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Quantitative autoradiography of [^{3}H]-MK-801 binding sites in mammalian brain

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1 An *in vitro* receptor autoradiography procedure is described for visualizing binding sites for the excitatory amino acid antagonist radiolabelled MK-801, in rat and gerbil brain sections.

2 Ten micron sections were labelled by incubation at room temperature for 20 min in 30 nM [^{3}H]-MK-801. This was followed by 2 rinses for 20 s in fresh buffer solution. Specifically bound ligand determined with 100 μM unlabelled MK-801 amounted to 55–60% of total.

3 Phencyclidine, (±)-SKF 10047, ketamine and 2-aminophosphonovaleric acid (APV) (all 100 μM) prevented the specfic binding of [^{3}H]-MK-801. L-Glutamate and N-methyl D-aspartate (NMDA) (100 μM) had no effect. However, L-glutamate prevented the inhibition by APV.

4 The highest concentrations of [^{3}H]-MK-801 binding sites occurred in the hippocampal formation, cerebral cortex, olfactory bulb and thalamus. Very low levels were detected in the brain stem and cerebellum.

5 The distribution of [^{3}H]-MK-801 binding sites was comparable to that of NMDA sites and phencyclidine sites (labelled with [^{3}H]-TCP) but not with high-affinity σ sites labelled with [^{3}H]-3-PPP.

6 The density of [^{3}H]-MK-801 binding sites in the gerbil hippocampus was examined 1, 2, 6 and 22 days after unilateral carotid artery occlusion for 10 min. Only at 6 and 22 days was the binding reduced (by 36% and 46% respectively) in the CA1 region whereas a significant neuronal loss was apparent at day 2. In CA2 a decrease in binding was only evident at day 22.

7 These results indicate that binding sites for [^{3}H]-MK-801 can be detected in mammalian brain sections by receptor autoradiography. Their distribution supports an association with the NMDA receptor complex and the loss in the hippocampus after carotid artery occlusion indicates their presence on pyramidal cells is vulnerable to ischaemic insult.

Introduction

Current evidence points to a heterogeneity of functional receptors for the excitatory amino acid neurotransmitter, L-glutamate. Three receptor types have been described and these have been defined on the basis of the agonists which selectively activate them, quisqualate, kainate and N-methyl D-aspartate (NMDA) (Watkins & Evans, 1981; McLennan 1983; Fagg *et al.*, 1986). The receptor subtypes cannot be classified so readily by specific antagonism although selective blockade of NMDA sites has been documented (e.g. Watkins & Evans, 1981; Evans *et al.*, 1982). Competitive antagonists for this receptor type have been described including 2-aminophosphonovaleric acid (APV), aminophosphonoheptanoic acid (APH) and 3-((+)2-carboxypiperazine-4-yl) propyl-1-phosphonic acid (CPP) (Perkins *et al.*, 1982; Olverman *et al.*, 1984; Harris *et al.*, 1986). In addition drugs classified as dissociative anaesthetics are also selective NMDA antagonists but they appear to act non-competitively and not directly at the NMDA recognition site (Anis *et al.*, 1983; Harrison & Simmonds, 1985). A compound found recently to be in this class is the novel anticonvulsant MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) (Clineschmidt *et al.*, 1982) which is a potent 'use-dependent' antagonist of NMDA-mediated depolarizations in neurones of the mammalian central nervous system (Wong *et al.*, 1986; Kemp *et al.*, 1986). It produces a

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selective blockade *in vivo* as well as *in vitro* and is active after peripheral administration (Foster *et al.*, 1987). Binding sites for tritium-labelled MK-801 have been demonstrated in mammalian brain and the pharmacological specificity and affinity of these binding sites correlates with that of the antagonist site detected by electrophysiological experiments (Wong *et al.*, 1986; Wong & Woodruff, 1986).

The primary purpose of the present study was to determine in detail the location of these binding sites for [^{3}H]-MK-801 within the mammalian brain and to establish their pharmacological relevance by making comparison with the distribution of NMDA sites. To achieve this an *in vitro* quantitative receptor autoradiography technique has been developed using rat and gerbil brain sections. The effects of neuronal damage produced by unilateral occlusion of the carotid artery on the distribution of [^{3}H]-MK-801 binding sites in the gerbil hippocampus was also investigated.

Part of this work has been previously communicated to the British Pharmacological Society (Bowery & Hudson, 1986).

Methods

Receptor autoradiography

Sprague-Dawley rats (200–250 g) or mongolian gerbils (50–70 g) were culled by cervical dislocation or perfused-fixed with 0.1% paraformaldehyde in 0.01 N phosphate buffered saline under pentobarbitone anaesthesia (40 mg kg^{-1}, i.p.). Ten micron cryostat sections of each brain were mounted on glass slides and stored frozen at −20°C for up to 2 weeks. Thawed sections were rinsed in Tris-HCl buffer (pH 7.4, 50 mM containing 190 mM sucrose) and allowed to air dry. After drying 100 µl of Tris buffer solution containing 30 nM [^{3}H]-MK-801 (22.5 Ci mmol^{-1}) with or without unlabelled drug was applied over each section at 23°C. This was left in contact for 20 min unless stated otherwise. After incubation the radiolabelled solution was rapidly aspirated off and the section rinsed twice for 2 × 20 s duration in 250 ml fresh Tris buffer solution at 23°C.

The dried sections were then placed in contact with LKB tritium-sensitive Ultrofilm for 3–4 weeks at 4°C. Non-specific binding was determined by addition of 100 µM unlabelled MK-801 to the incubation solution. To determine the optimal conditions for binding, sections were immersed in Hydrofluor scintillation solution after labelling with [^{3}H]-MK-801 and the bound radioactivity measured by scintillation spectrometry.

Densitometric analysis of autoradiograms

The photographic images obtained on LKB Ultrofilm were analysed by use of a computerized routine on a Quantimet 920 image analyser (Cambridge Instruments Plc). Regional densities were converted to the corresponding ligand concentration by reference to tritium brain paste standards prepared according to the method of Unnerstall *et al.* (1982).

[^{3}H]-MK-801 binding to synaptic membranes

Cerebral cortices from male Sprague-Dawley rats were homogenized in 9 volumes of ice-cold sucrose solution (0.32 M) by 10 strokes in a Potter-Elvejhem homogenizer. The homogenate was centrifuged for 10 min at 1000 *g* and the supernatant recentrifuged at 10000 *g* for 20 min at 4°C. The pellet was then suspended in Tris-HCl buffer (50 mM pH 7.4) and incubated for 20 min at 23°C before centrifuging at 10000 *g* for 20 min at 4°C. The pellet was resuspended in Tris-HCl buffer (1 g original tissue equivalent/55 ml). Aliquots (750 µl) of the membrane suspension (~0.5 mg protein) were mixed with 250 µl buffer solution containing [^{3}H]-MK-801 (final concentration 5 nM) with or without unlabelled displacer. The mixture was incubated at 23°C for 60 min. The incubation was terminated by rapid filtration through Whatman GF/B filters and washed twice with 5 ml aliquots of fresh ice-cold buffer. Radioactivity associated with the filters was determined by liquid scintillation spectrometry. Non-specific binding was determined with 100 µM MK-801.

Ischaemia-induced neuronal damage in gerbils

Gerbils were anaesthetized with halothane (2% in nitrous oxide, oxygen mixture) and the left carotid artery exposed. A clip was applied over this artery to occlude the blood flow for 10 min. After removal of the clip the incision was sealed with suture clips and the animal allowed to recover. The animals were killed 1, 2, 6 or 22 days later and 10 µm transverse sections cut at −15°C. Sections were either labelled with [^{3}H]-MK-801 as described above or stained with cresyl fast violet to check the degree of neuronal damage. The hippocampal staining or binding densities on the occluded left side were expressed as a percentage of that on the control right side in the same section. No statistically significant difference was observed between the staining or binding densities in the left and right hippocampi of the same section from sham-operated animals. Statistical analyses (Student's *t* test) were performed on density measurements before expressing the data as percentage of control side.

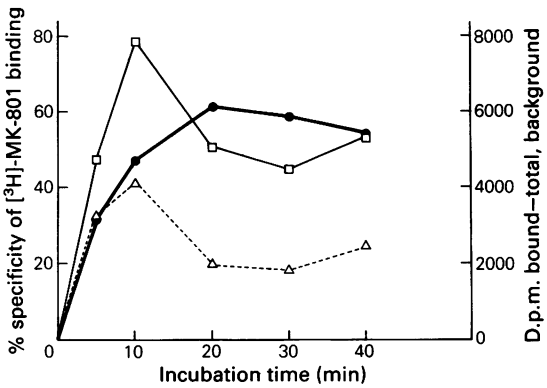

Figure 1 Time course of the binding of [^{3}H]-MK-801 to transverse sections of rat brain. Ten micron sections were incubated at 23°C in [^{3}H]-MK-801 (30 nM) for the time periods indicated on the abscissa scale. At the end of the incubation period the sections were rinsed twice for 20 s in fresh buffer solution at 20°C. The radioactivity associated with the sections was then assayed by liquid scintillation spectrometry. Each point represents the mean value from 3 batches of 4 sections. The s.e. mean was <5% of the mean in all cases. Total binding (□) was determined in [^{3}H]-MK-801 alone whereas the background values (△) were determined in the additional presence of unlabelled MK-801 (100 μM). The values representing specific binding (●) (total − background) have been calculated as a percentage of total binding.

Materials

[^{3}H]-MK-801 was prepared by tritium-halogen exchange of the 7-bromo derivative of (±)-MK-801. Phencyclidine and (±)-SKF 10047(N-allylnormetazocine) were gifts from the National Institute of Drug Abuse (Baltimore, MD) and ketamine was obtained from Parke Davis. Chemicals were obtained from commercial services.

Results

Optimizing the experimental conditions for [^{3}H]-MK-801 receptor autoradiography

To establish the incubation and rinse conditions required to maximize the detection of specific [^{3}H]-MK-801 binding, sections were analysed by liquid scintillation spectrometry. Varying the incubation time between 5 and 40 min at 23°C showed that maximal specific binding was obtained by 20 min as illustrated in Figure 1. No further increase in binding occurred after this time and it was therefore chosen for all subsequent incubations. The optimal rinse time was 2 periods of 20 s in Tris-HCl buffer at 23°C. After this time specifically bound ligand amounted to 55–60% of the total radioactivity associated with each section. Specific binding could still be detected even after 2 × 60 s rinses but this was approximately 50% of that achieved after 2 × 20 s and amounted to 45% of total binding. Periods less or greater than 2 × 20 s reduced the specific:background ratio still further. Rinsing the sections in ice-cold buffer failed to improve the binding ratio.

A reduction in the Tris-HCl buffer concentration from 50 mM to 5 mM doubled the total binding of [^{3}H]-MK-801 (30 nM) to sections at all rinse times. However, the background levels were also greatly enhanced. Thus a low Tris concentration offered no advantage.

Table 1 Inhibition of [^{3}H]-MK-801 binding to fresh cerebral cortex synaptic membranes from rat brain

	K_i (nM)	*nH*	n
MK-801	15.0 ± 2.2	0.93 ± 0.04	3
Phencyclidine	269 ± 13.7	0.96 ± 0.03	3
Ketamine	1740 ± 160	0.95 ± 0.02	3
(±)-SKF 10047	2544 ± 88	0.99 ± 0.02	3

Membranes were incubated with 5 nM [^{3}H]-MK-801 as described in Methods. Results are presented as mean ± s.e. mean of *n* determinations. Potencies of inhibition (IC_{50}) and Hill coefficients were measured from data obtained using at least five concentrations of drug (in duplicates), by computer-assisted curve fitting. K_i values were calculated from IC_{50} values based on the Chang-Prusoff (1973) equation.

Pharmacology of [^{3}H]-MK-801 binding sites in brain sections and homogenates

The specific binding of [^{3}H]-MK-801 to brain sections was inhibited by the addition of phencyclidine (PCP), (±)-SKF 10047, ketamine or 2-aminophosphonovaleric acid (APV; all 100 μM) to the incubation solution. L-Glutamate and N-methyl D-aspartate (NMDA; 100 μM) failed to reduce the binding of [^{3}H]-MK-801 but the presence of L-glutamate did prevent the inhibition by APV.

These data compare favourably with those obtained using fresh cerebral cortex synaptic membranes in 50 mM Tris-HCl solution. MK-801 was the most potent inhibitor followed by PCP, ketamine and (±)-SKF 10047 (Table 1). Neither L-glutamate nor NMDA (100 μM) altered the binding of [^{3}H]-MK-801 to the synaptic membranes. However, if

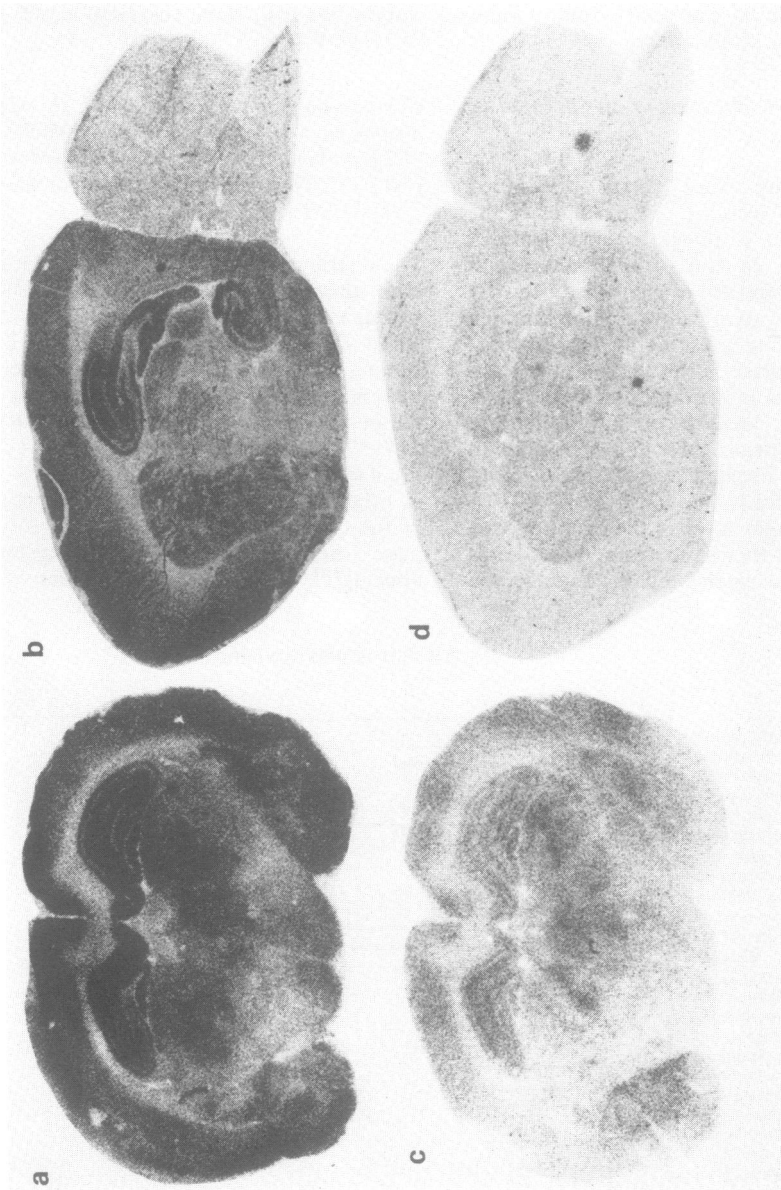

Figure 2 Receptor autoradiographs of [^{3}H]-MK-801 binding to rat brain sections. Transverse (a and c) or parasagittal (b and d) sections were incubated in 30 nM [^{3}H]-MK-801 for 20 min at 23°C. Sections (c and d) were incubated additionally in 100 μM unlabelled MK-801 to obtain background levels. Note the high density binding in the hippocampus in particular the CA1 and dentate gyrus molecular layer.

[^{3}H]-MK-801 binding was first lowered by a high concentration of APV (IC_{50}: 250 μM) this apparent inhibition was reversed by L-glutamate in a dose-dependent manner.

Similar pharmacological characteristics were observed in membranes prepared from hippocampus, striatum and medulla-pons.

Distribution of [^{3}H]-MK-801 binding sites in rat and gerbil brain

[^{3}H]-MK-801 binding sites were distributed unevenly in rat brain sections. An example of the pattern of distribution is illustrated in Figure 2. Total binding (Figure 2a and b) and background levels obtained in the presence of unlabelled MK-801 (100 μM) (Figure 2c and d) are shown. Note the high concentration of binding sites in the hippocampal formation, cerebral cortex, striatum and thalamus with much lower levels in the brain stem and cerebellum. Densitometric analysis of autoradiograms from serial sections (transverse and parasagittal) obtained from 3 rats indicated that the highest concentration of specifically bound [^{3}H]-MK-801 occurred in the molecular layer of the dentate gyrus (173 fmol mg^{-1} tissue). By contrast the lowest levels were detected in more caudal regions such as the pons and central grey (10 and 15 fmol mg^{-1} respectively). A summary of the mean data from 26 brain regions is shown in Figure 3. Clearly the hippocampus is richly endowed with MK-801 binding sites throughout its structure and this focal concentration has also been observed in the gerbil brain (see Figure 7).

Comparison of the distribution of [^{3}H]-MK-801 binding sites with those of NMDA (labelled with [^{3}H]-glutamate) phencyclidine (labelled with [^{3}H]-TCP) and high-affinity σ opiate (labelled with [^{3}H]-3-PPP) sites

The distributions of NMDA and phencyclidine sites have already been shown by others (Monaghan & Cotman 1985; Gundlach *et al.*, 1986a; Largent *et al.*, 1986; Contreras *et al.*, 1986). Table 2 provides a summary of their distributions together with those obtained in the present study. In order to compare the published data with the present values for [^{3}H]-MK-801 the density of binding in the CA1 radiatum in all studies has been designated 100%. The density in other brain regions was then expressed as a % of the CA1 value. The distributions of [^{3}H]-glutamate and [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]-TCP) were very similar. This is illus-

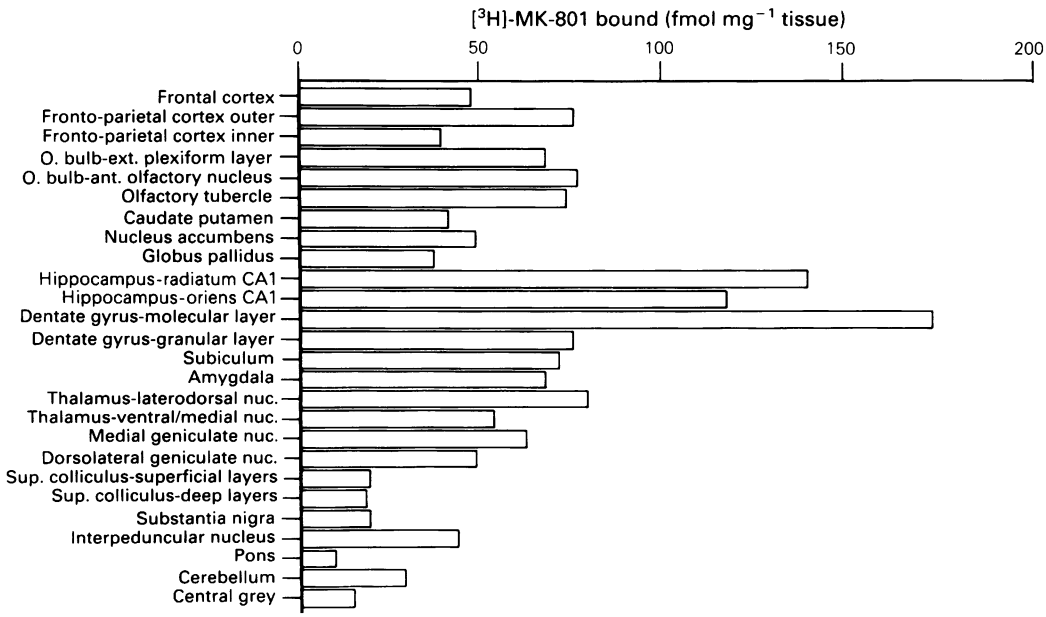

Figure 3 Comparative regional densities of [^{3}H]-MK-801 binding sites in rat brain sections. Density values for each brain region were determined from 3 brain sections each from 3 rats. The values were measured by densitometric analysis with reference to brain paste standards apposed to the same films. Background binding was subtracted in all cases.

Table 2 Relative distributions of [^{3}H]-MK-801, [^{3}H]-glutamate (NMDA) and [^{3}H]-3-PPP (high-affinity σ opiate) binding sites in mammalian brain

Region	*Reference number Figures 4, 5 & 6*	[^{3}H]-*MK-801*	[^{3}H]-*glutamate*	[^{3}H]-*TCP*	[^{3}H]-*3-PPP*
Frontal cortex	1	34.1	65.8	45.7	184.8
Frontoparietal cortex					
outer	2	53.7	65.2	60.2	204.3
inner	3	27.4	40.4	42.9	
Olfactory bulb					
external plexiform layer	4	48.3	33.9	32.4	NA
anterior olfactory nucleus	5	54.8	77.7	41.1	NA
Olfactory tubercle	6	52.3	59.3	41.3	NA
Caudate putamen	7	28.7	50.7	30.1	159.3
Nucleus accumbens	8	35.0	61.3	35.4	152.2
Globus pallidus	9	26.5	8.2	15.8	167.4
Hippocampus					
CA1 radiatum	10	*100*	*100*	*100*	*100*
Dentate gyrus					
molecular layer	11	123.7	78.2	79.8	NA
granular layer	12	53.7	NA	28.2	274.0
Subiculum	13	50.8	34.4	31.8	313.1
Amygdala	14	48.0	50.9	48.2	232.6
Thalamus					
laterodorsal nucleus	15	56.8	31.2	27.3	217.4
ventral/medial nucleus	16	38.4	26.6	25.7	202.0
Medial geniculate nucleus	17	44.5	37.5	41.7	NA
Dorsolateral geniculate nucleus	18	35.1	35.4	36.4	NA
Superior colliculus					
superficial layer	19	13.4	18.0	28.0	345.6
deep layer	20	12.8	10.2	14.0	NA
Substantia nigra	21	13.2	8.1	16.9	373.9
Interpeduncular nucleus	22	31.2	13.5	6.7	263.0
Pons	23	6.9	7.2	2.8	671.7
Cerebellum	24	20.8	15.0	11.5	300.0
Central grey	25	10.5	13.6	14.3	582.6

Data for [^{3}H]-glutamate derives from Monaghan & Cotman (1985).
Data for [^{3}H]-TCP ([^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine) derives from Gundlach *et al.* (1986a).
Data for [^{3}H]-3-PPP((+)-[^{3}H]-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine) derives from Gundlach *et al.* (1986b).
[^{3}H]-MK-801, [^{3}H]-glutamate and [^{3}H]-TCP data are from rat brain sections whereas [^{3}H]-3-PPP data are from guinea-pig brain. However, the distribution of [^{3}H]-3-PPP sites in rat brain is identical (Gundlach *et al.*, 1986b).
Values are expressed as a % of the ligand density in the CA1 radiatum reported for each study: 100 for [^{3}H]-MK-801 = 141 pmol g^{-1} tissue, [^{3}H]-glutamate = 1.29 pmol mg^{-1} protein, [^{3}H]-3-PPO = 0.046 pmol mg^{-1} protein, [^{3}H]-TCP = 0.525 pmol mg^{-1} protein.
NA = not available.

trated more clearly in Figures 4 and 5 in which the relative densities from a sample of 25 brain regions have been plotted as correlelograms. Figure 4 demonstrates the comparison between NMDA and MK-801 sites and Figure 5 MK-801 and phencyclidine (labelled with [^{3}H]-TCP) sites giving correlation coefficients for the regression lines of 0.78 and 0.85, respectively.

By comparison the distribution of σ opiate sites as labelled by (+)-[^{3}H]-SKF 10,047 and (+)-[^{3}H]-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ([^{3}H]-3-PPP; Gundlach *et al.*, 1985; 1986b) did not correlate with that of [^{3}H]-MK-801 (Table 2 and Figure 6). Although the [^{3}H]-3-PPP values were derived from guinea-pig brain sections the values for rat brain are apparently the same (Gundlach *et al.*, 1986b).

Effect of ischaemic insult on hippocampal levels of [^{3}H]-MK-801 binding sites

The density of [^{3}H]-MK-801 binding sites in hippocampus was examined in gerbil brain sections 1, 2, 6 and 22 days after unilateral carotid artery occlusion

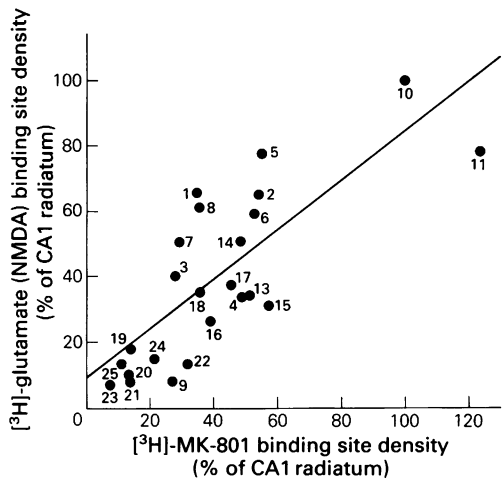

Figure 4 Comparison of the distributions of binding sites for [^{3}H]-MK-801 and [^{3}H]-glutamate (labelling N-methyl D-aspartate (NMDA) sites) in rat brain sections. The values for each brain region derive from columns 3 and 4 of Table 2. The numbers associated with each symbol corresponds to the individual brain regions indicated in column 2 of Table 2. The line has been fitted by regression analysis. The correlation coefficient (r) = 0.78. The data for [^{3}H]-glutamate are from Monaghan & Cotman (1985).

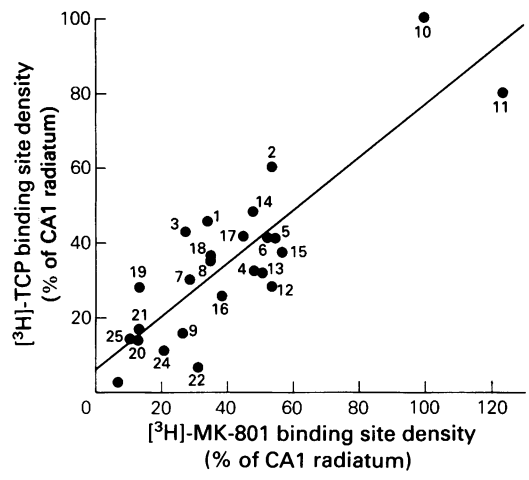

Figure 5 Correlation between the regional distributions of [^{3}H]-MK-801 and [^{3}H]-TCP binding sites in rat brain sections. The binding values expressed as a percentage of the CA1 radiatum level derive from columns 3 and 5 of Table 2. The correlation coefficient (r) for the regression line is 0.85. The data for [^{3}H]-TCP are from Gundlach et al. (1986a).

for 10 min. A summary of the results is shown in Table 3. Neuronal loss, determined by comparing the stain (cresyl fast violet) densities on the occluded and normal sides, was significantly reduced by day 2 in the CA1 and CA2 regions and was still evident after 6 (Figure 7c) and 22 days. There was a significant loss of neurones in CA3 and CA4 at 2 days but this was not apparent at 6 and 22 days.

A significant decrease in [^{3}H]-MK-801 binding occurred only 6 days (in CA1) (Figure 7a) and 22 days (in CA1) after surgery (Table 3). The apparent loss of neurones after 2 days in CA3 and CA4 was reflected by decreases in [^{3}H]-MK-801 binding at this time. However, after 22 days the density of binding sites in CA3 and CA4 was no longer significantly different from control.

Discussion

Binding sites for [^{3}H]-MK-801 have been detected in rat brain sections by a quantitative receptor autoradiography technique. These sites exhibited the same pharmacological specificity as observed in synaptic membranes prepared from rat brain (Wong et al., 1986). The inability of NMDA to displace the binding of [^{3}H]-MK-801 in brain sections supports the notion that the MK-801 and NMDA receptor recognition sites are pharmacologically distinct (Wong et al., 1986, 1988; Foster & Wong, 1987). However, NMDA receptor activation seems to be necessary for the binding of [^{3}H]-MK-801 to occur (Foster & Wong, 1987) and is consistent with the use-dependent nature of the blocking action of MK-801 (Kemp et al., 1986). The apparent inhibition of [^{3}H]-MK-801 binding in brain sections by APV could be explained therefore by antagonism of the stimulatory effect of an endogenous agonist(s) for the NMDA recognition site.

[^{3}H]-MK-801 binding sites were detected in many locations within the brain but their distribution was heterogeneous. The hippocampal formation contained the highest concentration of MK-801 binding sites notably in the dentate gyrus and CA1 region. In this latter region binding was localized over the dendritic layers of the radiatum and oriens with much lower levels over the pyramidal cell layer. This high level in the hippocampus concurs with the previous observations in membranes prepared from rat brain regions (Wong et al., 1986, 1988). The overall pattern of distribution was comparable to that described for other ligands of the NMDA receptor complex, i.e. [^{3}H]-L-glutamate, [^{3}H]-(±)-CPP and D-[^{3}H]-APV (Olverman et al., 1984; Monaghan & Cotman, 1985; Monaghan et al., 1985; Murphy et al., 1987). It has previously been pointed out by Maragos et al. (1986) that the distributions of NMDA and PCP/TCP

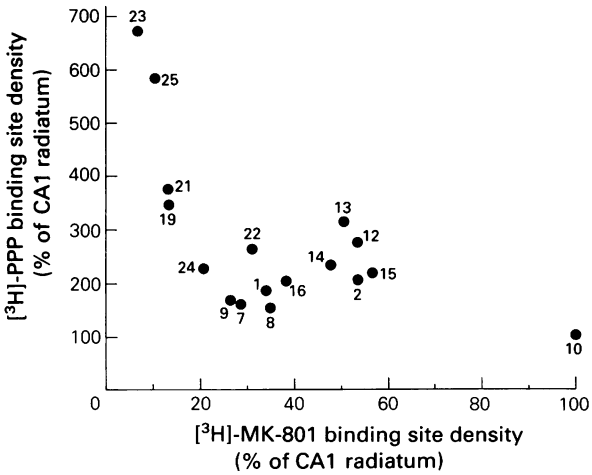

Figure 6 The lack of correlation between the distributions of [^{3}H]-MK-801 binding sites and those for the high-affinity σ opiate ligand [^{3}H]-3-PPP. The values derive from columns 3 and 6 of Table 2. The values for [^{3}H]-3-PPP were obtained from Gundlach *et al.* (1986b) who used guinea-pig brain sections. However, the pattern of binding is apparently the same as in rat brain sections (Gundlach *et al.*, 1986b).

receptors are the same in rat forebrain and sites labelled by the competitive NMDA receptor antagonist, D-[^{3}H]-APV, also show the same pattern (Monaghan *et al.*, 1984). Other autoradiographic studies using [^{3}H]-TCP are in agreement with this observation (Contreras *et al.*, 1986; Gundlach *et al.*, 1986a). Indeed, detailed pharmacological characterization of [^{3}H]-MK-801 and [^{3}H]-TCP binding sites in cortical membranes suggested an identical specificity (Wong *et al.*, 1987). Furthermore, both [^{3}H]-TCP and [^{3}H]-MK-801 binding are modulated by NMDA receptor activation (Loo *et al.*, 1986; Fagg, 1987; Foster & Wong, 1987). These similarities with [^{3}H]-MK-801 sites supports the contention that these sites (MK-801/TCP) are coupled to the NMDA receptor complex. In contrast there appears to be no relationship between [^{3}H]-MK-801 sites and the high-affinity haloperidol-sensitive σ opiate sites detected with [^{3}H]-3-PPP (Gundlach *et al.*, 1986b; Largent *et al.*, 1986). The lack of correspondence in regional distribution (Gundlach *et al.*, 1985), together with the dramatic difference in pharmacological specificity between the sites labelled by [^{3}H]-MK-801 and the σ ligand (+)-[^{3}H]-SKF 10,047 (Wong *et al.*, 1987), argues against the involvement of σ sites in the neuropharmacological action of MK-801.

The pattern of distribution of [^{3}H]-MK-801 sites in gerbil brain was similar to that observed in the rat with the highest density also occurring in the hippocampus. The gerbil provides a useful model for studying neuronal damage resulting from ischaemic insult since it lacks a Circle of Willis (Kahn, 1972). Thus unilateral occlusion of the carotid arterial blood supply produces ischaemic damage which is restricted to the occluded side (Harrison *et al.*, 1973; Ito *et al.*, 1975). The brain region which is most sus-

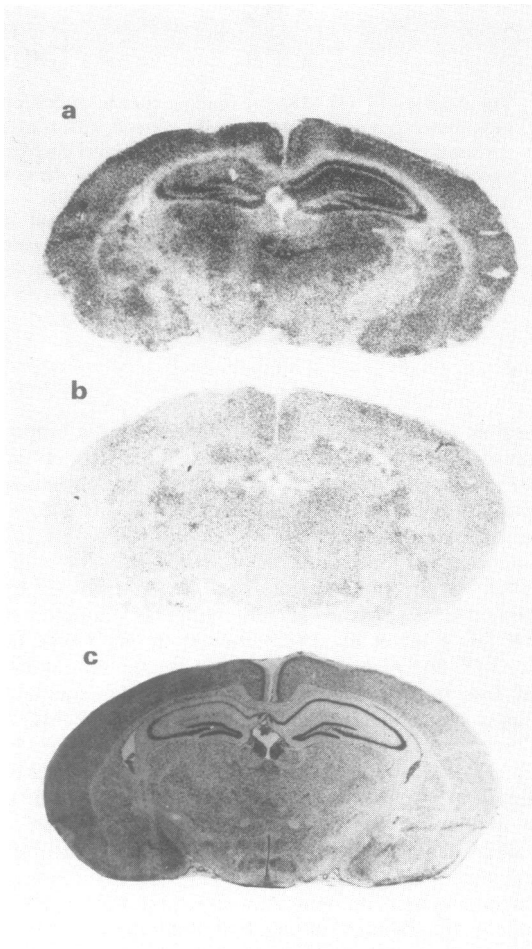

Figure 7 Effect of unilateral carotid artery occlusion on the distribution of binding sites for [^{3}H]-MK-801 in gerbil brain. The sections were obtained from a single gerbil 6 days after 10 min unilateral carotid artery occlusion. The artery supplying the left side of the brain sections shown was occluded. In (a) total [^{3}H]-MK-801 binding is shown, (b) background binding obtained in the presence of 100 μM MK-801 and (c) a section stained with cresyl fast violet to illustrate the decrease in hippocampal CA1 pyramidal cells.

Table 3 Binding of [^{3}H]-MK-801 in gerbil hippocampus following unilateral carotid artery occlusion

Post-surgery (days)	*Hippocampal region* CA1	CA2	CA3	CA4
1	99.5 ± 17.4 (101)	149.0 ± 71.5 (96)	101.6 ± 46.5 (104)	165.4 ± 96.3 (109)
2	92.4 ± 5.6 (67)	102.6 ± 14.1 (77)	58.3 ± 2.0** (74)	59.5 ± 3.9** (74)
6	64.5 ± 7.6* (45)	109.7 ± 8.4 (78)	83.6 ± 3.3 (120)	67.2 ± 1.7** (93)
22	53.7 ± 5.3** (75)	71.9 ± 8.1 (61)	72.7 ± 38.5 (103)	105.3 ± 23.5 (97)

The density of [^{3}H]-MK-801 binding sites in each hippocampal region was determined by image analysis of autoradiograms generated from 3 weeks contact with transverse brain sections. In each case the background density of corresponding sections incubated with unlabelled MK-801 was subtracted. The density of each region from the occluded side was expressed as a percentage of the control side in the same section. Mean values ± s.e. mean are from 3 sections from 3 animals at each time ($n = 9$).

The values in parentheses indicate the mean cresyl fast violet stain density of the pyramidal layer in the same sections with the occluded side again expressed as a percentage of the control side.

*$P < 0.05$; **$P < 0.01$, comparing binding density on lesion side with control side (Student's t test).

ceptible to ischaemic insult appears to be the hippocampus, in particular the CA1 region (Ito *et al.*, 1975). This was readily detected in the present study by histological staining. The neuronal loss is accompanied by an increase in Ca^{2+} deposition within the hippocampus (Sakamoto *et al.*, 1985; Bowery *et al.*, 1987). Both phenomena can be prevented by pretreatment of the gerbils with the antagonist MK-801 (Gill *et al.*, 1987; Bowery *et al.*, 1987). In fact MK-801 also significantly decreases the neuronal loss in rats even when neuronal damage was caused by direct intracranial injections of NMDA (Foster *et al.*, 1987). It was important therefore to determine how long [^{3}H]-MK-801 binding sites remain after carotid occlusion. Binding appeared to be unchanged for at least 2 days even though a marked decrease in neuronal staining was evident at that time. We assume that [^{3}H]-MK-801 binding sites are located on neurones within the CA1 region, since a decrease in binding had occurred by days 6 and 22 after ischaemia. It seems unlikely that glial cells provide the site of [^{3}H]-MK-801 binding. A proliferation of these cells would accompany the neuronal damage and this would produce a rise in the level of binding as observed in the microglial ligand [^{3}H]-Ro5-4864 in rat brain (Schoemaker *et al.*, 1982; Benavides *et al.*, 1987). However, no increase in [^{3}H]-MK-801 binding was observed. Despite a clear reduction in the CA1 neuronal population at 2 days the density of [^{3}H]-MK-801 binding sites was unchanged. Presumably the damaged neurones fail to take up the stain but their membranes, which contain the ligand binding sites, still retain the capacity to bind [^{3}H]-MK-801. Only when the membranes are later degraded does the binding site disappear. Thus the presence of the binding site would not necessarily indicate neuronal functionality if this explanation is correct. However, an alternative explanation might be that while a reduction in cell number occurs, the number of binding sites per individual neurone has increased in response to the ischaemic insult thereby producing little or no apparent change in the overall number of sites. It nevertheless does suggest that [^{3}H]-MK-801 binding might not be a sensitive marker for acute neuronal damage.

One theory of the neurotoxicity produced by ischaemia is that excitotoxins such as the excitatory amino acids are released in excess and this produces neuronal degeneration possibly through NMDA receptor activation (see Mayer & Westbrook, 1987). This could provide the rationale for the effectiveness of NMDA antagonists in preventing neuronal damage (Meldrum, 1985). Such a mechanism could produce a rapid change in the binding characteristics of the NMDA receptor complex. In particular an increase in the binding to modulatory sites would increase the effectiveness of NMDA receptor blockade. Further studies of the binding of [^{3}H]-MK-801 at times immediately after the neuronal insult might therefore provide some important information.

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