keuls test); ‡ $P < 0.05$ significantly different from cerebellum by Student's *t*-test.

significance. PCP and (±)-ketamine were not tested in striatum, and only PCP showed a significantly lower

affinity to cerebellar receptors although a smaller trend was apparent for (±)-ketamine. Memantine and amantadine showed no significant differences between the affinities in the different brain regions (Table 2).

Autoradiographic studies on rat brain sections

Distribution and density of total [³H]MK-801 binding using *in vitro* autoradiography was in good correlation to previous studies (Sakurai *et al.*, 1991). The highest density was detected in the CA1 region of the hippocampus with 624 ± 68 fmol/mg tissue, followed by dentate gyrus (577 ± 51 fmol/mg tissue), cortex (471 ± 28 fmol/mg tissue), CA3 of hippocampus (442 ± 63 fmol/mg tissue) and the lowest density in striatum (316 ± 52 fmol/mg tissue).

For calculation of K_i values analysis was limited to a single class of binding site. Under conditions where the radioligand [³H]MK-801 was displaced by increasing

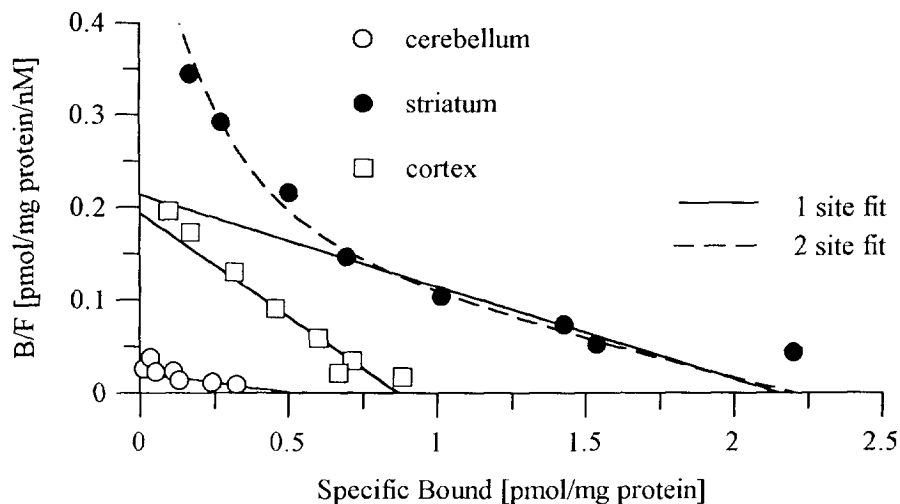


Fig. 2. Scatchard analysis of [³H]MK-801 binding from homogenized rat cerebellum, striatum, and cortex. Data are given as mean values from at least 3 experiments. Experiments were performed under equilibrium conditions in the presence of 10 μM glycine and 10 μM glutamate.

Table 3. Displacement of [³H]MK-801 binding (stimulated with 10 μM glycine and 10 μM L-glutamate) in rat brain sections with *in vitro* autoradiography. K_i values were calculated using the equation $K_i = IC_{50}/(1 + ([L]/K_D))$ (Cheng and Prusoff, 1973)

Brain region	(+)-MK-801 K_i [μM]	Competitor memantine K_i [μM]	amantadine K_i [μM]
Parietal cortex	0.0089 ± 0.0017	1.73 ± 0.245	56.0 ± 4.14*
<i>Hippocampus</i>			
CA1	0.0084 ± 0.0016	1.37 ± 0.246	56.1 ± 3.82*
CA3	0.0108 ± 0.002	1.32 ± 0.207	60.2 ± 5.17*
DG	0.0272 ± 0.0066	2.42 ± 0.271	90.1 ± 8.98
Striatum	0.0204 ± 0.013	1.78 ± 0.530	76.3 ± 9.19

* $P < 0.05$, significantly different from dentate gyrus by ANOVA (Student–Newman–Keuls test).

concentration of unlabeled (+)-MK-801 and assuming that both bind with the same affinity, K_D values were calculated according to the equation $K_D = IC_{50} - [L]$, where $[L]$ is the radioligand concentration (DeBlasi *et al.*, 1989).

In cortex and striatum we detected K_i values that were 2–4 times higher in autoradiographic studies as compared to those found in homogenized membrane preparations. Again there was a decrease of affinity of (+)-MK-801 to striatal membranes compared to cortical membranes, an effect not seen with memantine or amantadine. However, the differences were only close to significance if analyzed by the Student–Newman–Keuls test which is more appropriate for this kind of analysis (Table 3).

In hippocampal regions all compounds showed the lowest affinity in dentate gyrus but the affinity in CA1 and CA3 regions of the hippocampus was comparable to the affinities detected in cortex. Like Sakurai and coworkers (1991) using 5 nM [³H]MK-801 we were also unable to obtain sufficient specific binding in cerebellum to give reproducible values. This might also indicate the existence of a low affinity site in this structure.

However, our assay conditions in autoradiographic experiments did not allow us to calculate Hill coefficients and to make any assumptions about high- and low-affinity binding. Furthermore the use of shorter rinse times would have been necessary to accurately assess a potential low affinity site. It therefore did not seem productive to calculate Hill coefficients or saturation curves for autoradiographic studies.

DISCUSSION

The NMDA receptor–channel complex exists in multiple forms which probably have different physiological and pharmacological properties and are differentially distributed throughout the brain (e.g. Hollmann and Heinemann, 1994). The total of NMDAR1 subunits which are suggested to be necessary to produce functional NMDA receptor ion-channels are expressed ubiquitously in all neural cells throughout the rat brain with particularly high levels in cerebellum, hippocampus, cerebral cortex and olfactory bulb (Moriyoshi *et al.*, 1991; Nakanishi, 1992). *In situ* hybridization revealed

different expression for NMDAR2 mRNA and the subunits have been found to be more restricted to certain brain areas. NMDAR2A mRNA is distributed uniformly like the total of NMDAR1 subunits with highest densities occurring in hippocampal regions and NMDAR2B is expressed predominantly in forebrain but not in cerebellum where NMDAR2C predominates (Meguro *et al.*, 1992; Monyer *et al.*, 1992; Watanabe *et al.*, 1993). The NMDAR2D subunits were found to be most abundant in cerebellum, brain stem and olfactory bulb (Nakanishi, 1992).

Receptor binding studies on homogenized membranes of different brain regions and autoradiographic techniques on brain sections were used to evaluate further this concept of different NMDA receptor subtypes. Results from saturation experiments on homogenized membranes with [³H]MK-801 revealing single classes of binding sites in cortex and cerebellum were in accordance with findings described previously (Wong *et al.*, 1988; Ebert *et al.*, 1991; Reynolds and Palmer, 1991). The finding of this study that [³H]MK-801 binds with significantly lower affinity to cerebellum than other brain regions is also in agreement with findings previously reported and further supports the existence of pharmacologically distinct receptor subpopulations (Ebert *et al.*, 1991; Beaton *et al.*, 1992). Competition studies on rat cortical membranes revealed affinities comparable to those described in earlier studies (Wong *et al.*, 1988; Danysz *et al.*, 1994) and to those detected in human cortical membranes (Kornhuber *et al.*, 1991). The relatively weak displacement of [³H]MK-801 binding in cerebellum by a variety of dissociative anesthetics may add support for the existence of a distinct low-affinity subpopulation of NMDA receptors in cerebellum. In contrast, for memantine or amantadine unchanged binding properties were obtained in this brain region as compared to cortex. Assay conditions applied for autoradiographic studies using 5 nM [³H]MK-801 were unfavorable to detect sufficient specific binding in cerebellum (see also Sakurai *et al.*, 1991). However, even this observation does not preclude the existence of a low-affinity subpopulation of NMDA receptors in the cerebellum. As mentioned above the application of *in situ* hybridization techniques has revealed that especially NMDAR2C and NMDAR2D subunits are expressed in the cerebellum (Nakanishi, 1992). Taking this into consideration, memantine and amantadine may bind with the same or even higher affinity to these subunits than to those preferentially expressed in the forebrain whereas (+)-MK-801 may bind better to the subunits abundant in the forebrain. Indeed, for (+)-MK-801 this suggestion is supported by a recent study of Yamakura and co-workers (1993). Using mouse NMDA receptor subunits expressed in *Xenopus* oocytes they could demonstrate that $\epsilon 1/\zeta 1$ and $\epsilon 2/\zeta 1$ channels (rat counterparts are the heteromeric channels NMDAR2A/NMDAR1 and NMDAR2B/NMDAR1, respectively) are more sensitive to (+)-MK-801 than $\epsilon 3/\zeta 1$ and $\epsilon 4/\zeta 1$ (NM-

DAR2C/NMDAR1 and NMDAR2D/NMDAR1). Furthermore it has been suggested that the observation that a few low-affinity channel-blockers like amantadine and memantine which bind with similar affinity to cortical and cerebellar binding sites in contrast to high-affinity antagonists like (+)-MK-801 may be attributable to preferential binding to certain receptor subtypes (Porter and Greenamyre, 1995). In addition, these findings may be the basis for the development of drugs that are therapeutically tolerated. Memantine and amantadine have been used therapeutically in man for many years and such clinical experience adds support to this hypothesis as both possess much less psychotomimetic activity than, e.g., PCP or ketamine. However, with the exception of a recent study by Prado de Carvalho and co-workers (1993), which may lead to the suggestion that memantine may be more potent at NMDAR1A/NMDAR2C and NMDAR1B/NMDAR2C subunit combinations expressed in oocytes as compared to other subunits, to date no data concerning the subunit specificity of memantine and amantadine are available.

Another common property of low-affinity NMDA receptor antagonists has previously been reported. Memantine and amantadine block and unblock the channel with faster kinetics than high affinity channel blockers like MK-801 and may thereby be able to selectively reduce pathological damage whilst leaving physiological processes essentially intact (Chen *et al.*, 1992; Parsons *et al.*, 1993, 1994).

The present receptor binding studies on membrane homogenates provide the first clear evidence for the existence of two binding sites for [³H]MK-801 in the striatum. This finding contrasts to those reported by others who detected a single class of binding sites in the striatum (e.g. Reynolds and Palmer, 1991; Porter and Greenamyre, 1995). The reason for this discrepancy may be the fact that these authors determined the saturation parameters (K_D and B_{max}) using a fixed radioligand concentration, a condition which may be unfavorable to determine a high- and low-affinity binding site (DeBlasi *et al.*, 1989).

The differences in the relative potencies of (+)-MK-801, amantadine and memantine in striatum and hippocampus were only small and differ somewhat to those reported in some previous studies (Parsons *et al.*, 1994; Porter and Greenamyre, 1995). As such it would be unreasonable to draw any firm conclusions as to their possible relevance. In the present study in hippocampal regions all compounds exhibit the lowest affinity to the PCP binding site in the dentate gyrus. Since adding 10 μ M glycine and 10 μ M L-glutamate to the incubation medium in autoradiographic studies should saturate the agonistic binding sites of the NMDA receptor complex equally throughout the brain the reported observations should not be due to heterogeneous distribution of endogenous ligands. K_i values found for memantine and (+)-MK-801 in rat hippocampal brain regions are in

good correlation to those recently reported for human hippocampus by Berger and co-workers (1994).

Interestingly, the K_i values obtained with autoradiographic studies on brain sections were in general higher than those detected in receptor binding studies. This finding may further support the existence of an endogenous inhibitor of [³H]MK-801 binding which may still be present in autoradiographic experiments where shorter rinsing times are applied (Porter and Greenamyre, 1994).

In conclusion, the present results indicate the presence of two [³H]MK-801 binding sites in the striatum and single, but different, low-affinity sites in cerebellum and cortex which suggest heterogeneity of NMDA receptors. Considering the different affinities of the uncompetitive NMDA receptor antagonists in the various brain regions, their different pharmacological properties could be due to preferential binding to distinct subunit compositions of the NMDA receptor complex. The decreased affinity of high-affinity channel blockers in cerebellar membranes could be related to a decreased affinity to NMDA receptors containing NMDAR2C and/or NMDAR2D subunits which are abundant in this structure (Nakanishi, 1992). This is clearly not the case for memantine and amantadine which bind with the same affinity to this brain region. Considering that distinct subtypes of the NMDA receptor-channel complex are unevenly distributed throughout the brain and may be differentially involved in neurological disorders, it could be of crucial importance for the development of antagonists specific for receptor subtypes. Moreover, the differences in the subunit selectivity of 1-amino-adamantanes and various psychotomimetic agents may provide important new insights into the development of safe therapeutic agents.

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