Impact of Polyamine Analogues on the NMDA Receptor

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Several N,N′-terminal dialkylated homologs of the tetraamine spermine exhibit a pronounced biphasic activity at the *N*-methyl-D-aspartate (NMDA) receptor–channel complex in rat cerebral cortex membranes in the presence of 100 μM L-glutamate and 100 μM glycine. At low micromolar polyamine concentrations, these analogs enhance binding of [^{3}H]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ([^{3}H]MK-801) similar to spermine (SPM). At higher concentrations (e.g., ≥10 μM), the analogs are antagonists of [^{3}H]MK-801 binding. The most potent analog, N^1,N^{14}-bis(1-adamantyl)homospermine, is almost totally devoid of agonist activity and is a potent antagonist at concentrations ≥5 μM. Three structural features of the tetraamines studied appear to correlate with potency of inhibition: (1) N-terminally alkylated polyamines > terminal primary amines (e.g., SPM); (2) length of the polyamine backbone, e.g., DMHSPM > DMNSPM; and (3) size of the terminal alkyl groups, i.e., adamantyl > *tert*-butyl > ethyl > methyl. These findings emphasize the potential of the tetraamine backbone as a pharmacophore to modulate NMDA receptor–channel function.

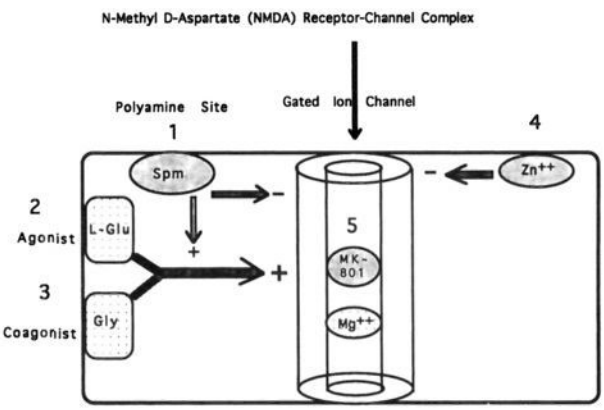
Introduction

N-Alkylated polyamines, especially terminally dialkylated analogs and homologs of the naturally occurring tetraamine spermine developed in these laboratories, exhibit antineoplastic activity against a number of murine and human tumor lines both *in vitro* and *in vivo*.[1,2] One of these analogs, N^1,N^{11}-diethylnorspermine (DENSPM), is currently in clinical trials as an antineoplastic. A second member of this series, N^1,N^{14}-diethylhomospermine (DEHSPM), was found to be a potent inhibitor of gastrointestinal motility during early clinical evaluation as an antineoplastic. Subsequent electrophysiological studies in rats and isolated guinea pig ileum strips showed that DEHSPM was able to abolish the waves of myoelectric activity associated with peristalsis.[3] These observations have led to an ongoing clinical evaluation of DEHSPM to control chronic diarrhea. The potent effects of DEHSPM on neuromuscular activity in the gut emphasize the potential of the tetraamine backbone as a pharmacophore to modulate neural transmission.

The *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor is a ligand-gated ion channel involved in excitatory neurotransmission in the mammalian central nervous system (CNS). Activation of the NMDA receptor–channel complex has been implicated in several physiological phenomena important to higher order CNS functions including long-term potentiation[4] and neuronal plasticity.[5] Overstimulation of the NMDA receptor results in neuronal excitotoxicity important in the pathophysiology of epilepsy[6] and ischemia-induced neuron death.[7]

The function of the NMDA receptor–channel complex, as measured by biochemical binding assays or electrophysiologically, is subject to a profound degree of regulation by a number of ligands, some of which are illustrated in Scheme 1. Separate regulatory sites include the binding site for the agonist L-glutamate ("2" in scheme); a high-affinity binding site for the obligatory co-agonist, glycine[8,9] ("3" in scheme); a site where Zn^{2+} acts to allosterically inhibit the agonist-induced response independently of membrane potential[10,11] ("4" in scheme); a site(s) within the channel[12,13] where Mg^{2+} and phencyclidines (phencyclidine (PCP), (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801), N-(1-[2-thienyl]cyclohexyl)piperidine (TCP), and ketamine) bind[14–16] to produce a voltage-dependent open channel block ("5" in scheme); and a distinct binding site for the polyamines, spermine, and spermidine which modulate NMDA receptor function[17,18] ("1" in scheme).

Scheme 1. Diagram Depicting the Mammalian *N*-methyl-D-aspartate (NMDA) Receptor Complex and Some of the Ligand Binding Sites Important to Receptor Function



As indicated in Scheme 1, the polyamine site may exert either agonistic or antagonistic modulatory activity depending on the polyamine concentration as well as on the chemical structure of the polyamine analogs.[19,20] *Agonists* like spermine allosterically facilitate function of the L-Glu and Gly sites resulting in increased binding of [^{3}H]MK-801 compared to that seen in the presence of maximal amounts of L-Glu and Gly alone ("100% Control"). Some polyamine analogues competitively inhibit spermine binding to the polyamine

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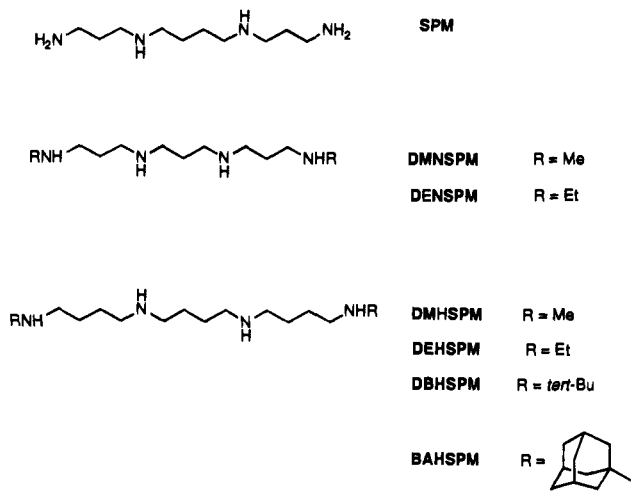


Figure 1. Structures of spermine (SPM) and its terminally dialkylated analogs.

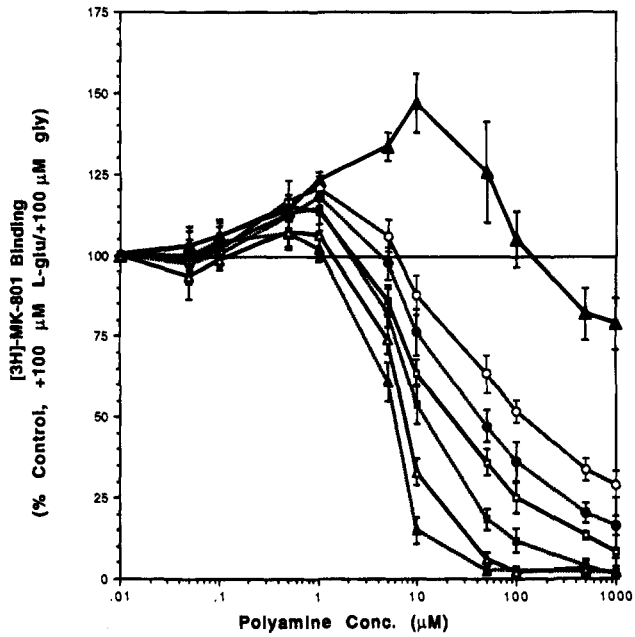
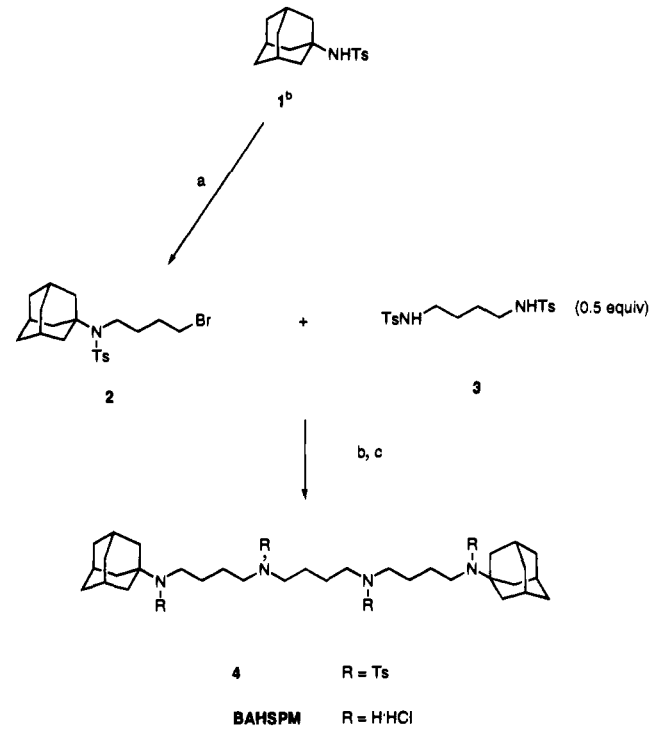


Figure 2. Modulation of [^{3}H]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ([^{3}H]MK-801, 2 nM) binding to extensively-washed rat cortical membranes (ca. 300 μg protein/mL) in the presence of excess L-glutamate (100 μM) and glycine (100 μM) by the endogenous tetraamine spermine and terminally N-alkylated analogues. Each experiment was done in triplicate for each concentration. Data are presented relative to "100% Control" binding observed in the presence of L-Glu and Gly coagonists, but absent added polyamine modulator. Standard deviation of the "100% Control" binding was typically about ±6%. Data are plotted as means ± standard deviation for four separate experiments on each tetraamine: (−▲−) SPM (n = 4), (○) DMNSPM (n = 4), (●) DMHSPM (n = 4), (□) DENSPM (n = 4), (■) DEHSPM (n = 4), (△) DBHSPM (n = 4), and (- -▲- -) BAHSPM (n = 4).

site thus reducing binding of [^{3}H]MK-801 to that seen in "100% Control". *Inverse agonist* action results in reduced binding of [^{3}H]MK-801 compared to "100% Control". Some have proposed that this action is a form of negative allosterism, but more recent evidence suggests that "inverse agonists" may directly produce a fast channel block to prevent binding of [^{3}H]MK-801.[21,22] It is noteworthy that spermine has these multiple actions at the NMDA receptor and that the site(s) responsible for both agonist and antagonist activities reside on a single subunit of the NMDA receptor−channel complex.[23] There is particular interest in the polyamine site(s) since several compounds which protect neurons from excitotoxicity appear to act as antagonists at this site(s).[24,25] Thus the polyamine site on the NMDA receptor−channel complex is a potential target for development of drugs which prevent excitotoxic or ischemic neuron cell death.[26]

Scheme 2. Synthesis of BAHSPM[a]



[a] (a) 1,4-Dibromobutane/NaH/DMF; (b) NaH/DMF; (c) Na/NH_3/THF, HCl. [b] Ts = *p*-tolylsulfonyl.

We now report a series of N-terminally dialkylated tetraamines which exhibit a pronounced biphasic action on NMDA receptor function displaying agonist activity similar to spermine at lower amine concentrations, but inhibition of [^{3}H]MK-801 binding in a manner similar to that described for inverse agonists at higher concentrations.

Synthesis

The symmetrically alkylated linear polyamine DENSPM, Figure 1, was accessed by bis-alkylation[1] of the tetrasulfonamide of norspermine, which has a 3-3-3 methylene arrangement. However, the terminally dialkylated homospermine (4-4-4 configuration) derivatives DEHSPM, DMHSPM, and N^1,N^{14}-di-*tert*-butylhomospermine (DBHSPM) were accessed by the "fragment synthesis" of polyamine analogs described in earlier works.[1,2] In these cases the central diamine segment as its N,N'-bis-sulfonamide was alkylated at each end with the fragmenting reagent, N-(ω-halomethylene)-N-alkylsulfonamide. This less direct method was employed for these compounds because homospermine is not commercially available and because attempted alkylation of the tetrasulfonamide dianion with, e.g., *tert*-butyl bromide leads instead to elimination.

The synthesis of N^1,N^{14}-bis(1-adamantyl)homospermine (BAHSPM) provides another example of the "fragment synthesis" of polyamine analogs. The generation of polyamine segmenting reagent **2**, Scheme 2, began by reacting 1-adamantanamine, a hindered primary amine, with toluenesulfonyl chloride under Schotten−

Baumann conditions. The resulting *N*-alkylsulfonamide **1** was deprotonated (NaH/DMF), followed by alkylation with 1,4-dibromobutane in excess to generate synthon **2**. The framework of BAHSPM was completed by alkylation of *N,N'*-ditosylputrescine (**3**) with >2 equiv of electrophile **2**. The sulfonamides of **4** were cleaved using sodium and liquid ammonia in THF to provide BAHSPM, a homospermine derivative with a highly bulky, and tertiary, alkyl group at each terminal nitrogen.

Results and Discussion

The spermine (SPM) curve is a biphasic "bell shape" with increased binding of [^{3}H]MK-801 relative to the "100% Control" observed at spermine concentrations between 1 and 100 μM (Figure 2). At 500 and 1000 μM SPM exhibits moderate antagonism of [^{3}H]MK-801 binding relative to the "100% Control": 500 μM SPM (82.0 ± 8.1) and 1000 μM SPM (78.9 ± 8.3). At 1 μM all of the polyamines except DBHSPM and BAHSPM displayed clear agonist activity: SPM (123.2 ± 5.0), DMNSPM (120.6 ± 3.1), DMHSPM (117.8 ± 8.1), DENSPM (113.7 ± 5.9), DEHSPM (114.3 ± 4.5), DBHSPM (106.5 ± 8.0), and BAHSPM (101.9 ± 4.0). At 10 μM all the analogues displayed significant ($p < 0.01$) antagonist activity relative to the "100% Control": DMNSPM (87.9 ± 6.0), DMHSPM (76.3 ± 7.0), DENSPM (63.7 ± 5.9), DEHSPM (53.9 ± 6.0), DBHSPM (33.0 ± 4.0), and BAHSPM (15.0 ± 4.0). Thus the potency as an antagonist correlates to the size of the terminal alkyl groups, i.e., adamantyl > *tert*-butyl > ethyl > methyl. The physiological significance of the agonist/antagonist properties of these molecules of course depends on the locally attainable tissue levels. For example the antitransit properties of DEHSPM observed in rodents is in keeping with the agonist behavior of the analog observed at low concentration.[3] In fact, in these experiments antitransit effects were seen at drug levels <5 mg/kg.

Potentiation at spermine concentrations between 1 and 250 μM and inhibition at higher concentrations (≥1 mM) have been reported in binding studies of [^{3}H]MK-801 as well as in electrophysiological assays of NMDA receptor function.[21-23] Structure–activity studies comparing di-, tri-, and tetraamines suggest that linear tetraamines exhibit the most potent agonist activity at the polyamine recognition site on the NMDA receptor.[20] A previous report suggested N-alkylation of the terminal amino groups alters the action at the polyamine recognition site. 1,3-Diaminopropane is a weak agonist, while the N^1,N^3-dimethyl analog is an inverse agonist without any agonist activity, but the N^1,N^1,N^3,N^3-tetramethyl analog is an agonist like the parent compound.[18] These diamine analogs only show activity at concentrations ≥100 μM. Moreover, most of the polyamine analogs previously reported exhibit one activity or the other, i.e., are agonists or antagonist/inverse agonist, but lack the biphasic behavior of spermine. A notable exception is the wasp venom toxin, philanthotoxin-433, a tetraamine derivative that at low concentrations (1–10 μM) enhances [^{3}H]MK-801 binding but at higher concentrations (20–1000 μM) exhibits potent inverse agonist activity.[27] Electrophysiological studies suggest that the inhibition observed at high spermine concentrations is due to a direct block of the ion channel,[21,22] that potentiation and block are mechanistically distinct processes, and that "inverse agonists" are acting as direct channel blockers. Given the potent antagonistic activity of BAHSPM, it is of interest that other 1-aminoadamantanes, amantadine and memanine, produce an open channel block to inhibit NMDA receptor function.[28] Three structural features of the tetraamines studied appear to correlate with potency of inhibition: (1) N-terminally alkylated polyamines > terminal primary amines (e.g., SPM); (2) length of the polyamine backbone, e.g., DMHSPM > DMNSPM; and (3) size of the terminal alkyl groups, i.e., adamantyl > *tert*-butyl > ethyl > methyl. We also hope to examine structural determinants of the agonist activity and the question of the degree to which it might be separated from antagonism in drug design. We are currently conducting experiments to further elucidate the mechanism(s) of action of *N,N'*-dialkyltetraamines and related polyamines at the NMDA receptor–channel complex. These will include a more extensive structure–activity study of a large number of tetraamine analogs and homologs previously synthesized as antineoplastics.[1]

Experimental Section

DMNSPM, DMHSPM, DENSPM, DEHSPM, and DTBHSPM were previously prepared in these laboratories.[1] Spermine tetrahydrochloride (SPM) and other reagents were purchased from Aldrich Chemical Co. Reactions using hydride reagents were run in distilled DMF under a nitrogen atmosphere. THF was distilled from sodium and benzophenone. Fisher Optima grade solvents were routinely used, and organic extracts were dried with sodium sulfate. Silica gel 60 (70–230 mesh) obtained from EM Science (Darmstadt, Germany) was used for column chromatography. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Proton NMR spectra were run at 90 MHz in $CDCl_3$ (not indicated) or at 300 MHz in D_2O with chemical shifts given in parts per million downfield from tetramethylsilane or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt, respectively. Coupling constants (J) are in hertz. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

Treatment of the appropriate amine with *p*-toluenesulfonyl chloride under biphasic conditions furnished *N*-(1-adamantyl)-*p*-toluenesulfonamide (**1**), mp 166 °C, mp (lit.[29] mp 166 °C) and *N,N'*-bis(*p*-toluenesulfonyl)-1,4-butanediamine (**3**) mp 138–140 °C, mp (lit.[30] mp 139 °C).

***N*-(1-Adamantyl)-*N*-(4-bromobutyl)-*p*-toluenesulfonamide (2).** NaH (80%, 2.0 g, 67 mmol) was added in portions to **1** (1.7 g, 5.6 mmol) in DMF (100 mL). The mixture was stirred at room temperature for 1 h, and 1,4-dibromobutane (62 g, 0.29 mol) was added at 0 °C. The mixture was stirred at room temperature overnight and heated at 85 °C for 2 h. The reaction mixture was cooled to 0 °C and the reaction cautiously quenched with water, followed by removal of solvents under high vacuum. Aqueous $NaHCO_3$ was added to the residue, followed by extraction with $CHCl_3$. Solvent removal gave **2** in quantitative yield: NMR δ 1.5–1.7 (m, 6 H), 1.74–2.15 (m, 13 H), 2.38 (s, 3 H), 3.23–3.54 (m, 4 H), 7.21 (d, 2 H, J = 8), 7.68 (d, 2 H, J = 8). Anal. ($C_{21}H_{30}BrNO_2S$) C, H, N.

***N*^{1},*N*^{5},*N*^{10},*N*^{14}-Tetrakis(*p*-toluenesulfonyl)-*N*^{1},*N*^{14}-bis(1-adamantyl)homospermine (4).** NaH (80%, 1.0 g, 33 mmol) was added in portions to **3** (3.86 g, 9.74 mmol) in DMF (90 mL). The mixture was stirred at room temperature for 1 h, and **2** (8.82 g, 20.0 mol) in DMF (10 mL) was added at 0 °C. The mixture was heated at 85 °C for 3 h. The reaction mixture was cooled to 0 °C and the reaction cautiously quenched with water, followed by removal of solvents under high vacuum. Water was added to the residue. Solid was filtered and then purified by column chromatography on silica gel, eluting with 6% EtOAc/$CHCl_3$ to produce 8.30 g (76%) of **4** as a solid: mp 208–209 °C; NMR δ 1.4–2.1 (m, 42 H), 2.38 (s, 12 H), 2.8–3.5 (m, 12 H), 7.10–7.34 (m, 8 H), 7.64 (d, 8 H, J = 8). Anal. ($C_{60}H_{82}N_4O_8S_4$) C, H, N.

N^1,N^{14}-Bis(1-adamantyl)homospermine Tetrahydrochloride (BAHSPM). Sodium (4.6 g, 0.20 mol) was added to liquid NH_3 (200 mL) at −78 °C. After the solution was stirred at −78 °C for 2 h, a suspension of **4** (8.30 g, 7.43 mmol) in THF was slowly added. The reaction mixture was stirred at −78 °C for an additional 1 h and at room temperature overnight to evaporate the ammonia. After the reaction was quenched with EtOH (200 mL), solvents were removed by rotary evaporation. The residue was diluted with H_2O and extracted with Et_2O (2×). The organic extracts were shaken with 1 N HCl, which was further extracted with Et_2O. The aqueous HCl layer was basified with aqueous NaOH. Solid was filtered and then purified by column chromatography on silica gel eluting with 20% concentrated NH_4OH/CH_3OH. After solvent removal, product was converted to its tetrahydrochloride, which was recrystallized with 10% aqueous EtOH to produce 1.72 mg (36%) of BAHSPM as a white solid: NMR (D_2O) δ 1.62−2.00 (m, 36 H), 2.16−2.25 (m, 6 H), 3.02−3.18 (m, 12 H). Anal. ($C_{32}H_{62}Cl_4N_4$) C, H, N.

Membrane Preparation and Binding Assay. The procedure for measuring specific binding of [^{3}H]MK-801 to the *N*-methyl-D-aspartate receptor complex was a modification of the method of Ransom and Stec.[17] Cerebral cortices from young male Sprague−Dawley rats (200−300 g) were homogenized with 10 volumes of ice-cold 0.32 M sucrose using a motor-driven glass/Teflon homogenizer. The homogenate was centrifuged at 1000*g* for 10 min. The 1000*g* pellet was resuspended in buffer A (5 mM Tris-HCl, pH 7.7 at 4 °C) and centrifuged at 8000*g* for 20 min. The supernatant and upper buffy coat of the pellet were combined and centrifuged at 50000*g* for 20 min. The pellet was then resuspended in 10 volumes of buffer A and homogenized using high-intensity ultrasound and centrifuged at 50000*g*, discarding the supernatant. The pellet was washed in this manner an additional three times and stored as a frozen suspension at −80 °C for at least 18 h, but no longer than 2 weeks before use.

For binding experiments, frozen membranes were thawed, pelleted at 50000*g* for 20 min, and washed as described above, except that 20 volumes of buffer A were used for resuspension, for a total of five times. The final pellet was resuspended in buffer B (5 mM Tris-HCl, pH 7.5 at 23 °C). The binding assay mixture was 1.00 mL of buffer B containing 200−300 μg of membrane protein (Lowry method[31]), 100 μM L-glutamate, 100 μM glycine, 2 nM [^{3}H]MK-801, and tetraamine at the following concentrations: 0 ("100% + L-Glu + Gly Control") and 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, and 1000 μM. Nonspecific binding was determined using 100 μM MK-801.

Binding assays were performed in triplicate at 23 °C for 1 h and were terminated by filtration through Whatman GF/B glass fiber filters followed by three 5.0 mL rinses of ice-cold buffer B using a Brandel M-48 cell harvester. Each tetraamine was tested on at least three different preparations of rat cortical membranes always with comparable results.

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