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Effects of Polyamines on the Binding of [^{3}H]MK-801 to the *N*-Methyl-D-aspartate Receptor: Pharmacological Evidence for the Existence of a Polyamine Recognition Site

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SUMMARY

A heat-stable factor of low molecular weight that increases the binding of [^{3}H]MK-801 to rat brain membranes in the presence of maximally effective concentrations of L-glutamate and glycine was purified from bovine brain by reverse phase and ion-exchange high pressure liquid chromatography. The stimulatory activity was due to the presence of spermidine in the active fractions. Polyamines including spermine and spermidine are found in high concentrations in mammalian tissue. These compounds increase the affinity of *N*-methyl-D-aspartate (NMDA) receptors for [^{3}H]MK-801 when assays are carried out in the presence of 100 μM L-glutamate and 100 μM glycine. At concentrations of 1 to 300 μM, a number of di- and triamines, including $NH_2(CH_2)_3NH_2$, $NH_2(CH_2)_3NH(CH_2)_2NH_2$, and $NH_2(CH_2)_3NH(CH_2)_3NH_2$, have partial or full agonist-like activity similar to that of spermidine. Other polyamines, including putrescine, cadaverine, $NH_2(CH_2)_2NH(CH_2)_2NH_2$, and $CH_3NH(CH_2)_3NHCH_3$, at concentrations of 1 to 100 μM, inhibited the binding of [^{3}H]MK-801 in the presence of spermine, L-glutamate, and glycine but not in the presence of only L-glutamate and glycine. It is concluded that these compounds are selective antagonists of the effects of spermine at the NMDA receptor. These results suggest that there may be a polyamine recognition site on the NMDA receptor complex.

Receptors for excitatory amino acids have been classified as NMDA, quisqualate, and kainate subtypes (1, 2). The NMDA receptor is associated with a cation-selective ion channel that gates Na^+ and Ca^{2+} ions and is blocked in a voltage-dependent manner by Mg^{2+} (2, 3). The receptor is thought to contain separate recognition sites for glutamate and for glycine. Glycine is an allosteric modulator that has been shown to enhance the effects of glutamate in electrophysiological assays (4) and in binding assays with [^{3}H]MK-801 (5). A number of compounds, including phencyclidine, ketamine, and MK-801, are functional antagonists of the effects of glutamate at the NMDA receptor. These compounds bind to a third site that is distinct from the two amino acid binding sites (5–7). Results of electrophysiological studies suggest that this site is within or closely associated with the ion channel of the receptor complex (8). Binding of [^{3}H]MK-801 to well washed membranes prepared from brain is greatly enhanced by amino acids that act at the glutamate or glycine recognition sites (5, 6). It appears that [^{3}H]MK-801 binds specifically to an activated state of the receptor at a site within the ion channel of the receptor complex. Binding of [^{3}H]MK-801 can also be stimulated or inhibited by divalent cations (9) and is markedly inhibited by millimolar concentrations of many other ions. Ransom and Stec (10) recently reported that spermine and spermidine increase the affinity of the NMDA receptor for [^{3}H]MK-801.

Putative endogenous modulators of a number of ion channels and receptors for neurotransmitters have been identified. For example, an inhibitor of the binding of [^{3}H]diazepam (11) interacts with the benzodiazepine binding site of the γ-aminobutyric $acid_A$ receptor, and a number of steroids interact with the barbiturate recognition site on the same receptor (12). A low molecular weight factor that inhibits the binding of [^{3}H]nitrendipine to voltage-dependent calcium channels has been partially purified from rat brain (13). We have examined extracts of brain to try to identify endogenous factors that interact with the NMDA receptor complex. The use of a radioligand binding assay with [^{3}H]MK-801 may permit identification of compounds that act at the amino acid recognition sites, at the [^{3}H]MK-801 binding site, or at other sites on the receptor complex. We report here the purification of a factor from brain that enhances the binding of [^{3}H]MK-801 to the NMDA receptor in the presence of maximally effective concentrations of L-glutamate and glycine. This factor has been identified as the

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high pressure liquid chromatography; SPM, synaptic plasma membrane.

polyamine spermidine. As a further step towards characterizing the site and mechanism of action of spermidine and spermine, we have studied a number of polyamines to try to define structure-activity relationships of polyamines that affect binding of [^{3}H]MK-801 to the NMDA receptor. Studies of the effects of polyamines on the binding of [^{3}H]MK-801 led to the conclusion that this class of compounds includes agonists, antagonists, and partial agonists.

Materials and Methods

Preparation of membranes from rat brain. A SPM fraction was prepared from rat brain for use in binding assays with [^{3}H]MK-801. Membranes were prepared using either fresh brains from male Sprague-Dawley rats (Charles River Laboratory) or frozen rat brains (Pel-Freez Biologicals, Rogers, AR). No differences in the binding of [^{3}H]MK-801 were observed between membranes prepared from fresh or frozen brains. Unless otherwise stated, all procedures were carried out at 4°. Brains from 25 rats (minus cerebellum and brainstem) were chopped with scissors and then homogenized with a glass/Teflon homogenizer in 300 ml of 0.32 M sucrose containing 5 mM K-EDTA (pH 7.0). The homogenate was centrifuged for 10 min at 1,000 × *g* and the supernatant was removed and centrifuged at 30,000 × *g* for 30 min. The resulting pellet was resuspended in 250 ml of 5 mM K-EDTA (pH 7.0), stirred on ice for 15 min, and then centrifuged at 30,000 × *g* for 30 min. The pellet was resuspended in 90 ml of 5 mM K-EDTA (pH 7.0), and 15-ml aliquots were layered over discontinuous sucrose gradients made up of 10 ml each of 0.9 M and 1.2 M sucrose. The gradients were centrifuged at 95,000 × *g* for 90 min, and the SPM fraction at the 0.9 M/1.2 M sucrose interface was collected. Membranes were washed by resuspension in 500 ml of 5 mM K-EDTA (pH 7.0), incubated at 32° for 30 min, and centrifuged at 100,000 × *g* for 30 min. The wash procedure, including the 30-min incubation, was repeated three times. The final pellet was resuspended in 60 ml of 5 mM K-EDTA (pH 7.0) and stored in aliquots at −80°.

Binding assays with [^{3}H]MK-801. Aliquots of SPMs were thawed, washed once by incubation at 32° for 30 min, and centrifuged at 100,000 × *g* for 30 min. Membranes were resuspended in buffer A (20 mM K-HEPES, 1 mM K-EDTA, pH 7.0). Binding assays were carried out in polypropylene test tubes containing membranes (80–100 μg of protein, except for saturation binding curves, which used 25 μg of protein) and [^{3}H]MK-801. The concentration of [^{3}H]MK-801 was 7 nM, except for saturation binding curves where eight concentrations (1 to 100 nM) were used. A concentration of 7 nM [^{3}H]MK-801 is below the K_D value for this radioligand in the presence of 100 μM L-glutamate and glycine, and effects of polyamines can be easily detected when assays are carried out under these conditions. L-Glutamate, glycine, and polyamines were added and the final incubation volume was 200 μl. Duplicate samples were incubated at 32° for 3 hr. Nonspecific binding was determined in the presence of 10 μM (+)-MK-801. At a concentration of 7 nM [^{3}H]MK-801, nonspecific binding was ≤10% of total binding. Assays were terminated by the addition of 10 ml of ice-cold buffer A, followed by filtration over glass-fiber filters (Schleicher & Schuell No. 30). The filters were washed with another 10 ml of buffer A and radioactivity was determined by scintillation counting at an efficiency of 35–40% for ^{3}H.

Preparation of factor from bovine brain. Bovine brain (Pel-Freez Biologicals) was thawed and homogenized in an equal volume (w/v) of 10 mM K-EDTA (pH 7.0). After centrifugation (30,000 × *g*, 30 min), the supernatant was removed and the pellet was re-extracted in 10 mM K-EDTA and centrifuged as before. The supernatants were combined, heated to 95° for 15 min, and then centrifuged at 100,000 × *g* for 30 min. The resulting supernatant was passed through a 0.22-μm filter and an Amicon YM10 membrane (molecular weight cutoff = 10,000). The filtrate was concentrated in a rotary evaporator and stored at 4° for up to 3 days. Aliquots of the concentrated material were chromatographed on a reverse phase HPLC column (Dynamax-300A C18; Rainin, Woburn, MA). The starting buffer was 0.1% trifluoroacetic acid in water, and material was eluted using a linear gradient of acetonitrile (0–80%). Fractions containing material that increased the binding of [^{3}H]MK-801 were pooled, concentrated by lyophilization, and subjected to cation-exchange HPLC using a Hydropore SCX-column (Rainin). The starting buffer was 5 mM ammonium acetate, and elution was obtained with a gradient of ammonium acetate (5 mM to 2 M). Fractions containing stimulatory activity were pooled and concentrated by lyophilization. Thin layer chromatography was carried out using alumina GF analytical plates (Analtech, Newark, DE), with a mobile phase consisting of *n*-butanol/acetic acid/water (7:1:1). The plates were exposed to 2% ninhydrin in ethanol and heated at 60° for visualization of amines.

Materials. (+)-[3-^{3}H]MK-801 (specific activity, 15 to 18 Ci/mmol) was purchased from New England Nuclear (Wilmington, DE). (+)-MK-801 was a gift from Merck & Co. Inc. (West Point, PA). L-Glutamate and glycine were purchased from Sigma Chemical Co. (St. Louis, MO). All polyamines were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Results

Activity of bovine brain extracts. Extracts of bovine brain were examined to try to identify endogenous modulators of the NMDA receptor. A YM10 filtrate of a boiled extract of homogenized bovine brain was chromatographed on a C18 reverse phase HPLC column. Fractions were lyophilized, resuspended in buffer A, and assayed for effects on the binding of [^{3}H]MK-801 in the presence of maximally effective concentrations of L-glutamate (100 μM) and glycine (100 μM). Addition of higher concentrations of L-glutamate and/or glycine had no effect on the binding of [^{3}H]MK-801. Three distinct peaks of activity were detected (Fig. 1). One peak of material that inhibited binding of [^{3}H]MK-801 was eluted in the void volume of the column, as are many of the salts present in the brain extract. Binding of [^{3}H]MK-801 is markedly inhibited by these salts. A second broad peak of inhibitory material was eluted with a concentration of 60–75% acetonitrile. This activity was nonspecific, in that it inhibited the specific binding of radioligands to a variety of different types of neurotransmitter receptors (results not shown). Activity that increased the binding of [^{3}H]MK-801 above the levels seen with 100 μM L-glutamate and glycine was eluted from the C18 column after the salts in the void volume but before the onset of the acetonitrile gradient (Fig. 1). The active fractions were pooled and chromatographed on an SCX cation-exchange HPLC column. Active material was eluted with ammonium acetate and was separated from most of the material that absorbs at 280 nm (Fig. 2). One half of the pooled active fractions from an SCX chromatograph was analyzed by thin layer chromatography on alumina. A ninhydrin-positive spot that co-migrated with spermidine was detected. The intensity of this spot was equivalent to 20–40 nmol of spermidine (Fig. 2). The other half of the pooled active material was used in a binding assay with [^{3}H]MK-801. This material enhanced binding of [^{3}H]MK-801 to about the same extent as 30 nmol of spermidine. [^{3}H]Spermidine also co-migrates with the active fractions on reverse phase and SCX HPLC, whereas [^{14}C]spermine is eluted before the active material on reverse phase HPLC (Fig. 2) but elutes after the active material on SCX HPLC.

Spermine and spermidine increased the binding of [^{3}H]MK-801 in the presence of maximally effective concentrations of L-glutamate and glycine (Fig. 3). This effect was optimal at concentrations of polyamine between 10 and 100 μM and the

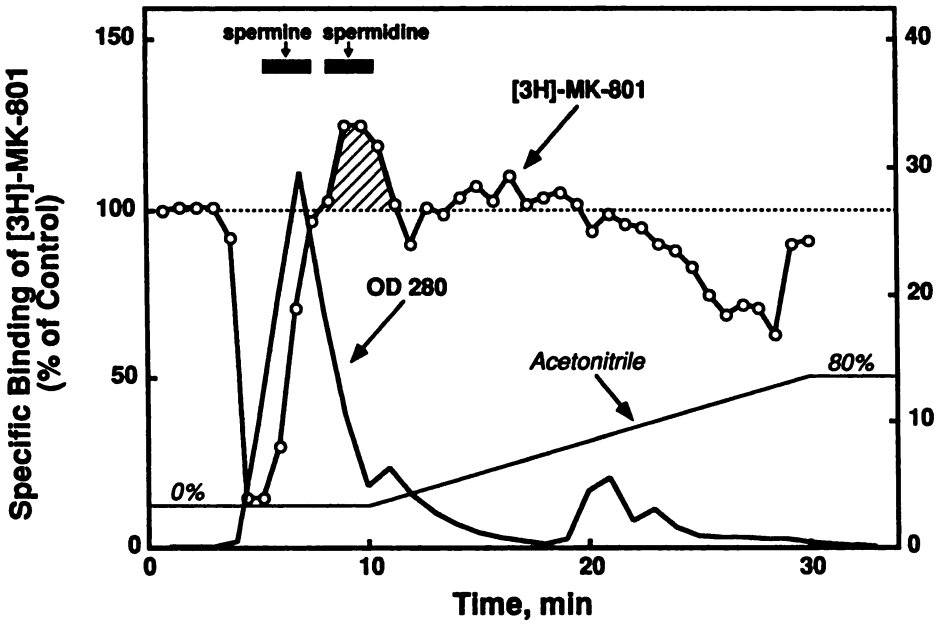

Fig. 1. Reverse phase HPLC of an extract of bovine brain. A YM10 filtrate of a boiled extract of bovine brain was chromatographed on a C18 column that was eluted (1 ml/min) with a linear gradient of 0–80% acetonitrile in 0.1% trifluoroacetic acid. Fractions (0.75 min) were collected, lyophilized, and resuspended in buffer. Aliquots of each fraction were tested for effects on the binding of [^{3}H]MK-801 in the presence of 100 μM L-glutamate and glycine. Fractions that increased the binding of [^{3}H]MK-801 above control values (*shaded area*) were pooled for further study. When [^{3}H]spermidine and [^{14}C]spermine were added to an extract before chromatography, these standards eluted in the positions indicated by the *horizontal bars*.

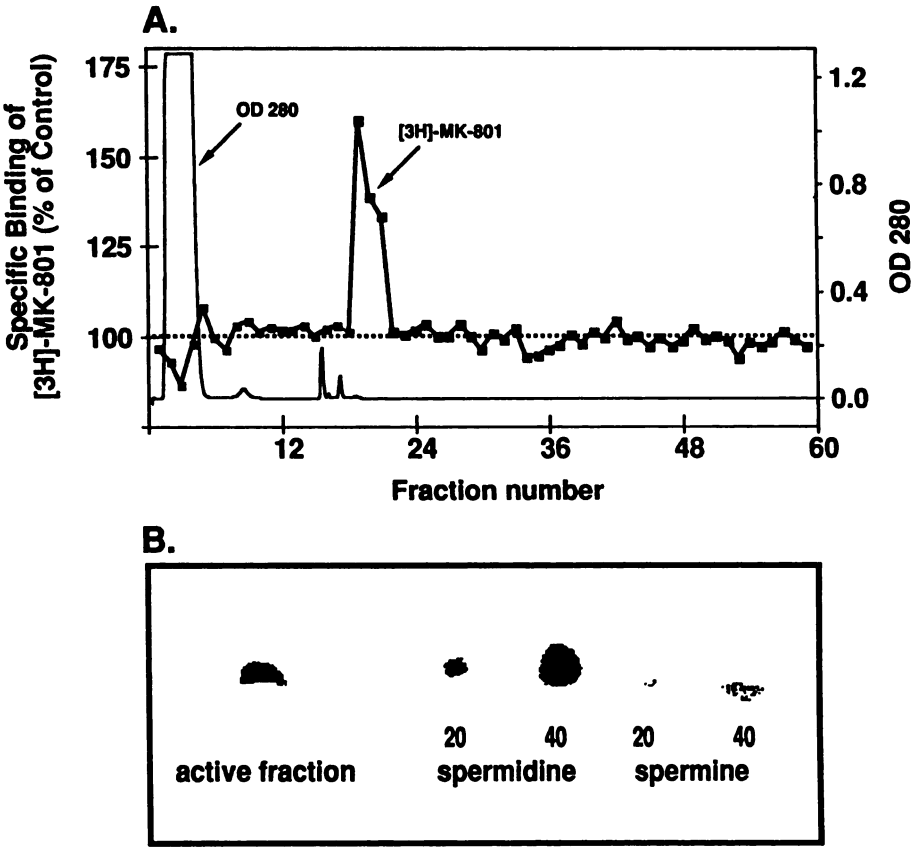

Fig. 2. Cation-exchange HPLC and thin layer chromatography of bovine brain factor. A, Active fractions from reverse phase HPLC (Fig. 1) were chromatographed on an SCX column that was eluted (1 ml/min) with a linear gradient (5 mM to 2 M) of ammonium acetate. Fractions were lyophilized, resuspended, and assayed for effects on the binding of [^{3}H]MK-801 in the presence of 100 μM L-glutamate and glycine. The absorbance at 280 nm was also measured. B, Active fractions from an experiment similar to that shown in A were pooled, concentrated, and run on an alumina thin layer chromatography plate. Also included on the plates were 20 and 40 nmol of spermidine and spermine. The chromatogram was exposed to 2% ninhydrin in ethanol.

effect declined at concentrations above 100 μM. Because of the co-migration of the stimulatory activity purified from bovine brain with spermidine and the presence of spermidine in our most purified fractions in sufficient amounts to account for their activity, we conclude that this activity is due to spermidine.

Activity of polyamine analogues. The possibility that the effects of spermine and spermidine may be mediated by an interaction of the polyamines with a distinct recognition site on or associated with the NMDA receptor complex was investigated. A number of di- and triamines were examined for effects on the binding of [^{3}H]MK-801 to membranes prepared from rat brain. Binding assays were carried out in the presence of 100 μM L-glutamate and 100 μM glycine, in the absence or presence of 10 μM spermine. When spermidine was used instead of spermine in similar experiments, equivalent results were obtained.

The effects of compounds having two primary amines separated by differing numbers of methylene groups were determined. 1,3-Diaminopropane (compound **DA3**) at concentra-

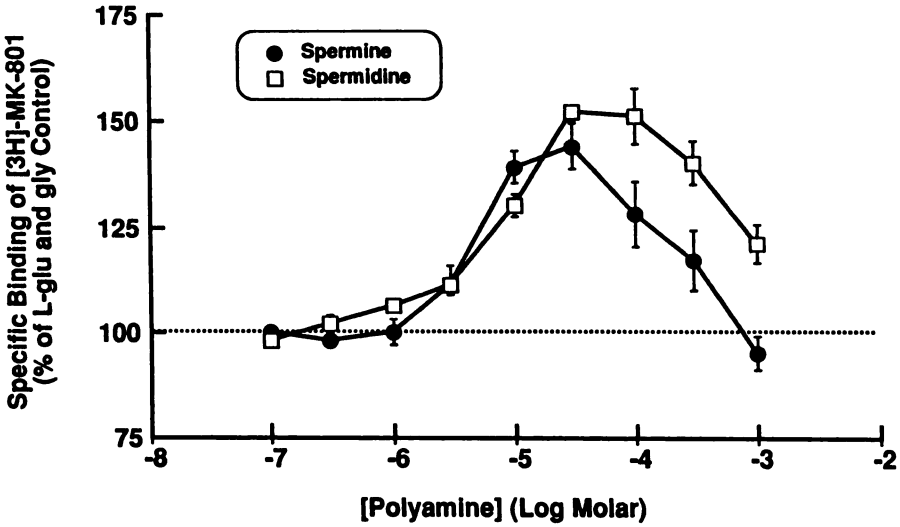

Fig. 3. Effects of spermine and spermidine on the binding of [^{3}H]MK-801 to SPMs prepared from rat brain. All samples contained 100 μM L-glutamate and 100 μM glycine. Values are means ± standard errors from four (spermine) or five (spermidine) experiments. Control binding (100%) (*broken line*) was 154 ± 13 fmol (spermine) and 157 ± 10 fmol (spermidine).

tions above 30 μM increased binding of [^{3}H]MK-801 in the presence of L-glutamate and glycine (Fig. 4A). At concentrations above 100 μM, **DA3** inhibited binding in the presence of spermine (Fig. 4A). Thus, **DA3** can be regarded as a weak agonist whose activity is similar to that of spermine and spermidine. Addition of one or two methylene groups between the primary amines of **DA3** gives rise to the endogenous amines putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane). Putrescine and cadaverine did not affect binding of [^{3}H]MK-801 in the presence of L-glutamate and glycine but attenuated the increase in the binding of [^{3}H]MK-801 produced by 10 μM spermine (Fig. 4, B and C). These compounds, therefore, selectively antagonize the effects of spermine on the binding of [^{3}H]MK-801. 1,6-Diaminohexane and 1,7-diaminoheptane had effects similar to putrescine and cadaverine (data not shown). Diamines having 8, 9, 10, or 12 methylene groups separating the primary amino groups inhibited binding in the presence or absence of spermine (shown for 1,10-diaminodecane in Fig. 4D). The similarity in the concentration-effect curves in the presence or absence of spermine suggests that this effect is nonspecific or is due to direct inhibition at the binding sites for glutamate, glycine, or MK-801.

The activities of triamines were also investigated. In this series, differences in activity were seen when the number of methylene groups separating one or both of the primary amines from the secondary amine was altered (Fig. 5). Diethylene triamine (compound **I**) is a selective inhibitor of the stimulatory effects of spermine (Fig. 5A). Compared with spermidine (Fig. 5D), *N*-(2-aminoethyl)-1,3-propanediamine (compound **II**) is a partial agonist (Fig. 5B) and 3,3′-iminobispropylamine (compound **III**) is a full agonist (Fig. 5C) for increasing the binding of [^{3}H]MK-801 in the presence of L-glutamate and glycine. The potency as an antagonist of compound **I** (Fig. 5A) is similar to that of cadaverine (Fig. 4C). In both of these polyamines, two primary amines are separated by a chain of five atoms.

The effects of dimethylated and tetramethylated derivatives of compound **DA3** (Fig. 6A) were determined. Over a concentration range of 3–100 μM, compound **VI** (*N*,*N*′-dimethyl-1,3-propanediamine) selectively inhibited the effect of 10 μM spermine (Fig. 6B). At 300 μM, compound **VI** inhibited the binding of [^{3}H]MK-801 in the presence or absence of spermine, suggesting that nonspecific inhibitory effects occur at this concentration. In contrast, the tetramethylated derivative *N*,*N*,*N*′*N*′-tetramethyl-1,3-propanediamine (compound **XV**) was a weak partial agonist (Fig. 6C).

Compounds that did not affect the binding of [^{3}H]MK-801 in the presence or absence of spermine included L-ornithine (the endogenous precursor of spermine and spermidine in eukaryotic cells), *N*-ethyl-ethylenediamine, and *N*-methyl-1,3-propanediamine at concentrations up to 300 μM. Spermine and spermidine did not affect the binding of [^{3}H]*N*-methylscopolamine to muscarinic acetylcholine receptors or the binding of [^{3}H]PN200-110 to voltage-dependent Ca^{2+} channels (data not shown).

The effects of spermine on the binding of [^{3}H]MK-801 are due to an increase in the apparent affinity of the binding site for [^{3}H]MK-801, with no change in the total number of binding sites (B_{max}) (Fig. 7). Values for B_{max} and the apparent equilibrium dissociation constant (K_D) were, for control, B_{max} = 2.9 ± 0.07 pmol/mg of protein and K_D = 10.7 ± 0.12 nM; in the presence of 10 μM spermine, B_{max} = 3.0 ± 0.16 pmol/mg of protein and K_D = 5.5 ± 0.32 nM (mean ± standard error; three experiments). Compound **I** (0.3 mM) greatly attenuated the effects of 10 μM spermine on the affinity of the binding site for [^{3}H]MK-801 (Fig. 7).

Discussion

Endogenous modulators of a number of neurotransmitter receptors and ion channels have been purified from extracts of brain (e.g., Refs. 11 and 13). Binding of [^{3}H]MK-801 to the NMDA receptor can be regulated by amino acids that act at two distinct recognition sites on the receptor complex (5, 14) and by divalent cations that may act at sites distinct from either of the amino acid binding sites (7, 9). It is possible that other, hitherto unidentified, modulators of the NMDA receptor exist in brain. Extracts of bovine brain were used to try to isolate and identify factors that may be endogenous modulators of the NMDA receptor. A factor that increases binding of [^{3}H]MK-801 above the levels seen with maximally effective concentrations of L-glutamate and glycine was identified and purified. This factor co-chromatographs with spermidine in a number of chromatographic systems and its effect on the binding of [^{3}H]MK-801 is the same as that of spermidine. It is concluded that the activity of the endogenous factor is accounted for by the

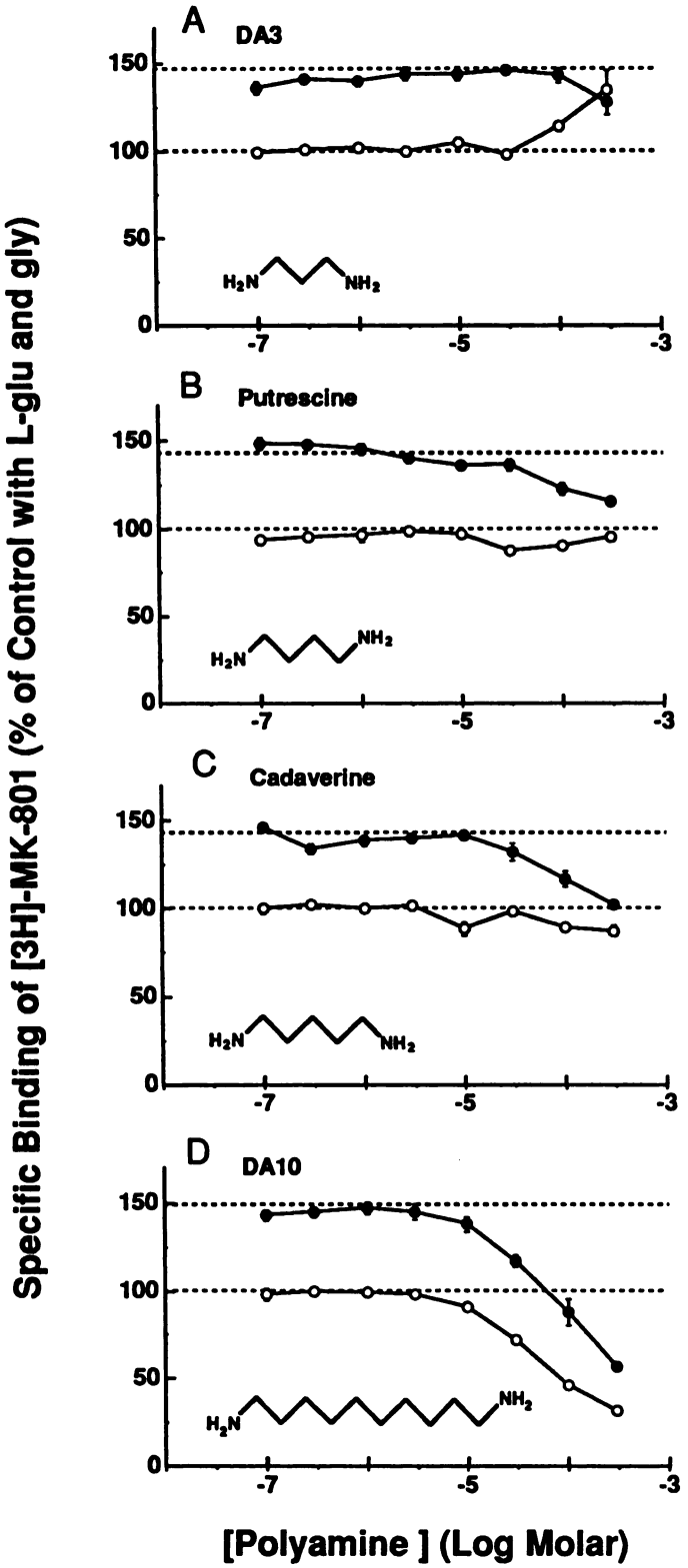

Fig. 4. Effects of diamines on the binding of [^{3}H]MK-801. **DA3** (A), putrescine (B), cadaverine (C), and **DA10** (D) were studied. The binding of [^{3}H]MK-801 was measured in the presence of 100 μM L-glutamate and glycine (○) and in the presence of 100 μM L-glutamate and glycine plus 10 μM spermine (●). *Broken lines* show control binding in the presence of L-glutamate and glycine (100%) or in the presence of L-glutamate and glycine plus spermine. The structure of each polyamine is shown at the *bottom* of each *panel*. Values are means ± standard errors from three experiments. Control (100%) values were 151 ± 13 fmol (A), 157 ± 10 fmol (B and C), and 158 ± 3 fmol (D).

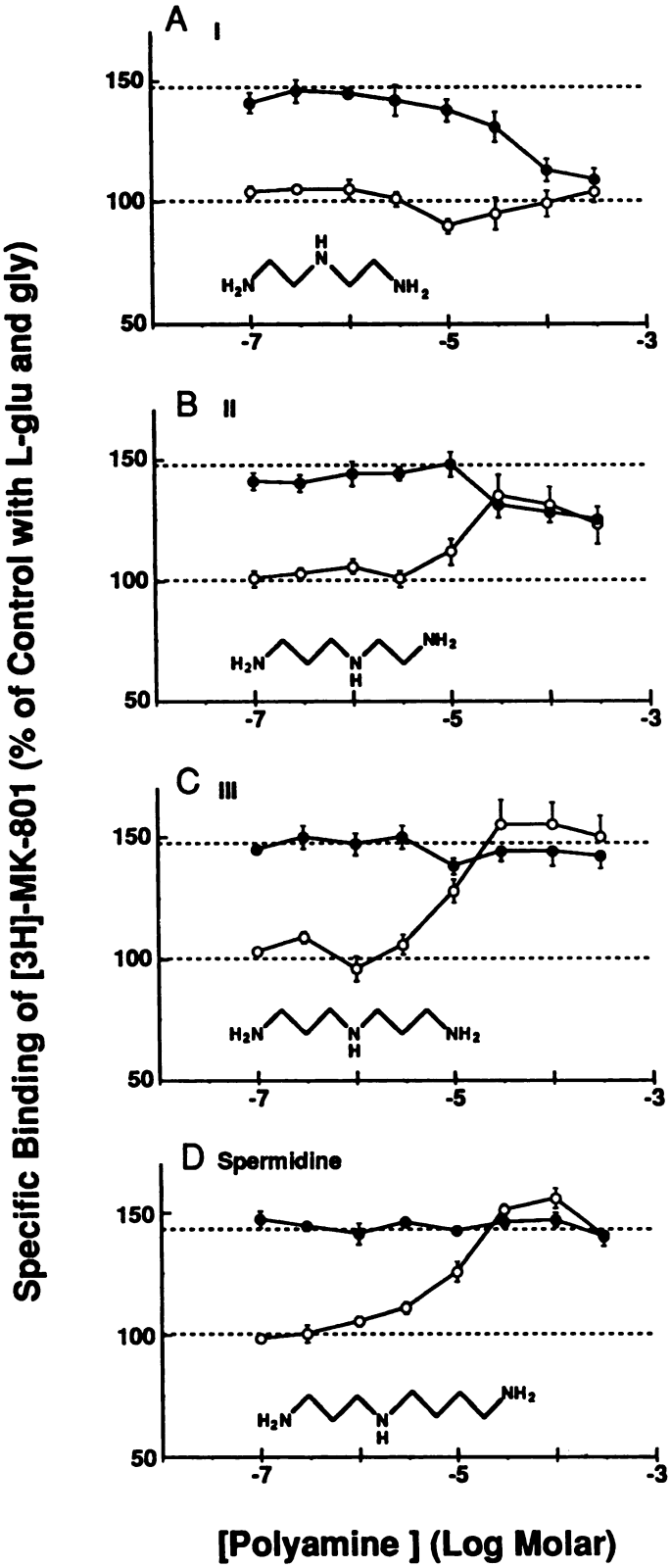

Fig. 5. Effects of triamines on the binding of [^{3}H]MK-801. Compounds **I** (A), **III** (B), and **II** (C) and spermidine (D) were studied. The binding of [^{3}H]MK-801 was measured in the presence of 100 μM L-glutamate and glycine (○) and in the presence of 100 μM L-glutamate and glycine plus 10 μM spermine (●). Results are expressed as in Fig. 4. Values are means ± standard errors from three experiments. Control (100%) values were 151 ± 13 fmol (A, B, and C) and 157 ± 10 fmol (D).

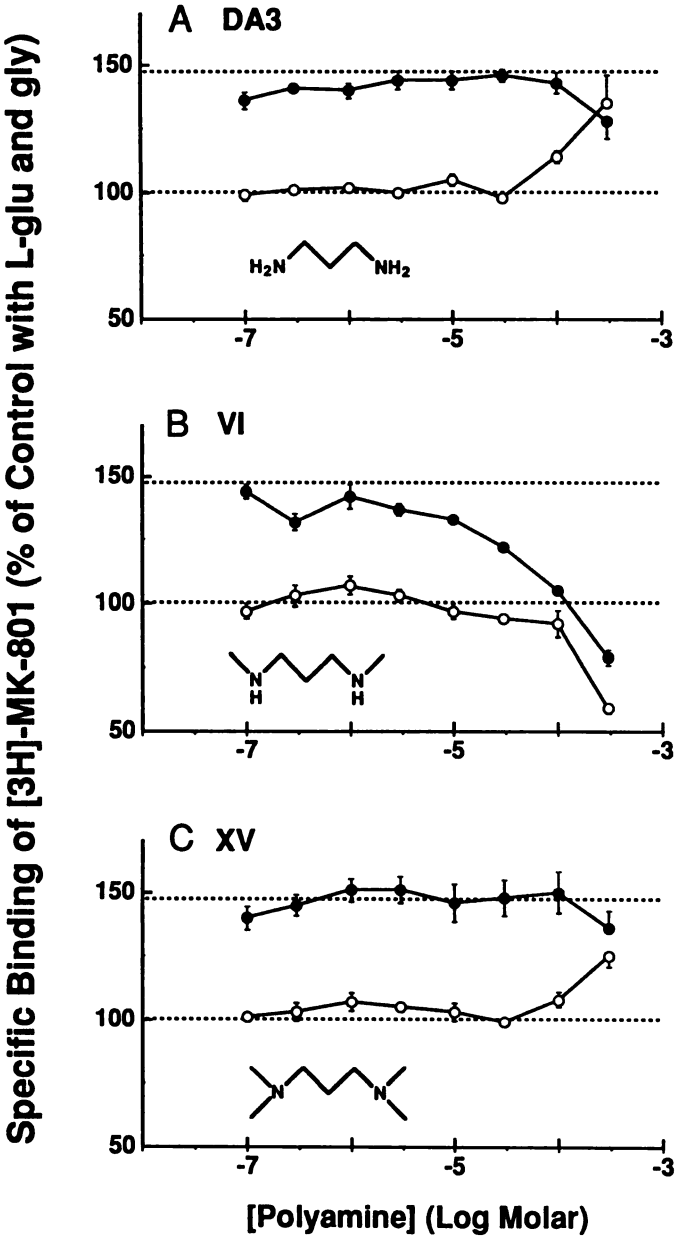

Fig. 6. Effects of diamines on the binding of [^{3}H]MK-801. **DA3** (A) and compounds **VI** (B) and **XV** (C) were studied. The binding of [^{3}H]MK-801 was measured in the presence of 100 μM L-glutamate and glycine (○) and in the presence of 100 μM L-glutamate and glycine plus 10 μM spermine (●). Results are expressed as in Fig. 4. Values are means ± standard errors from three experiments. Control (100%) values were 151 ± 13 fmol.

presence of spermidine, derived from bovine brain, in the active fractions.

The polyamines spermine and spermidine increase binding of [^{3}H]MK-801 above the levels seen with maximally effective concentrations of L-glutamate and glycine. This effect is due to an increase in the affinity of the binding site for [^{3}H]MK-801. These data are consistent with the findings of Ransom and Stec (10), who showed that spermine and spermidine increased the affinity of a binding site for [^{3}H]MK-801 when experiments were carried out in the presence of 50 μM L-glutamate and 30 μM glycine. These authors also described the effects of polyamines on the binding of [^{3}H]MK-801 in the

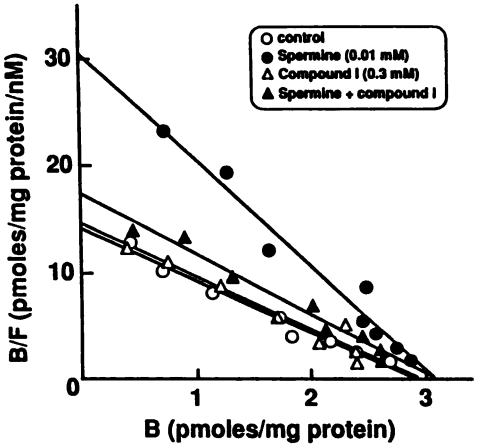

Fig. 7. Scatchard analyses of the binding of [^{3}H]MK-801. Assays were carried out in the presence of 100 μM L-glutamate and glycine. Spermine (10 μM) or compound I (0.3 mM) were included as shown. The results are from one experiment, representative of three such experiments.

absence of exogenous amino acids (10). The effects of L-glutamate and glycine on the binding of [^{3}H]MK-801 (15, 16) and of [^{3}H]*N*-(1-[thienyl]cyclohexylpiperidine) (17) are complex and can be accounted for in part by changes in the rate constants of binding. When no amino acids, or only low concentrations, are added to well washed brain membranes, the binding of [^{3}H]MK-801 at 25–37° may take 12–24 hr to reach equilibrium. Furthermore, in the absence of added amino acids, binding of [^{3}H]MK-801 is influenced by the amounts of endogenous amino acids that remain in washed membranes. Data obtained from experiments carried out in the absence of added amino acids can be difficult to interpret. In the absence of added amino acids, the effects of putative modulators of the NMDA receptor could be due, for example, to production of amino acids by increased proteolytic degradation of membrane components. In the present report, the effects of polyamines on the binding of [^{3}H]MK-801 were examined in the presence of 100 μM L-glutamate and glycine. Under these conditions, the binding of [^{3}H]MK-801 reaches equilibrium within 2–2.5 hr at 32° (data not shown). The 100 μM concentrations of L-glutamate and glycine are 10-fold higher than the concentrations needed to maximally increase binding of [^{3}H]MK-801 in the absence of other modulators. Because the enhancing effects of spermine and spermidine occur in the presence of 100 μM L-glutamate and 100 μM glycine, they are likely to be mediated at a site other than the amino acid binding sites. This conclusion is supported by the discovery of a number of polyamines that inhibit the binding of [^{3}H]MK-801 in the presence of L-glutamate, glycine, and spermine but not in the presence of only L-glutamate and glycine. These include two naturally occurring diamines, putrescine and cadaverine, and compounds **I** and **VI**. Ransom and Stec (10) reported that putrescine and cadaverine did not affect the binding of [^{3}H]MK-801. However, these authors did not examine the effects of putrescine and cadaverine on the enhancement of binding of [^{3}H]MK-801 caused by spermine. Our data suggest that putrescine, cadaverine, and compounds **I** and **VI** are selective inhibitors of the effects of polyamines that enhance the binding of [^{3}H]MK-801.

A number of diamines and triamines that are structurally related to spermidine and spermine had effects on the binding of [^{3}H]MK-801. Of the series of diamines having structures $NH_2(CH_2)_xNH_2$, only **DA3** (where $x = 3$) increased the binding

of [^{3}H]MK-801 (Fig. 4), whereas putrescine ($x = 4$) and cadaverine ($x = 5$) were antagonists. In the triamine series having structures $NH_2(CH_2)_xNH(CH_2)_yNH_2$, compounds **II** ($x = 3$, $y = 2$) and **III** ($x = 3$, $y = 3$), but not compound **I** ($x = 2$, $y = 2$), increased the binding of [^{3}H]MK-801. This suggests that the presence of two primary amines (**DA3**) or a primary and a secondary amine (**II, III**, spermidine, and spermine) separated by three methylene groups is sufficient for the stimulatory activity of the polyamines. In this regard, it is of interest that the dimethylated derivative of **DA3** (compound **VI**) is an inhibitor of the effects of spermine but a tetramethylated derivative (compound **XV**) has activity similar to **DA3**. This suggests that a terminal primary or tertiary amine is required for agonist activity. Compound **I** has only antagonist activity, whereas compound **II** is a partial agonist and compound **III** is a full agonist. The ethylenediamine portion of compound **II** may account for inhibitory activity and the propylenediamine portion for stimulatory activity.

Spermine and spermidine are present in high concentrations (20–100 μg/g of wet weight) in the central nervous system (18). There is evidence for a role of polyamines in cellular growth and development (19, 20). However, the functions of polyamines in both the brain and periphery remain obscure. The present report and that of Ransom and Stec (10) suggest that spermidine and spermine might be involved in modulation of the NMDA receptor. The effects of polyamines on the binding of [^{3}H]MK-801 may be mediated by a distinct recognition site on the NMDA receptor complex. Divalent cations, including Mg^{2+}, can increase the binding of [^{3}H]MK-801 (7, 9). It is not known whether this is related to the ability of higher concentrations of Mg^{2+} to inhibit the binding of [^{3}H]MK-801 (7, 9) or to the voltage-dependent blockade of the ion channel by Mg^{2+} (2). It is possible that polyamines act at the same site or sites at which Mg^{2+} acts to enhance or inhibit the binding of [^{3}H]MK-801. However, because Mg^{2+} does not enhance the binding of [^{3}H]MK-801 above the level observed in the presence of 100 μM L-glutamate and glycine, it is unlikely that the stimulatory effects of polyamines are mediated through an interaction at the Mg^{2+} binding site. The apparent nonspecific inhibitory effects of high concentrations of some polyamines could be mediated by an interaction of these compounds with the site at which Mg^{2+} acts to inhibit binding of [^{3}H]MK-801. Another possibility is that the effects of polyamines are not mediated by a direct interaction with a recognition site on the NMDA receptor complex. Spermine and spermidine can bind to membrane phospholipids (19) and can function to stabilize membranes (19, 20). The effects of polyamines on the binding of [^{3}H]MK-801 could be due to an interaction with membrane lipids.

We have described the activities of compounds that selectively antagonize the effects of spermine and spermidine at the NMDA receptor. These compounds should prove to be useful in attempts to identify the site and mechanisms of action of polyamines at the NMDA receptor and for studies of the physiological significance of these mechanisms.

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