Pergamon

Biochemical Pharmacology, Vol. 47, No. 6, pp. 961–967, 1994.

0006-2952/94 $6.00 + 0.00

0006-2952(93)E0075-I

EFFECT OF CHEMICAL MODIFICATION OF ARGINYL AND HISTIDYL RESIDUES ON [^{3}H]MK-801 BINDING TO BRAIN SYNAPTIC MEMBRANES

CAROLINE T. CRILLEY* and ANTHONY J. TURNER†

Department of Biochemistry and Molecular Biology, Leeds University, Leeds LS2 9JT, U.K.

(*Received* 12 *October* 1993; *accepted* 19 *November* 1993)

Abstract—Chemical modification of the *N*-methyl-D-aspartate (NMDA) receptor was investigated in a thoroughly washed Triton-treated pig forebrain membrane preparation. Modification of arginyl residues using phenylglyoxal significantly reduced activation of the NMDA receptor as measured by specific binding of [^{3}H]MK-801 [(+)-5-methyl-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5,10-imine]. The reduction was due to a decrease in the affinity of MK-801 for its binding site from 3.2 ± 0.5 nM to 22 ± 8 nM. Protection studies indicated that alteration of the glutamate or glycine binding sites is not directly responsible for the effect. Diethyl pyrocarbonate treatment also reduced [^{3}H]MK-801 binding, by modification of histidyl residues. The binding affinity was reduced to 8.3 ± 1.4 nM whereas the B_{max} was unchanged. Protection studies indicated that the modified histidine is unlikely to be a component of the glutamate, glycine or MK-801 binding sites. However, the accessibility of the modified histidine seems to be partly dependent on the activation state of the receptor.

Key words: NMDA receptors; diethyl pyrocarbonate; phenylglyoxal; excitatory amino acids; neurotransmitter receptors; glutamate

NMDA‡ receptors mediate excitatory synaptic transmission in the central nervous system. The receptors are involved in learning and memory, and dysfunction may cause neuropathologies such as neurodegeneration and epilepsy. As a result of their vital role in the brain this class of excitatory receptors has been extensively studied. Characterization by electrophysiology and pharmacology has revealed a highly complex receptor [1]. Briefly, the receptor is a ligand-gated cation channel. Binding of glutamate and glycine causes channel opening. A Mg^{2+} binding site is responsible for voltage-dependent block of receptor activation [2]. Activation can also be modulated by the binding of a variety of other ligands. These include Zn^{2+} [3] and H^+ [4], which cause inhibition by non-competitive antagonism. There is also a polyamine/divalent cation binding site at which the ligands induce potentiation of receptor activation at low concentrations and inhibition at high concentrations [5]. The PCP/MK-801 binding site is significant in terms of biochemical studies and has also been exploited in an attempt to treat over-activation of the receptors [6]. PCP and MK-801 are thought to cause blockade by binding to the open channel [2]. Thus, experimentally, activation of the NMDA receptor can be inferred from the extent of their binding.

The aim of this study was to investigate which amino acids in the NMDA receptor are responsible for ligand binding. This would complement the characterization of the ligand features that determine activity at the receptor [7], and may suggest which amino acids would be interesting to alter by site directed mutagenesis. Specific chemical modification of amino acids has been successfully used to probe ligand interactions with the $GABA_A$ benzodiazepine receptor. Arginine has been shown to play a critical role in the binding of muscimol/GABA [8] and histidine appears to be involved in binding of diazepam/flunitrazepam [9].

Some limited studies on modification of NMDA receptors have been reported. Reduction of disulphide bonds by dithiothreitol increases activation of the NMDA receptor in a DTNB reversible manner. DTNB alone also causes a decrease in activation. These studies have been carried out using whole cell [10] and patch-clamp recordings [11] and MK-801 binding assays [12]. The alteration in binding could not be accounted for by modification of a specific ligand binding site on the NMDA receptor. Modification of carboxyl residues using EDAC markedly reduced MK-801 binding activity [13]. This was apparently via alteration of a Mg^{2+} binding site within the channel domain of the NMDA receptor. This correlated with the proposal that the six amino acids at the base of the putative channel domain TM2 are responsible for Mg^{2+} binding [14].

In this study, modification of argininyl and histidyl residues was investigated as both glutamate and glycine contain carboxylate residues which may interact with these residues in the receptor. Receptor

* Present address: Clinical Research Institute of Montréal, Montréal, Canada H2W 1R7.

† Corresponding author. Tel. (0532) 333131; FAX (0532) 423187.

‡ Abbreviations: DEP, diethyl pyrocarbonate; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); EDAC, 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide; GABA, gamma-aminobutyric acid; MK-801, (+)-5-methyl-10,11-dihydro-dibenzo[*a*,*d*]cyclohepten-5,10-imine; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; PMSF, phenylmethylsulphonylfluoride.

activation was monitored using a [^{3}H]MK-801 binding assay.

MATERIALS AND METHODS

(+) [^{3}H]MK-801 (28.8 Ci/mmol) was supplied by New England Nuclear Du Pont Ltd (Stevenage, U.K.). Glass fibre filters were from Whatman Biochemicals Ltd (Kent, U.K.). (+)MK-801 was donated by Dr L. L. Iversen, Merck, Sharp & Dohme Neuroscience Research Centre (Harlow, U.K.). All other chemicals were from the Sigma Chemical Co. (Poole, U.K.).

Fresh pig brains were provided by Asda Farm Stores (Lofthousegate, U.K.) and were obtained within 10 min of death. These were kept on ice and used within 1 hr or frozen at −70°.

Tissue preparation

Crude synaptic membranes were prepared by an adaptation of the methods of Ransom and Stec [5] and Yoneda and Ogita [15]. Briefly, 25 g of pig forebrain were homogenized in 20 vol. 0.32 M sucrose, 50 mM Tris–HCl pH 7.4 in the presence of a cocktail of protease inhibitors, 3 mM EDTA, 5 mg/mL pepstatin A, 0.1 mM PMSF and 5 U/mL trypsin inhibitors II-O and II-S. The homogenate was centrifuged at 1000 *g* for 10 min. The supernatant was removed and centrifuged at 20,000 *g* for 20 min. The pellet was then resuspended using a teflon–glass homogenizer in approximately 10 vol. 20 mM Tris–HCl pH 7.4 containing 2 mM EDTA, 5 mg/mL pepstatin A, 0.1 mM PMSF and 5 U/mL trypsin inhibitors II-O and II-S. The pellet was washed three times by resuspension in 10 vol. 5 mM Tris–HCl pH 7.4 (buffer A) using the teflon–glass homogenizer and centrifugation at 50,000 *g* for 20 min. Finally the pellet was resuspended in approximately 3 vol. of the original homogenization buffer. This suspension was stored at −70° for up to 4 months with no loss of activity.

On the day of the experiment the thawed membranes were subjected to further washing before use. The suspensions were diluted 10 times in buffer A and then centrifuged at 50,000 *g* for 20 min. The pellet was resuspended in buffer A to a protein concentration of 0.35 mg/mL and Triton X-100 was added to a final concentration of 0.04%. After gentle stirring for 10 min at 2° the treatment was terminated by centrifugation at 50,000 *g* for 20 min. These pellets were washed once more in buffer A before use.

Treatment with modifying reagents

2,3-Butanedione [8]. Washed crude synaptic membranes were resuspended in 50 mM Na borate buffer (pH 8.2) at a protein concentration of 1.25 mg/mL. Modification was carried out with 2,3-butanedione (20 mM) freshly prepared in 50 mM Na borate buffer at 25° for 1 hr. The reaction was terminated by centrifugation and resuspension in fresh buffer. Aliquots were assayed for MK-801 binding as described but 50 mM Na borate (pH 8.2) was used instead of buffer A.

Phenylglyoxal [16]. A 20 mM solution of phenyl glyoxal was prepared in 0.1 M $NaHCO_3$ (pH 8.0). Washed crude synaptic membranes were resuspended in 0.1 M $NaHCO_3$ (pH 8.0) at a protein concentration of 2.5 mg/mL. Modification was performed for 40 min at 25°. The reaction was terminated by centrifugation and resuspension in buffer A. After washing in buffer A the membranes were assayed for specific MK-801 binding.

DEP [9]. Washed crude synaptic membranes were resuspended in 20 mM KH_2PO_4 pH 6 at a protein concentration of 0.5 mg/mL. DEP was freshly prepared in ethanol at a concentration of 200 mM. The membranes were treated with 1–3 mM DEP for 30 min at room temperature. The reaction was stopped by dilution with ice-cold buffer A followed by centrifugation at 50,000 *g* for 20 min. The pellets were resuspended in buffer A and the centrifugation was repeated. MK-801 binding was assessed after resuspension of the pellets in buffer A.

DTNB [12]. A stock solution of DTNB was prepared in 5 mM Tris–HCl (pH 7.5). DTNB was included in the MK-801 binding assay medium.

Control. All the control tissue homogenates were treated in an identical manner but without the modifying reagent.

Protection experiments. For protection experiments, the tissue suspension was pre-incubated with various ligands at room temperature for 30 min, followed by treatment with the protein modifying reagents, as described above.

MK-801 binding assay

[^{3}H]MK-801 binding was measured using a filtration assay as described previously [17]. [^{3}H]-MK-801 was incubated with 50–150 mg/mL crude synaptic membranes in a final volume of 1 mL for 3.5 hr at room temperature. Non-specific binding was defined as that remaining in the presence of 100 μM PCP. The reaction was terminated by dilution with 3 mL ice-cold buffer A and rapid filtration through GF-B glass fibre filters presoaked in 0.05% polyethyleneimine (PEI). Each filter was rinsed four times with 3 mL ice-cold buffer A in less than 15 sec. Emulsifier Safe (15 mL) was added to each filter and the radioactivity was measured by liquid scintillation spectrometry at a counting efficiency of 38%. Each determination was measured in triplicate.

(+)[^{3}H]MK-801 was used at a concentration of 3–5 nM. High concentrations of MK-801 in equilibrium saturation studies were achieved using a mixture of unlabelled and labelled MK-801. Equilibrium binding was achieved by 3.5 hr with all membrane preparations (data not shown). Modulators (50 μM glutamate, 30 μM glycine) were also included in the incubation medium, except in the initial membrane washing studies and where stated.

Equilibrium saturation binding data were analysed by non-linear regression using the Enzfitter program (Sigma) on the IBM PS-2 computer. Statistical analysis was carried out using the Student's *t*-test.

Protein determination

Protein concentration was determined either by the method of Lowry *et al.* [18] or the bicinchoninic acid (BCA) assay [19] using BSA as the standard.

RESULTS

MK-801 binding to washed membranes was

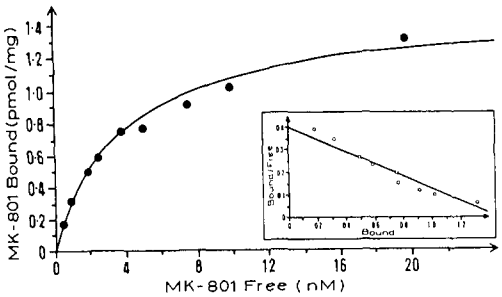

Fig. 1. Saturation analysis of [^{3}H]MK-801 binding to adult pig forebrain membranes in the presence of glutamate and glycine. Triton-treated adult pig forebrain membranes (0.1–0.2 mg/mL) were assayed for specific [^{3}H]MK-801 binding with a range of radioactive ligand concentrations. Non-specific binding was detected using 0.1 mM PCP. The figure is representative of three independent experiments. Each data point is the mean of triplicate determinations. The saturation curve is shown and the inset is the Scatchard transformation.

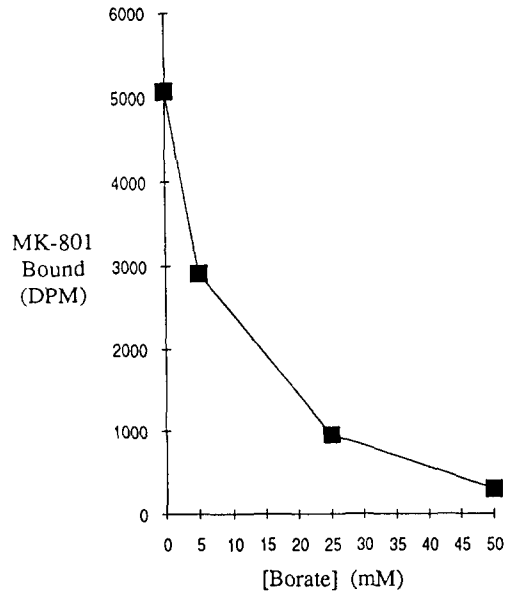

Fig. 2. The effect of borate buffer on [^{3}H]MK-801 binding to adult pig forebrain membranes. Adult pig forebrain membranes (0.1–0.2 mg/mL) were assayed for specific [^{3}H]MK-801 (2 nM) binding in borate buffer (pH 8.2) of 0–50 mM. The figure is representative of two independent experiments. Each data point is the mean of triplicate determinations.

assayed in the presence and absence of various modulators to check the effectiveness of the washing procedure described above. The binding relative to control was 217 ± 17% in the presence of glutamate, 109 ± 4% in the presence of glycine and 190 ± 38% with both present. [^{3}H]MK-801 bound to a single class of sites with a K_d of 3.2 ± 0.5 nM and B_{max} of 1.9 ± 0.6 pmol/mg (N = 3) in washed membranes with glutamate and glycine included in the assay buffer (Fig. 1).

Arginine modification

Arginine modification was initially attempted using 2,3-butanedione treatment [8]. Specific MK-801 binding was, however, substantially reduced when membranes were resuspended in borate buffer (Fig. 2). This agrees with other studies in which it has been shown that MK-801 binding varies depending on the type and strength of buffer used [20]. Butanedione was therefore an unsuitable modification reagent as the presence of borate buffer is necessary for stabilization of the reaction product [21].

Arginine modification was therefore attempted using phenylglyoxal treatment [16]. Phenylglyoxal treatment caused a time- (Fig. 3) and concentration-dependent decrease in MK-801 binding. Binding was reduced to a minimum of 20% over the course of a 50 min incubation with 10 mM phenylglyoxal.

Protection experiments were carried out to determine the site of the modified arginine (Fig. 4). Glutamate (1 mM), glycine (1 mM) or a combination of the two could not prevent the effects of phenylglyoxal on MK-801 binding. Pre-incubation with PCP also had no effect. Pre-incubation of the membranes with any of the modulators did not affect binding to untreated membranes (data not shown). Equilibrium saturation assays showed that in the modified membrane preparation MK-801 bound to a single site with an affinity of 22 ± 8 nM and a capacity of 2.3 ± 0.3 pmol/mg (N = 3, Fig. 5).

Histidine modification

DEP has been shown to modify histidyl residues [22]. Incubation of Triton-treated membranes with DEP caused a 40% decrease in specific MK-801 binding. This effect was time and concentration dependent.

Data from equilibrium saturation binding assays best fitted a one-site model with an affinity of 8.3 ± 1.4 nM and a capacity of 1.6 ± 0.3 pmol/mg (N = 3, Fig. 6). Pre-incubation of the membranes with 1 mM glutamate and 1 mM glycine partially protected against modification (Fig. 7). However, neither amino acid gave protection alone.

Since DEP under certain conditions may also modify sulfhydryl residues [22], the subsequent effect of modification of sulfhydryl residues using DTNB was investigated (Fig. 8). Inclusion of 0.5 mM DTNB in the assay medium reduced binding to control membranes by 23 ± 3% and DEP treated membranes by 19 ± 3%. Thus DEP caused a 38% decrease in MK-801 binding to membranes assayed in the presence of DTNB ($P > 0.5$ that the per cent decrease is significantly different to that measured in the absence of DTNB).

DISCUSSION

The removal of endogenous modulators during membrane preparation is necessary to ensure that modifying reagents have access to amino acids within ligand binding sites. The enhancement of MK-801

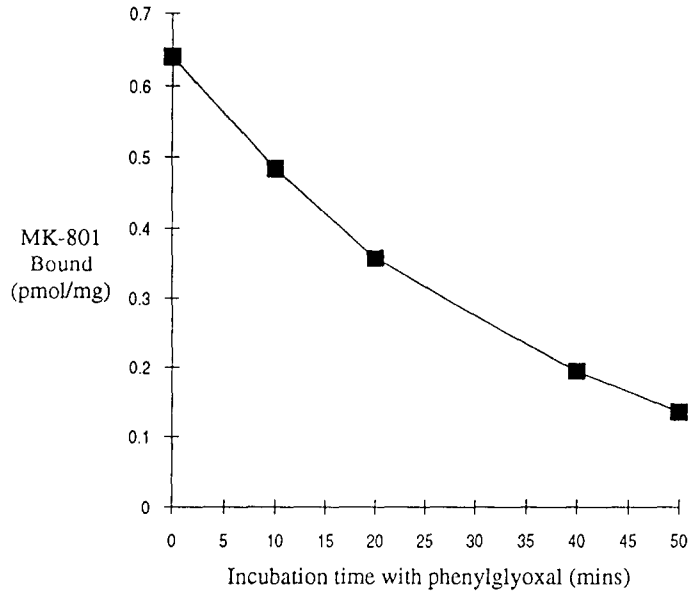

Fig. 3. Time course of phenylglyoxal effect on [^{3}H]MK-801 binding to adult pig forebrain membranes. Triton-treated membranes prepared from adult pig forebrain were resuspended in 0.1 M $NaHCO_3$ pH 8 (2.5 mg/mL) and treated with 20 mM phenylglyoxal for 0–50 min as described in Materials and Methods. After centrifugation and resuspension in buffer A, specific [^{3}H]MK-801 binding was assayed. The figure is representative of two independent experiments. Each data point is the mean of triplicate determinations.

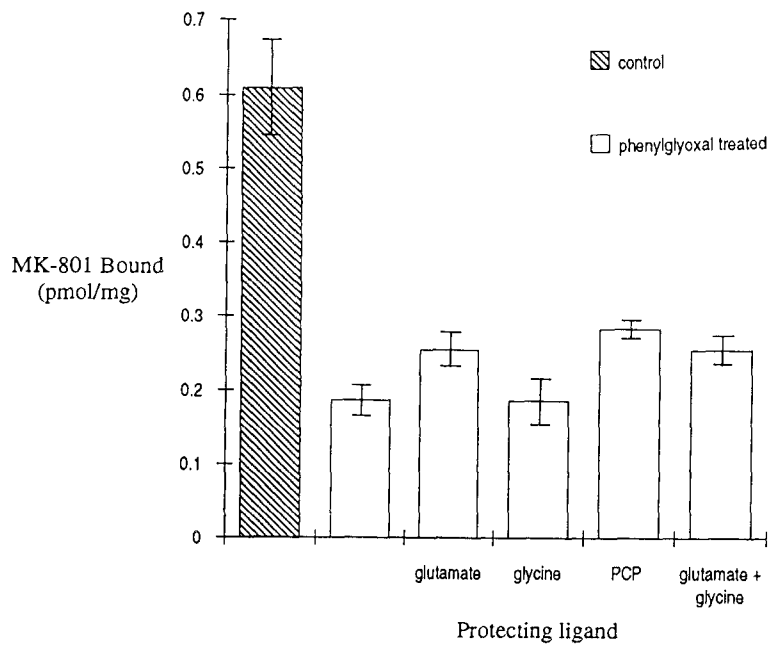

Fig. 4. Protection against phenylglyoxal effect by NMDA receptor ligands. Triton-treated membranes resuspended in 0.1 M $NaHCO_3$ pH 8 (2.5 mg/mL) were prepared as in Fig. 4. After pre-incubation with various ligands (glutamate, 1 mM; glycine, 1 mM; PCP, 0.1 mM) for 30 min at room temperature, the membranes were treated with 20 mM phenylglyoxal. Values are the means ± SD for three independent experiments in which each data point is the mean of triplicate determinations.

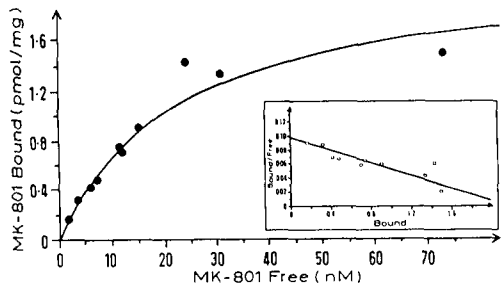

Fig. 5. Saturation analysis of [^{3}H]MK-801 binding to phenylglyoxal-treated adult pig forebrain membranes. Triton-treated membranes prepared from adult pig forebrain were treated with 20 mM phenylglyoxal as described in Materials and Methods. Saturation analysis was carried out as described in Fig. 2. The figure is representative of three independent experiments. Each data point is the mean of triplicate determinations. The saturation curve is shown and the inset is the Scatchard transformation. (Probability that K_d is the same as with control membranes < 0.05.)

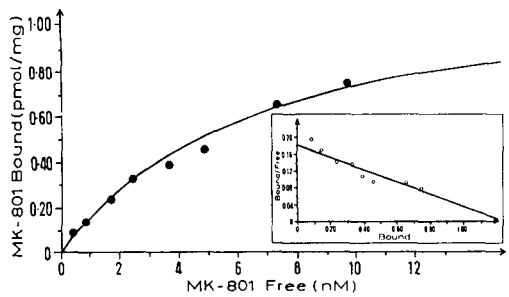

Fig. 6. Saturation analysis of [^{3}H]MK-801 binding to DEP-treated adult pig forebrain membranes. Triton-treated membranes prepared from adult pig forebrain were resuspended in 20 mM KH_2PO_4 pH 6 at a protein concentration of 0.5 mg/mL and treated with 3 mM DEP as described in Materials and Methods. After centrifugation and resuspension in buffer A, specific [^{3}H]MK-801 binding was assayed as described in Fig. 2. The figure is representative of three independent experiments. Each data point is the mean of triplicate determinations. The saturation curve is shown and the inset is the Scatchard transformation. (Probability that K_d is the same as with control membranes < 0.07.)

binding by glutamate and glycine confirms that Triton treatment followed by washing by centrifugation and resuspension is appropriate for modification studies.

Arginine modification was attempted using butanedione treatment. This reaction reverses rapidly unless maintained in 50 mM borate buffer [21]. As borate buffer itself caused a substantial decrease in MK-801 binding in our hands this treatment could not be used to assess the effects of arginine modification. However, Chazot *et al.* [13] have reported that butanedione had no effect on [^{3}H]MK-801 binding when assayed in 0.2 M borate buffer.

Modification of arginyl residues using phenylglyoxal drastically reduces MK-801 binding. The reduction is not due to direct modification of the MK-801 binding site as the number of MK-801 binding sites is unchanged. The decrease in binding affinity suggests an alteration in conformation of the NMDA receptor. This does not seem to be due to specific modification of either the glutamate or glycine binding sites.

Modification of histidyl residues using DEP reduced MK-801 binding. DEP is not completely specific for histidyl residues [22]. At pH 6 it is possible that sulfhydryl residues are also affected. As previous studies have shown that sulfhydryls can alter MK-801 binding, further studies were undertaken. DTNB, which modifies sulfhydryl residues, was included in the MK-801 binding assay medium. This caused a 20% decrease in binding, in agreement with the results of Reynolds *et al.* [12]. In contrast, Chazot *et al.* [13] reported that DTNB had no effect on binding, perhaps because DTNB was not present during the binding assay. Alternatively, the differences in membrane preparation could be responsible. DEP treatment caused a 38% reduction in binding compared to control membranes. Therefore these data implicate histidine as the residue modified during DEP treatment.

Equilibrium saturation experiments indicate that DEP causes a decrease in the binding affinity of MK-801 without altering the number of binding sites. Glutamate and glycine together were found to protect partially against modification. This could be due to various possibilities. Both sites could be modified by DEP, necessitating the presence of both amino acids for protection. If this were the case, MK-801 binding would be expected to be reduced by more than 40%. Receptor heterogeneity with respect to the effects of DEP could account for anomalies. Alternatively, the modified histidine may not be a component of the glutamate and glycine binding sites. This would suggest that the accessibility of the modified histidine may be partly dependent on the activation state of the receptor. An interesting possibility is that the histidine is part of a Zn^{2+} binding site. Recently Hollmann *et al.* [23] have shown that Zn^{2+} potentiates [^{3}H]MK-801 binding to certain variants of the NMDAR1 subunit as well as causing the more characteristic inhibition [24]. Thus modification of a Zn^{2+} binding site could reduce [^{3}H]MK-801 binding if a proportion of [^{3}H]MK-801 bound to homomeric NMDAR1 receptors. It has also been reported that DEP reduces Zn^{2+} binding to the NMDA receptor in well washed membranes, although this resulted in relief of Zn^{2+} inhibition [24]. It is possible that Triton treatment encourages removal of higher concentrations of NMDA receptor modulators, therefore unmasking the stimulatory effects of Zn^{2+} (EC_{50} = 0.5 μM, IC_{50} = 8.3 μM [23]).

As the receptor is a complex oligomer with multiple allosteric sites, modifications at distant sites could well affect MK-801 binding. However, although the existence of histidyl or arginyl residues at the ligand binding sites is not indicated, it cannot be ruled out as the conformation of the receptor may render the amino acids inaccessible to the modifying reagents.

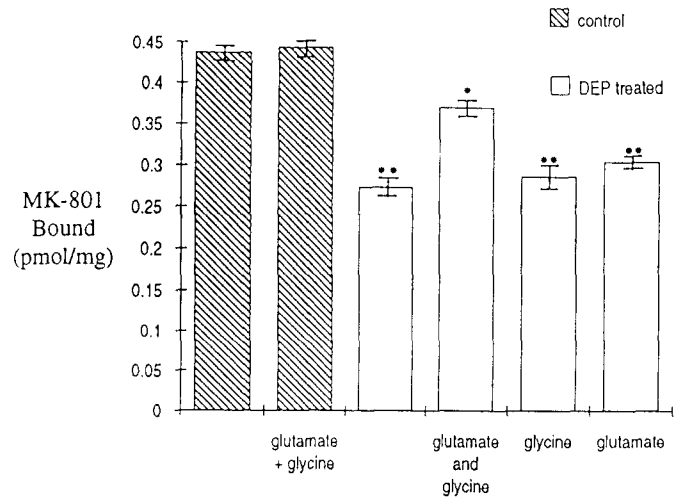

Fig. 7. Protection against DEP effect by NMDA receptor ligands. Triton-treated membranes prepared from adult pig forebrain were resuspended in 20 mM KH_2PO_4 pH 6 at a protein concentration of 0.5 mg/mL. After pre-incubation with various ligands (glutamate, 1 mM; glycine, 1 mM; PCP, 0.1 mM) for 30 min at room temperature, the membranes were treated with 3 mM DEP. Control incubations in the absence of DEP were also performed. After centrifugation and resuspension in buffer A, specific [^{3}H]MK-801 binding was assayed. Values are the means ± SD for four independent experiments in which each data point is the mean of triplicate determinations. $^*P < 0.05$ compared to control (untreated membranes), $P < 0.02$ compared to control (DEP-treated membranes) and $P < 0.05$ compared to pre-incubation with glutamate or glycine (DEP-treated membranes). $^{**}P < 0.01$ compared to control membranes.

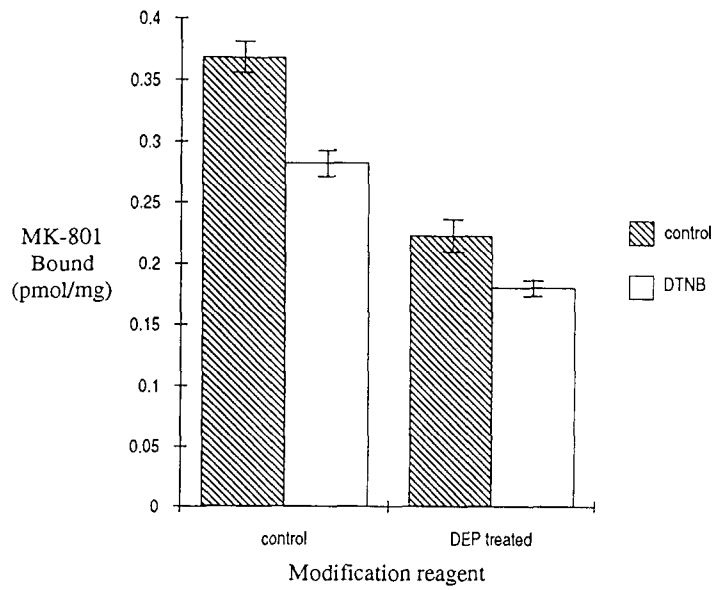

Fig. 8. Effect of DTNB on modification of specific [^{3}H]MK-801 binding by DEP. Triton-treated membranes were treated with DEP as described in Fig. 7. Control incubations in the absence of DEP were also performed. After centrifugation and resuspension in buffer A, treated and untreated membranes (0.1–0.2 mg/mL) were assayed for specific [^{3}H]MIK-801 binding in the presence or absence of DTNB (0.5 mM). Values are the means ± SD for three independent experiments in which each data point is the mean of triplicate determinations.

By analogy to other receptors in the Class I superfamily of ligand-gated ion channels, the NMDA receptors are likely to be heterogeneous, i.e. there are a number of different combinations of subunit which associate to form the receptor complex. This is substantiated by the results of cloning of the NMDA receptor from which eight isoforms of the NMDAR1 subunit [23] and five variants of the NMDAR2 subunit have been identified [25]. The results appear to indicate that all the NMDA receptor complexes in the forebrain are sensitive to chemical modification to a similar extent. However, slight differences in MK-801 binding or the presence of small populations of distinct activities may not be detectable.

Acknowledgements—We thank Mrs L. Backhouse for secretarial assistance. C.T.C. was supported by the U.K. Medical Research Council.

REFERENCES

1. Abbott A, Lodge DL and Collingridge GL (Eds.), *The Pharmacology of Excitatory Amino acids.* Trends in Pharmacological Sciences (Special report), Elsevier Books, Amsterdam, 1991.
2. MacDonald JF and Nowak LM, Mechanisms of blockade of excitatory amino acid receptor channels. *Trends Pharmacol Sci* **11**: 167–171, 1990.
3. Enomoto R, Ogita K, Han D and Yoneda Y, Differential modulation by divalent cations of [^{3}H]MK-801 binding in brain synaptic membranes. *J Neurochem* **59**: 473–482, 1992.
4. Traynelis SF and Cull-Candy SG, Proton inhibition of NMDA receptors in cerebellar neurons. *Nature* **345**: 347–349, 1990.
5. Ransom RW and Stec NL, Co-operative modulation of [^{3}H]MK-801 binding to the NMDA receptor-ion channel complex by L-glutamate, glycine and polyamines. *J Neurochem* **51**: 830–836, 1988.
6. Willetts J, Balster RL and Leander JD, The behavioural pharmacology of NMDA receptor antagonists. *Trends Pharmacol Sci* **11**: 423–428, 1990.
7. Cordi AA, Monahan JB and Reisse J, A topological model of the NMDA-glycine receptor model. *J Receptor Res* **10**: 299–315, 1990.
8. Widdows KB, Kirkness EF and Turner AJ, Modification of the GABA/benzodiazepine receptor with the arginine reagent, 2,3-butanedione. *FEBS Lett* **222**: 125–128, 1987.
9. Maksay G, Modification of benzodiazepine receptors supports the distinctive role of histidine residues. *Eur J Pharmacol* **227**: 57–62, 1992.
10. Aizenman E, Lipton SA and Loring RH, Selective modulation of NMDA responses by reduction and oxidation. *Neuron* **2**: 1257–1263, 1989.
11. Tang LH and Aizenman E, The modulation of *N*-methyl-D-aspartate receptors by redox and alkylating agents in rat cortical neurons *in vitro*. *J Physiol* **465**: 303–323, 1993.
12. Reynolds IJ, Rush EA and Aizenman E, Reduction of NMDA receptors with DTT increases [^{3}H]MK-801 binding and NMDA induced Ca^{2+} fluxes. *Br J Pharmacol* **101**: 178–182, 1990.
13. Chazot PL, Fotherby A and Stephenson FA, Evidence for the involvement of a carboxyl group in the vicinity of the MK-801 and magnesium ion binding site of the *N*-methyl-D-aspartate receptor. *Biochem Pharmacol* **45**: 605–610, 1993.
14. Miller RJ and Oliva AA Jr, Excitatory amino acids provide the stimulus. *Current Biol* **2**: 174–176, 1992.
15. Yoneda Y and Ogita K, Labeling of NMDA receptor channels by [^{3}H]MK-801 in brain synaptic membranes treated with Triton X-100. *Brain Res* **499**: 305–314, 1989.
16. Cheung S-T and Fonda ML, Reaction of phenylglyoxal with arginine. The effect of buffers and pH. *Biochem Biophys Res Commun* **90**: 940–947, 1979.
17. Keana JFW, Scherz MW, Quarum M, Sonders MS and Weber E, Synthesis and characterization of the PCP/NMDA receptor ligand (+)MK-801 with high specific activity. *Life Sci* **43**: 965–973, 1988.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Smith PK, Krohn RI, Hermanson GI, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchoninc acid. *Anal Biochem* **150**: 76–85, 1985.
20. Wong EHF, Knight AR and Woodruff GN, [^{3}H]MK-801 labels a site on the NMDA receptor–channel complex in rat brain membranes. *J Neurochem* **50**: 274–281, 1988.
21. Riordan JF, Arginyl residues and anion binding sites in proteins. *Mol Cell Biochem* **26**: 71–91, 1979.
22. Miles EW, Modification of histidyl residues in proteins by DEP. *Methods Enzymol* **47**: 431–442, 1977.
23. Hollmann M, Boulter J, Maron C, Beasley L, Sullivan J, Pecht G and Heinemann S, Zinc potentiates agonist-induced currents at certain splice variants of the NMDA receptor. *Neuron* **10**: 943–954, 1993.
24. Reynolds IJ, Interactions between zinc and spermidine on the *N*-methyl-D-aspartate complex: clues to the mechanism of action of 1,10-bis(guanido)decane and pentamidine. *J Pharmacol Exp Ther* **263**: 632–638, 1992.
25. Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M and Nakanishi S, Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. *J Biol Chem* **268**: 2836–2843, 1993.