Polyamine Analogue Regulation of NMDA MK-801 Binding: A Structure−Activity Study

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A series of analogues and homologues of spermine were synthesized, and their impact on MK-801 binding to the *N*-methyl-D-aspartate (NMDA) receptor was evaluated. These tetraamines encompass both linear and cyclic compounds. The linear molecules include norspermine, N^1,N^{11}-diethylnorspermine, N^1,N^{12}-bis(2,2,2-trifluoroethyl)spermine, homospermine, and N^1,N^{14}-diethylhomospermine. The cyclic tetraamines consist of the piperidine analogues N^1,N^3-bis(4-piperidinyl)-1,3-diaminopropane, N^1,N^4-bis(4-piperidinyl)-1,4-diaminobutane, N^1,N^4-bis(4-piperidinylmethyl)-1,4-diaminobutane, and N^1,N^4-bis[2-(4-piperidinyl)ethyl]-1,4-diaminobutane and the pyridine analogues N^1,N^3-bis(4-pyridyl)-1,3-diaminopropane, N^1,N^4-bis(4-pyridyl)-1,4-diaminobutane, N^1,N^4-bis(4-pyridylmethyl)-1,4-diaminobutane, and N^1,N^4-bis[2-(4-pyridyl)-ethyl]-1,4-diaminobutane. This structure−activity set makes it possible to establish the importance of charge, intercharge distance, and terminal nitrogen substitution on polyamine-regulated MK-801 binding in the NMDA channel. Four families of tetraamines are included in this set: norspermines, spermines, homospermines, and tetraazaoctadecanes. Calculations employing a SYBYL modeling program revealed that the distance between terminal nitrogens ranges between 12.62 and 19.61 Å. The tetraamines are constructed such that within families cyclics and acyclics have similar lengths but different nitrogen pK_a's and thus different protonation, or charge, states at physiological pH. The pK_a values for all nitrogens of each molecule and its protonation state at physiological pH are described. The modifications at the terminal nitrogens include introduction of ethyl and β,β,β-trifluoroethyl groups and incorporation into piperidinyl or pyridyl systems. The studies clearly indicate that polyamine length, charge, and terminal nitrogen substitution have a significant effect on how the tetraamine regulates MK-801 binding to the NMDA receptor. Thus a structure−activity basis set on which future design of MK-801 agonists and antagonists can be based is now available.

Introduction

Recently a great deal of attention has been focused on the *N*-methyl-D-aspartate (NMDA) receptor as a target for the development of central nervous system (CNS) related therapeutics. The NMDA subtype of the glutamate receptor is a ligand-gated ion channel involved in excitatory neurotransmission in the mammalian CNS.[1] Activation of the NMDA receptor−channel complex has been implicated in several physiological phenomena important to higher order CNS functions including long-term potentiation[2] and neuronal plasticity.[3] Overstimulation of this receptor results in an inflow of Ca^{2+} and neuronal excitotoxicity important in the pathophysiology of epilepsy[4] and ischemia-induced neuron death.[5] This ligand-gated ionotropic glutamate receptor is subject to complex regulation by a number of ligands, some of which are illustrated in Figure 1.[6] Separate regulatory sites include the binding site (2) for the agonist *L*-glutamate; a high-affinity binding site (3) for the obligatory coagonist, glycine;[7,8] a site (4) where Zn^{2+} acts to allosterically inhibit the agonist-induced response independently of membrane potential;[9,10] a site(s) (5) within the channel[11,12] where Mg^{2+} and phencyclidine (PCP), (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (dizocilpine, MK-801), *N*-(1-[2-thienyl]cyclohexyl)-piperidine (TCP), and ketamine bind[13−15] to produce a voltage-dependent open channel block; and a distinct

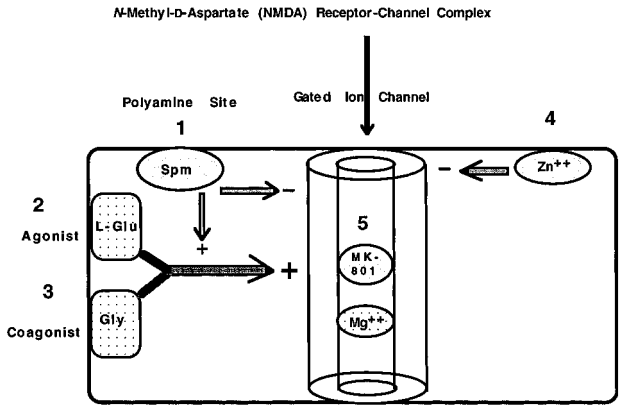

Figure 1. Diagram depicting the mammalian *N*-methyl-D-aspartate (NMDA) receptor complex and some of the ligand binding sites important to receptor function.

binding site (1) for the polyamines, spermine and spermidine, which modulate NMDA receptor function.[16,17]

Spermine and spermidine serve as potent allosteric effectors of the NMDA receptor. Both biochemical and electrophysiological studies support a "glycine independent" stimulation at saturating glycine concentrations and mild glycine dependent stimulation, in which a small spermine-dependent increase in glycine binding has been demonstrated.

Initial studies on the impact of these polyamines on dizocilpine binding to the NMDA receptor revealed a kind of biphasic polyamine action, which was agonistic

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at low concentration and antagonistic at high concentration.[6,18] Electrophysiological studies have suggested that the voltage dependent inhibition by spermine, or antagonistic effect, probably involves an open channel block and/or a screening of surface charges near the opening of the ion channel.[19] Thus it seems that potentiation and inhibition at least by spermine are consistent with two separate binding sites on the NMDA receptor−channel complex.[20]

Terminally N-dialkylated analogues and homologues of the naturally occurring tetraamine spermine exhibit a number of therapeutically useful properties. In fact phase I clinical trials are ongoing with DENSPM as an antineoplastic, and DEHSPM is in phase II clinical trials as an agent for the treatment of AIDS-related diarrhea (ARD).

The N-alkylated spermine analogues and homologues exhibit antineoplastic activity against a number of murine and human tumor lines both *in vitro* and *in vivo*.[21,22] These compounds have been shown to utilize the polyamine transport apparatus for incorporation,[23,24] to deplete polyamine pools,[25] to drastically reduce the level of ODC[26,27] and AdoMetDC activities,[28] and in some cases to upregulate SSAT.[29−33] Interestingly, very small structural alterations in these polyamine analogues can result in marked differences in their biological activities.[24] For example DENSPM stimulates SSAT by 1200-fold in MALME-3 cells, whereas DEHSPM stimulates SSAT by only 30-fold.[30,31,34] Differences in the behavior of these analogues are also reflected *in vivo* in terms of the therapeutic windows.[35,36]

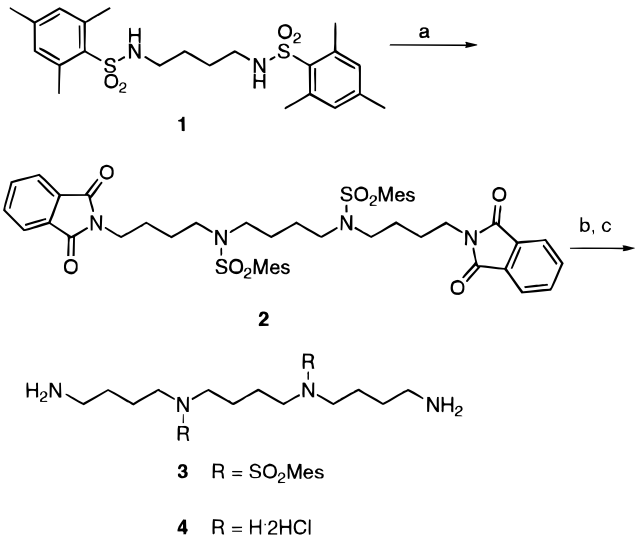
Interestingly, just as with the antineoplastics, small alterations in the polyamine backbone can have a tremendous impact on antitransit properties of the analogues. For example, DENSPM is 10 times less effective than DEHSPM as an antidiarrheal. This has been demonstrated in a number of animal models and recently in the clinic against ARD.[37]

In an earlier study we were able to demonstrate that a number of terminally dialkylated polyamine analogues had a significant impact on binding of MK-801 to the NMDA receptor in isolated rat brain cortical vesicles.[6] Of the analogues studied we were able to show a graded biphasic behavior. While none of the compounds investigated was as agonistic as spermine itself, they nevertheless all exhibited some agonistic behavior. This of course could translate to a potential toxicity problem if a polyamine analogue as a drug accumulates in the CNS to any significant extent.

In view of the biphasic behavior of the analogues investigated in the earlier study, we have elected to explore in a more systematic way how structural alterations of the polyamine analogues affect NMDA MK-801 binding. Clearly an understanding of such an SAR would help to facilitate the removal of potential neurotoxicities. Thus in the current study we evaluate the effects of chain length, charge, and terminal nitrogen substitution on the NMDA MK-801 binding properties.

Synthesis. Homospermine (HSPM), the predominant metabolic product from N^1,N^{14}-diethylhomospermine (DEHSPM),[36] was synthesized for testing in the NMDA receptor according to Scheme 1. N^1,N^4-Bis(mesitylenesulfonyl)putrescine (**1**)[38] was converted to its dianion (NaH/DMF) and alkylated with *N*-(4-bromobutyl)phthalimide (2 equiv), generating fully protected

Scheme 1.[a] Synthesis of Homospermine (HSPM) (**4**)



[a] Reagents: (a) *N*-(4-bromobutyl)phthalimide (2 equiv)/NaH/DMF; (b) $(NH_2)_2$/EtOH; (c) 30% HBr in HOAc/PhOH/CH_2Cl_2 then HCl.

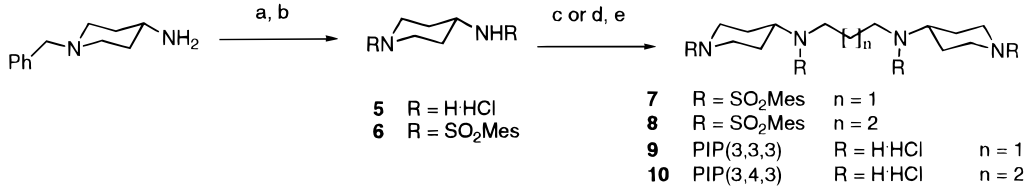
tetraamine **2**. Removal of the imide protecting groups of **2** with hydrazine hydrate in refluxing ethanol resulted in N^5,N^{10}-bis(mesitylenesulfonyl)homospermine (**3**). The internal sulfonyl groups were cleaved using 30% HBr in HOAc/PhOH to give HSPM (**4**) as its tetrahydrochloride salt. This method is a viable alternative to the literature preparation of HSPM,[39] in which the internal nitrogens were masked as their *N*-benzylamines.

The aliphatic cyclic tetraamines, N^1,N^3-bis(4-piperidinyl)-1,3-diaminopropane [PIP(3,3,3)] (**9**) and N^1,N^4-bis(4-piperidinyl)-1,4-diaminobutane [PIP(3,4,3)] (**10**), were also prepared utilizing the mesitylenesulfonyl amine protecting group and segmenting reagents (Scheme 2).[38,40] 4-Amino-1-benzylpiperidine was converted to 4-aminopiperidine dihydrochloride (**5**)[41] employing mild catalytic reduction conditions (Pd−C/methanolic HCl). Treatment of **5** with mesitylenesulfonyl chloride under biphasic conditions (NaOH/CH_2Cl_2) furnished *N*,*N*′-bis(mesitylenesulfonyl)-4-aminopiperidine (**6**). Alkylation of **6** with 1,3-dichloropropane (0.5 equiv)/NaH/DMF provided the tetrasulfonamide **7**, which was cleanly converted with 30% HBr in HOAc (PhOH/CH_2Cl_2) to bicyclic DENSPM analogue **9**. Reaction of **6** with 1,4-diiodobutane (0.5 equiv)/NaH/DMF gave masked polyamine **8**, which was deprotected as before to external dipiperidinyl DESPM analogue **10**.

N^1,N^3-Bis(4-pyridyl)-1,3-diaminopropane [PYR(3,3,3)] (**11**) and N^1,N^4-bis(4-pyridyl)-1,4-diaminobutane [PYR(3,4,3)] (**12**), DENSPM and DESPM analogues, respectively, in which the terminal ethyl groups have been tied back into the polyamine chain and the rings aromatized, have been synthesized by heating the hydrochloric acid salts of 4-bromopyridine and the appropriate diamine above 200 °C (Scheme 3).[42]

Molecular Geometry. In order to assess the significance of chain length in the polyamine regulation of MK-801 binding to the NMDA receptor, the distances between internal and external nitrogens were determined for each tetraamine using a SYBYL molecular modeling program (Table 1). Two distances were calculated: $N^{\alpha}-N^{\beta}$, the distance between the external

Scheme 2.[a] Synthesis of DIPIP(3,3,3) (**9**) and DIPIP(3,4,3) (**10**)



[a] Reagents: (a) H_2/10% Pd−C/CH_3OH/HCl; (b) mesitylenesulfonyl chloride (2 equiv)/NaOH/CH_2Cl_2; (c) 1,3-dichloropropane (0.5 equiv)/NaH/DMF; (d) 1,4-diiodobutane (0.5 equiv)/NaH/DMF; (e) 30% HBr in HOAc/PhOH/CH_2Cl_2 then HCl.

Scheme 3.[a] Synthesis of DIPYR(3,3,3) (**11**) and DIPYR(3,4,3) (**12**)

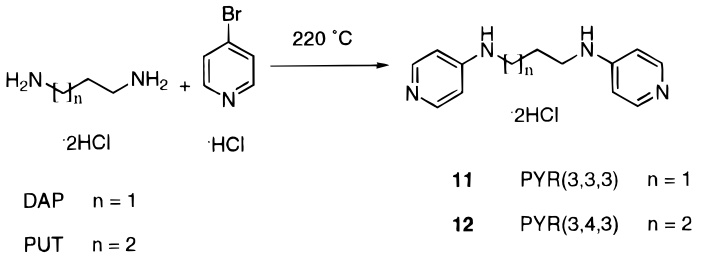


Table 1. SYBYL-Generated Distances between Nitrogens on Polyamine Analogues

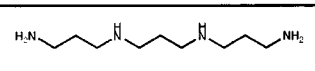
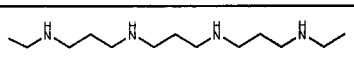
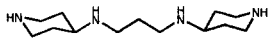
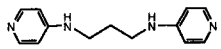
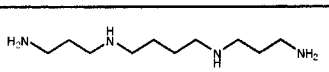
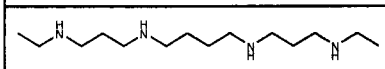
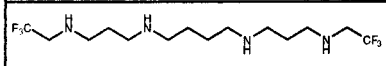
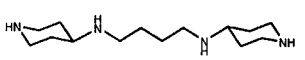
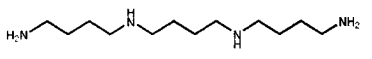
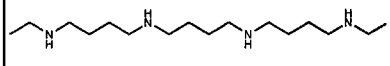
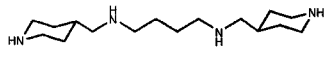
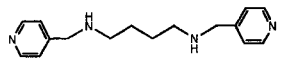
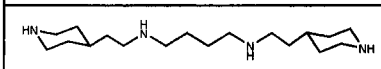
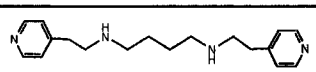
polyamines	$N^{\alpha}-N^{\beta}$ (Å)	$N^{\alpha}-N^{\alpha'}$ (Å)
NSPM(3,3,3)	5.028	15.093
DENSPM(3,3,3)	5.038	15.110
PIP(3,3,3)	4.333	13.164
PYR(3,3,3)	4.177	12.617
SPM(3,4,3)	5.029	16.406
DESPM(3,4,3)	5.038	16.425
FDESPM(3,4,3)	5.006	16.352
PIP(3,4,3)	4.368	14.089
PYR(3,4,3)	4.177	13.863
HSPM(4,4,4)	6.361	18.978
DEHSPM(4,4,4)	6.370	18.994
PIP(4,4,4)	5.377	17.035
PYR(4,4,4)	5.020	16.275
PIP(5,4,5)	6.777	19.609
PYR(5,4,5)	6.506	18.859

nitrogen and the first internal nitrogen and $N^{\alpha}-N^{\alpha'}$, the distance between the external nitrogens. In this calculation the charges set on the nitrogens were those calculated for the predominant species at physiological pH (Table 2). These cationic species were set in their lowest energy state, and the distances were measured. On the basis of previous studies carried out on various 4-amino-substituted pyridines,[43] we made the assumption that PYR(3,3,3) and PYR(3,4,3) are dications from protonation of the pyridine nitrogens.

Protonation State Calculations. One of the critical structure−activity issues is related to the significance of polyamine charge on polyamine regulation of MK-801 binding in the NMDA channel. Thus we designed a series of tetraamines which we anticipated would have similar dimensions but different protonation states, e.g., DESPM *vs* FDESPM or PIP(3,4,3) *vs* PYR(3,4,3) (Table 2). Polyamine hydrochlorides were first converted to free amines by addition of base, and titrations were carried out in 0.1 N KCl. The pK_a's were estimated from a computer fit of the titration curves utilizing an MINSQ.2.8 nonlinear parameter estimation. The fraction of various protonated species was calculated using the same program in the simulation mode given the measured pK_a values. However, in the case of PYR(3,4,3) insolubility of the free base at high pH precluded pK_a measurement. In the case of PYR(3,3,3), addition of base resulted in a very fine dispersion. However, the turbidity completely cleared just after the first equivalent of acid had been added during the titration (ca. pH 9.3), and exactly 2 equiv of acid were consumed prior to pH 8. There were only these two titratable groups in the pH range 2−12. The titration curve itself was very well-fitted to the theoretical with pK_a's of 9.55 and 9.14 as indicated; if there is any error due to solubility problems, these values are artificially *low*. The conclusion that PYR(3,3,3) exists almost exclusively as a bispyridinium dication at pH 7.4 with the charges separated by the entire length of the molecule is corroborated by previous determination of the pK_a's in 4-aminopyridine[43] where the pyridine nitrogen is quite basic (pK_{a1} = 9.15) and the primary amine is too weak to be titrated in aqueous solution (pK_{a2} = −6.60).

Norspermine and Analogues. Like spermine, norspermine (NSPM) is biphasic in its impact on MK-801 binding but has a more protracted concentration range in which it is agonistic (Figure 2a). The agonistic phase begins at 0.5 μM, peaks at 10 μM with a 33% binding increase and approaches an antagonistic frame around 1 mM. The agonistic behavior lasts over a 1 mM span. Although NSPM never really becomes an antagonist over the concentration evaluated, it is clearly moving in that direction. DENSPM also exhibits biphasic activity, but relative to NSPM the curve is shifted to the left. The agonistic behavior, albeit somewhat weak, begins at 0.1 μM, peaks at 1 μM with a 13% binding enhancement, and moves into an antagonistic mode above 2.0 μM. Thus the agonistic phase spans a 1.9 μM range. At 25 μM DENSPM, MK-801 binding is reduced by 50%. We shall refer to this as the antagonistic IC_{50} value (A IC_{50}). The piperidine analogue PIP(3,3,3) is the most potent agonist we have identified, increasing MK-801 by 100%. Its agonistic behavior begins at 0.05 μM, peaks at 500 μM, and begins to fall. Its antagonistic concentration curve is shifted to the right relative to NSPM. It seems clear that if indeed a concentration can be attained in which the tetraamine PIP(3,3,3) is an antagonist, it would require high millimolar concentrations. Finally, the pyridine norspermine analogue, PYR(3,3,3), while it may demonstrate minor, if any, agonistic behavior at low concentrations, is very similar

Table 2

Structure	Compound Abbreviation	pKa1	pKa2	pKa3	pKa4	Relative Abundance of Species vs. Charge (pH=7.4) 0	1+	2+	3+	4+
	NSPM(3,3,3)	10.61	9.8	8.35	7.51	0	0	4.7	41.6	53.7
	DENSPM(3,3,3)	10.83	9.91	8.51	7.68	0	0	2.6	33.5	63.9
	PIP(3,3,3)	11.18	10.67	8.88	7.14	0.0%	0.0%	2.1%	63.2%	34.7%
	PYR(3,3,3)	9.55	9.14	<2	<2	0.0%	1.8%	98.2%	0.0%	0.0%
	SPM(3,4,3)	10.86	10.05	8.82	7.95	0.0%	0.0%	1.0%	23.4%	75.6%
	DESPM(3,4,3)	11.20	10.77	9.42	8.25	0.0%	0.0%	0.1%	12.4%	87.5%
	FDESPM(3,4,3)	10.48	9.41	4.81	4.09	0.0%	1.0%	98.6%	0.3%	0.0%
	PIP(3,4,3)	11.40	10.68	9.02	7.60	0.0%	0.0%	0.9%	38.4%	60.7%
	HSPM(4,4,4)	11.25	10.40	9.57	8.72	0.0%	0.0%	0.0%	4.6%	85.4%
	DEHSPM(4,4,4)	11.10	10.81	9.73	8.91	0.0%	0.0%	0.0%	3.0%	97.0%
	PIP(4,4,4)	10.93	10.69	9.43	8.45	0.0%	0.0%	0.1%	8.2%	91.7%
	PYR(4,4,4)	9.23	7.64	4.43	3.77	0.7%	36.3%	63.0%	0.0%	0.0%
	PIP(5,4,5)	10.93	10.82	10.01	9.10	0.0%	0.0%	0.0%	2.0%	98.0%
	PYR(5,4,5)	9.81	8.85	5.69	4.93	0.0%	3.3%	94.7%	2.0%	0.0%

to DENSPM in its antagonistic behavior, which begins around 0.05 μM with a left shift in the concentration *vs* binding curve. It has an A IC_{50} of 11 μM.

Spermine and Analogues. Spermine (SPM) has clear biphasic behavior with an agonistic segment beginning at 0.1 μM and peaking at 11 μM with a 35% enhancement in MK-801 binding (Figure 2b). The antagonistic behavior begins at 300 μM. Thus the agonistic mode continues through a 300 μM concentration range. DESPM also shows biphasic activity, although not nearly as pronounced as the parent compound. The agonistic onset begins at 0.1 μM and lasts over a 1.9 μM range with a peak at 1 μM. The antagonistic phase begins at 2.0 μM with an A IC_{50} at 10 μM.

Initially PIP(3,4,3) behaves very much like SPM with onset of agonism beginning at 0.8 μM and peaking at 10 μM with a 45% increase in MK-801 binding. Its curve is thus shifted to the right relative to SPM. However, agonistic behavior spans at least into the millimolar range. In contrast PYR(3,4,3) is essentially a pure antagonist manifested at a very low concentration with an A IC_{50} of 8 μM and a clear left shift in the curve relative to SPM. Finally, over the concentrations studied FDESPM has little if any activity, similar to putrescine (PUT).[17] In both FDESPM and PUT the two point charges at a pH of 7.4 are separated by four methylenes.

Homospermine and Homologues. Homospermine (HSPM) shows much less of an exaggerated biphasic behavior than spermine (Figure 2c). Agonism begins at 0.5 μM and peaks at 7.0 μM with a 13% increase in MK-801 binding; antagonism begins at 10 μM. Thus the agonistic fragment of the curve persists over a 9.5 μM range. Of the three parent linear tetraamines, HSPM is clearly the most effective antagonist, with an 80 μM A IC_{50}. DEHSPM behaves like HSPM in its biphasic activity except that the curve is shifted to the left. The agonistic portion of the curve begins at 0.1 μM and peaks at 1 μM with a 13% increase in MK-801 binding. The antagonistic phase begins at 2 μM, with an A IC_{50} of 10 μM. Thus the agonistic phase runs over a 1.9 μM range. Of the HSPM analogues, it is the most potent antagonist. The homospermine piperidine ana-

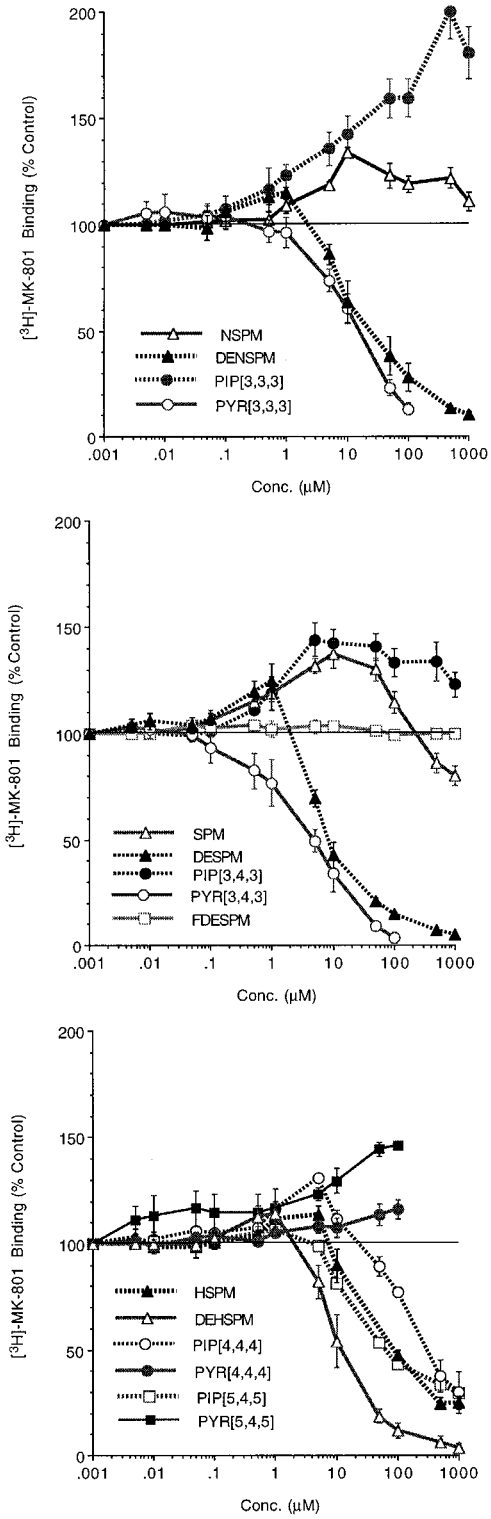


Figure 2. Modulation of [^{3}H]-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine ([^{3}H]MK-801, 2 nM) binding to extensively washed rat cortical membranes (ca. 300 μg of protein/mL) in the presence of excess L-glutamate (100 μM) and glycine (100 μM) by the tetraamines, norspermine (**2a**), spermine (**2b**), and homospermine (**2c**) and the corresponding terminally *N*-ethylated analogues, and bis(4-piperidinyl/pyridyl) homologues. 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 ±3%. Data are plotted as means ± standard deviation of four separate experiments ($N = 4$) for each tetraamine and each tetraamine analogue and homologue.

logue PIP(4,4,4) response curve is shifted to the right of HSPM itself with the agonistic component beginning at 0.5 μM and peaking at 8 μM with a 30% enhanced binding. The antagonistic phase begins at 12 μM. Thus the agonistic phase encompassed an 11.5 μM range with moderate antagonistic behavior beyond this point. The pyridine analogue PYR(4,4,4) is a weak agonist over the entire range studied beginning at 0.1 μM. However, MK-801 binding is only increased by 16% at 100 μM. The PYR(5,4,5) analogue is a pure agonist in the concentration range studied with a 50% binding enhancement at 100 μM with a clear left shift in the curve relative to HSPM. The agonistic behavior of this compound begins at very low concentration, <0.05 μM. Interestingly the corresponding piperidine analogue PIP(5,4,5) is weakly biphasic with a slightly left shift and an agonistic component beginning at 0.1 μM and peaking at 1 μM with a 10% MK-801 binding enhancement. The compound then moves into a potent antagonistic phase at 8 μM with a 50 μM A IC_{50}. The agonistic component lasts only through a 7.9 μM range.

Discussion

Earlier studies in our laboratories[6] and others have clearly demonstrated that polyamines[16,17] and polyamine analogues[44] have a profound impact on the NMDA receptor. Probably the most thoroughly studied of the polyamines, spermine, a naturally occurring linear tetraamine, has been shown to exhibit concentration dependent biphasic activity at the level of MK-801 binding in the NMDA channel. The inhibitory and potentiation properties of this tetraamine involve two distinct polyamine binding sites. At saturating concentrations of glycine, low concentrations of spermine produce a "glycine independent" increase in NMDA-induced whole cell currents and a voltage dependent inhibition at higher spermine concentrations. However, studies with various cloned NMDA heteromeric receptors strongly suggest that spermine-induced glycine dependent stimulation is mechanistically different from glycine independent stimulation.[45−48] It seems likely that the voltage dependent inhibition by spermine involves an open channel block. However, a still lingering alternative explanation involves the screening of surface charge on the NMDA receptor by the polyamine.[19]

An earlier structure−activity investigation of the effects of polyamine analogues on MK-801 binding in the NMDA channel suggested the key parameter in voltage dependent inhibition is the length of the polyamine chain rather than the nature of the terminal alkyl substituents.[44] The study included a series of ethylated spermine homologues and analogues, both tetraamines and pentaamines. However, while the results were very interesting, the program only focused on terminally ethylated polyamines, e.g., DENSPM, DEHSPM, and did not include the corresponding norspermine and homospermine controls. This exclusion makes it somewhat difficult to assess the significance of the alkyl groups within a family of particular chain length. For example, if polyamine chain length is indeed the most important structural feature in voltage block, then it becomes problematic to assess the contribution, if any, of terminal alkyl groups on norspermine when comparing the voltage blocking properties of an alkylated norspermine with those of an alkylated homospermine. The significance of these terminal alkyl

groups becomes particularly important in the design of polyamine analogue therapeutics which could impact on the NMDA receptor as either agonists or antagonists.[6] We have already demonstrated that polyamine analogues can be substantially less toxic than the parent amine.[27] We have also demonstrated that it is possible to metabolically program a polyamine analogue in such a way as to substantially reduce toxicity associated with its metabolites.[37]

Thus a consideration of how structural modifications affect a linear polyamine's impact on the NMDA receptor could be critical in the design of polyamine analogues. In this regard we have adopted a systematic approach to assessing the effects of charge, polyamine chain length, and terminal alkyl group size on the ability of polyamines to agonize or antagonize MK-801 binding.

We have chosen to evaluate a group of spermine homologues and analogues including norspermines (3,3,3), spermines (3,4,3), homospermines (4,4,4), and tetraazaoctadecanes (5,4,5). The terminal nitrogen substituents include ethyl and β,β,β-trifluoroethyl, as well as incorporation of the terminal nitrogens into a piperidine or pyridine ring.

Charge. Four structural alterations at the terminal nitrogens were used to control the charge, or protonation state, of the polyamine analogues (Table 2). The alterations were all made at the primary terminal nitrogen. The terminal primary amines of norspermine, spermine, or homospermine were either ethylated to DENSPM, DESPM, DEHSPM; incorporated into a piperidyl ring system PIP(3,3,3), PIP(3,4,3), PIP(4,4,4); or incorporated into a pyridyl system PYR(3,3,3), PYR(3,4,3), PYR(4,4,4). The terminal nitrogens of spermine were also β,β,β-trifluoroethylated. Finally in the tetraazaoctadecanes the terminal nitrogen was incorporated into either a piperidyl or pyridyl ring.

The trends in the pK_a values are in keeping with simple electrostatics (Table 2). In going from norspermine, a tetraamine in which all of the nitrogens are separated by three methylenes, through spermine, in which the terminal and internal nitrogens are separated by three methylenes and the internal nitrogens by four methylenes, to homospermine, in which successive nitrogens are separated by four methylenes, the fraction of tetracation at physiological pH goes from 54 to 76 to 85%. In each instance fixing an ethyl group to both of the external nitrogens increases the fraction of tetracation by about 10%. Interestingly in going from the terminal secondary amines DENSPM, DESPM, and DEHSPM to the corresponding secondary piperidyl systems, the fraction of +4 cation drops in each case for PIP(3,3,3), PIP(3,4,3), and PIP(4,4,4). The longer the molecule the smaller the decrease. This is in keeping with the idea that folding the ethyl groups of DENSPM, DESPM, and DEHSPM back into a piperidyl system brings the terminal secondary nitrogens even closer to the internal nitrogens. Thus in the tetracation the charges would be closer together for the piperidyl systems, an electrostatically less favorable situation. As predicted this effect becomes less significant the longer the methylene insulator between nitrogens, e.g., the decrease in the +4 cation ratio between DENSPM/PIP(3,3,3) and DEHSPM/PIP(4,4,4).

Substitution of the ethyl group of DESPM with a β,β,β-trifluoroethyl group reduces the pK_a of the two terminal nitrogens and thus the fraction of tetracation from 88% to essentially 0% while raising the level of dication from 0 to 99%. A similar situation exists for PIP(4,4,4) and the corresponding pyridyl compound PYR(4,4,4). The former tetraamine is 92% in the tetracationic state and nearly 0% in the dicationic state, while the latter compound is 63% dication and 36% monocation with no tetracationic form at physiologic pH. A similar scenario applies to the PIP(5,4,5) and the PYR(5,4,5). The PIP compound is 98% in the tetracationic state with no dicationic species. The PYR(5,4,5) presents almost exclusively as the dication (95%). The difference in the fraction of monocationic and dicationic species between PYR(4,4,4) and PYR(5,4,5) is likely related to the fact that the PYR(4,4,4) has only one methylene separating the two central nitrogens from each electron-withdrawing pyridine ring, while PYR(5,4,5) has two such insulators.

The most interesting pyridine analogues are PYR(3,3,3) and PYR(3,4,3). In both cases there is no insulator between the internal nitrogens and the aromatic ring. PYR(3,3,3) is almost exclusively in the dicationic state. On the basis of literature studies of similar molecules, it is likely that only the pyridine nitrogens of both analogues are protonated.[43] Because of this one would anticipate that PYR(3,3,3) and PYR(3,4,3) would behave much like the corresponding long-chain diamines 1,11-diaminoundecane and 1,12-diaminododecane, respectively.

The importance of charge to the tetraamine's biological activity is quite clear from the studies. In the norspermine series the best comparison is made between PIP(3,3,3) and PYR(3,3,3). The PIP(3,3,3) tetraamine is the most potent MK-801 binding agonist we have identified, while the aromatic tetraamine is a very potent antagonist much like 1,10-diaminodecane.[49,50] Again this is simply because PYR(3,3,3) has a charge disposition very much like the true diamine. In the spermine series the effect of substituting the ethyls of DESPM with β,β,β-trifluoroethyls is profound. The resulting FDESPM, a dicationic species at physiologic pH, is nearly devoid of any activity over the concentration range studied, while DESPM exhibits biphasic activity with a potent antagonistic component. In this tetraamine series the NMDA agonist and antagonist relationship between the PIP(3,4,3) and PYR(3,4,3) analogues is similar to that seen with PIP(3,3,3) and PYR(3,3,3). Thus the tetracation PIP(3,4,3) is a potent agonist albeit somewhat less active than the shorter PIP(3,3,3). The PYR(3,4,3) analogue is the most potent antagonist we have identified. Again, because of the terminal position of the charges in this molecule at physiological pH, this tetraamine behaves like 1,12-diaminododecane.[49,50]

In the homospermine family the significance of charge in MK-801 binding is further underscored. PIP(4,4,4) is biphasic, while the corresponding pyridyl compound PYR(4,4,4) has no antagonistic properties over the range investigated but is weakly agonistic. Recall that at physiological pH this PYR(4,4,4) has a fairly substantial fraction of monocation (36%) with 63% in the form of the dication. The PIP(5,4,5) compound is slightly biphasic but primarily an antagonist. However and more

notable is the fact that the PYR analogue is a pure agonist and its agonistic properties relative to PIP(5,4,5) are greater than those of PYR(4,4,4) relative to its analogue PIP(4,4,4). Recall that PYR(5,4,5) has a much higher fraction of dication than PYR(4,4,4) at physiological pH.

Chain Length. In designing these molecules a BIOSYM molecular modeling program was employed. The synthetic candidates were set in their potential minimum, and the through-space distances between the respective $N^{\alpha}-N^{\beta}$ nitrogens, the external nitrogen and closest neighboring nitrogen, were measured along with the $N^{\alpha}-N^{\alpha'}$ value the distance between the two external nitrogens (Table 1).

These calculations were carried out on the basis of the following assumptions about the charged state of the tetraamines at physiological pH (Table 2). On the basis of the pK_a studies, all of the parent amines are largely in the +4 charge state as are their ethylated analogues. PIP(3,3,3) is assumed to be predominately in the +3 cationic and PIP(3,4,3), PIP(4,4,4), and PIP(5,4,5) largely in the +4 cationic state. FDESPM and the pyridyl compounds are in the +2 cationic state. FDESPM is assumed to have a single charge on each of the central nitrogens. The pyridine compounds PYR(3,3,3) and PYR(3,4,3) are assumed to have a single charge on each of the terminal nitrogens.[43] While there was nothing unexpected about the results of the calculations, a few brief comments are in order. In each family of tetraamines-norspermine, spermine, homospermine, and tetraazaoctadecane—the $N^{\alpha}-N^{\alpha'}$ distances consistently decreased in going from the ethylated compounds through the piperidyl to pyridyl analogues. The tetraamines varied in length from 12.617 Å for PYR(3,3,3) to 19.609 Å for PIP(5,4,5). Finally DESPM and FDESPM are nearly identical in terms of their $N^{\alpha}-N^{\alpha'}$ and $N^{\alpha}-N^{\beta}$ values.

There is indeed a correlation between tetraamine length and impact on MK-801 binding. Looking at the concentration required to reduce MK-801 binding by 50%, antagonistic IC_{50} (A IC_{50}), homospermine is significantly more effective than either norspermine or spermine. Although PYR(3,3,3) and PYR(3,4,3) are shorter molecules than PYR(4,4,4) and PYR(5,4,5), it is critical to recall that when considering the overall length of the molecule, the important distance is between external cations. Both PYR(3,3,3) and PYR(3,4,3) are protonated on the terminal pyridine nitrogens at physiological pH, unlike PYR(4,4,4) and PYR(5,4,5), which are protonated on the two internal nitrogens. Thus from a length perspective PYR(3,3,3) and PYR(3,4,3) are more like 1,11-diaminoundecane and 1,12-diaminododecane,[49,50] while PYR(4,4,4) and PYR(5,4,5) are both like disubstituted putrescine. This thus explains the impressive antagonistic behavior of PYR(3,3,3) and PYR(3,4,3) relative to the lack of antagonism with PYR(5,4,5) and PYR(4,4,4). Thus it seems like these observations are in keeping with earlier suggestions that the length of the tetraamine is a major component contributing to its antagonistic behavior.

The agonistic concentration span is also inversely related to the length of the parent tetraamines. For example norspermine is agonistic over the largest concentration range, followed by spermine and homospermine. The same inverse relationship holds for the PIP compounds, with agonistic activity of PIP(3,3,3) > PIP(3,4,3) > PIP(4,4,4) > PIP(5,4,5). No such relationship between the concentration span of antagonism and PYR analogue structure is apparent.

The onset of antagonism also correlates inversely with the length of the tetraamine for both the parent polyamines and the piperidyl analogues. However, both PYR(3,3,3) and PYR(3,4,3) have very low antagonism onset values, while the corresponding PYR(4,4,4) and PYR(5,4,5) systems are pure agonists.

Nitrogen Substitution. For norspermine, spermine, and homospermine the overall trend in terminal nitrogen substitution seems clear. Addition of an ethyl group to the parent amine shifts the dose–response curves to the left, rendering the tetraamine better antagonists. However, incorporation of the terminal nitrogens into a piperidine ring system shifts the dose–response curves to the right, resulting in compounds which are better agonists. For both PYR(3,3,3) and PYR(3,4,3), where the terminal pyridine nitrogens are protonated, the curves shift to the left, resulting in compounds that are potent antagonists. In the PYR(4,4,4) case without terminal protonation at physiological pH, the curve is shifted right to a more agonistic direction. It is unfortunate that we cannot speak to the impact of PYR or PIP on the 5,4,5 system, but the parent tetraamine was not available.

Conclusion

It seems clear that the effectiveness with which the tetraamines described in the study antagonize MK-801 binding in the NMDA channel is related to their length and charge state. For example, when comparing tetracations at physiological pH, the longer molecules are the best antagonists. It is not enough to compare tetraamines unless the charge distribution is the same. Holding the geometry of the molecule essentially constant but changing the pK_a of the nitrogens and thus the protonation state has a profound effect on the tetraamine's activity. For example, FDESM is virtually identical to the potent tetraamine analogue, DESPM, except that the p*K*a's of the two external nitrogens are changed from strongly to weakly basic so that FDESM is charged only at the two internal nitrogens, resembling putrescine, and like putrescine is completely devoid of activity. In another example, PIP(3,3,3) has substantial tetracationic character at physiological pH and behaves as a short tetracation like norspermine, an agonist over a wide concentration range. However, the corresponding PYR(3,3,3) analogue is only charged at the terminal pyridinium nitrogens, resembling a long-chain diamine like 1,11-diaminoundecane. Not surprisingly, PYR(3,3,3) is a potent antagonist of MK-801 binding as is the true diamine.

Experimental Section

DENSPM, DESPM, FDESPM, DEHSPM, PIP(4,4,4), PYR(4,4,4), PIP(5,4,5), and PYR(5,4,5) were previously prepared in these laboratories.[21,38,40] NSPM, SPM, and other reagents were purchased from Aldrich Chemical Co. Reactions using hydride reagents were run in distilled DMF under a nitrogen atmosphere. Fisher Optima grade solvents were routinely used, and organic extracts were dried with sodium sulfate unless otherwise indicated. Silica gel 32–63 (40 μM "flash") from Selecto, Inc. (Kennesaw, GA) 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 300 MHz in $CDCl_3$ (not indicated) or 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. FAB mass spectra were run in a glycerol/trifluoroacetic acid matrix. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

Computer Modeling.[40] The distances between nitrogens on polyamine analogues were taken from their most stable conformations. The search for low-energy conformations of polyamine analogues was done using BIOSYM program 2.3.0 running on a Silicon Graphics Indigo2 workstation, and the results are similar to those using the conformational search program supported by Tripos Associates Inc. in SYBYL 6.02.

Potentiometric Measurements.[40] The amine tetrahydrochloride (0.300 mmol) was dissolved in carbon dioxide free 0.1 M KCl (20.0 mL) and converted to the free base by addition of a slight excess of 1 N NaOH (1.25 mequiv). This solution was then *immediately* titrated with 1 N HCl using a Radiometer-Copenhagen DTS833 digital titration system. The pK_a's were estimated from a computer fit of the titration data using MINSQ v. 2.0 nonlinear parameter estimation software (Micromath Scientific Software, Salt Lake City, UT). The relative abundance of each charged species present was calculated by MINSQ in the simulation mode given the four pK_a values.

Membrane Preparation and Binding Assay.[6] 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.[16] Cerebral cortices from young male Sprague−Dawley rats (175−200 g) were homogenized with 10 volumes 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 discarded, and the supernatant was centrifuged at 18000*g* for 20 min. After the supernatant was removed, the pellet was resuspended in 10 volumes of 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[51]), 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 4.0 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.

***N,N′*-Bis(4-phthalimidobutyl)-*N,N′*-bis(mesitylenesulfonyl)-1,4-butanediamine (2).** Sodium hydride (60%, 1.72 g, 43.0 mmol) was introduced into a solution of **1**[38] (7.59 g, 16.8 mmol) in DMF (125 mL) at 0 °C. After the mixture was stirred at room temperature for 35 min, *N*-(4-bromobutyl)phthalimide (10.20 g, 36.2 mmol) was added. The mixture was stirred at room temperature for 2 h and at 70 °C for 19 h and cooled, and the reaction was quenched with EtOH. Following removal of solvent under high vacuum, water was added, and product was extracted with EtOAc (3×). The combined organic layers were washed with water (2×) and brine, concentrated, and recrystallized from EtOAc to give 9.43 g (66%) of **2** as a white solid: mp 172−174 °C; ^{1}H NMR δ 1.3−1.7 (m, 12 H), 2.23 (s, 6 H), 2.55 (s, 12 H), 3.07−3.18 (m, 8 H), 3.54 (t, 4 H), 6.89 (s, 4 H), 7.68−7.86 (m, 8 H). Anal. ($C_{46}H_{54}N_4O_8S_2$) C, H, N.

***N*^{5},*N*^{10}-Bis(mesitylenesulfonyl)homospermine (3).** Hydrazine monohydrate (1.5 mL, 31 mmol) was added to a suspension of **2** (9.32 g, 10.9 mmol) in absolute EtOH (120 mL), and the mixture was heated at reflux for 2 h. After being cooled to room temperature, the mixture was filtered, and the white solid was washed with EtOH (2×). The filtrate was concentrated to furnish **3**, which was used directly in the next step. Anal. ($C_{30}H_{50}N_4O_4S_2$) C, H, N.

Homospermine Tetrahydrochloride (HSPM) (4). HBr in HOAc (30%, 50 mL) was added to a solution of **3** (7.28 g, 12.2 mmol) and phenol (46 g, 0.49 mol) in CH_2Cl_2 (100 mL) at 0 °C. After the mixture was stirred at room temperature overnight, water (100 mL) was added, and the layers were separated. The organic layer was washed with water (50 mL), and the combined aqueous portion was extracted with CH_2Cl_2 (3×). The aqueous portion was evaporated under high vacuum, treated at 0 °C with 2 N NaOH (10 mL) and 50% NaOH (25 mL), and extracted with $CHCl_3$ (8 × 50 mL). After solvent removal, the residue was taken up in EtOH (50 mL), acidified with concentrated HCl (10 mL), and evaporated. Recrystallization from aqueous EtOH gave 3.7 g of **4** (80%) as white crystals: ^{1}H NMR (D_2O) δ 1.71−1.86 (m, 12 H), 3.01−3.17 (m, 12 H). Anal. ($C_{12}H_{34}Cl_4N_4$) C, H, N.

***N,N′*-Bis(mesitylenesulfonyl)-4-aminopiperidine (6).** A solution of mesitylenesulfonyl chloride (14.86 g, 67.9 mmol) in CH_2Cl_2 (125 mL) was added to **5**[41] (5.44 g, 34.2 mmol) in 1 N NaOH (150 mL) at 0 °C. After addition was complete, the biphasic mixture was stirred overnight from 0 °C to room temperature. Water (100 mL) and $CHCl_3$ (200 mL) were added, the layers were separated, and the aqueous portion was extracted further with $CHCl_3$ (2 × 50 mL). The combined organic phase was washed with 100 mL of H_2O, 1 N HCl, and H_2O and evaporated *in vacuo*. Recrystallization from $CHCl_3$/EtOAc afforded 11.56 g (73%) of **6** as a crystalline white solid: mp 220−221 °C; NMR δ 1.35−1.83 (m, 4 H), 2.29 and 2.30 (2 s, 6 H), 2.56 and 2.62 (2 s, 12 H), 2.73−2.84 (m, 2 H), 3.17−3.49 (m, 3 H), 4.58 (d, 1 H, J = 8), 6.92 and 6.94 (2 s, 4 H). Anal. ($C_{23}H_{32}N_2O_4S_2$) C, H, N.

Tetrakis(mesitylenesulfonyl)-*N*^{1},*N*^{3}-bis(4-piperidinyl)-1,3-diaminopropane (7). Sodium hydride (60%, 0.55 g, 14 mmol) was added to **6** (4.65 g, 10 mmol) in DMF (80 mL) at 0 °C. The suspension was stirred at room temperature until a clear solution was obtained. 1,3-Dichloropropane (0.65 g, 5.75 mmol) was added, and the reaction mixture was heated at 85 °C overnight. Additional 60% NaH (0.2 g, 5 mmol) was added with ice cooling, and then the mixture was stirred for 2 h, followed by addition of more 1,3-dichloropropane (0.25 g, 2.2 mmol). The reaction mixture was heated at 85 °C for an additional 5 h. Solvent was removed *in vacuo*, and the residue was dissolved in CH_2Cl_2 (200 mL), which was washed with H_2O (4 × 50 mL) and dried over $MgSO_4$. The solvent was concentrated to about 50 mL and loaded onto a silica gel flash chromatography column, with 30% EtOAc/hexane as eluant, to provide 4.0 g (40%) of **7** as a white solid: mp 215 °C; ^{1}H NMR δ 1.30−1.65 (m, 4 H), 2.20−2.40 (m, 2 H), 2.40−2.80 (m, 28 H), 2.82−3.02 (m, 4 H), 3.40−3.66 (m, 6 H), 6.80−7.10 (m, 6 H). Anal. ($C_{49}H_{68}N_4O_8S_4$) C, H, N.

***N*^{1},*N*^{3}-Bis(4-piperidinyl)-1,3-diaminopropane Tetrahydrochloride [PIP(3,3,3)] (9).** HBr in acetic acid (30%, 75 mL) was added to a solution of **7** (3.82 g, 3.94 mmol) and phenol (14.2 g, 0.15 mmol) in CH_2Cl_2 (70 mL) at 0 °C. The reaction mixture was stirred overnight from 0 °C to room temperature. Distilled water (120 mL) was added at 0 °C, followed by extraction with CH_2Cl_2 (3 × 100 mL). The aqueous layer was evaporated under high vacuum. The residue was mixed with absolute ethanol (200 mL) to promote precipitation. The solid was filtered and redissolved in water (10 mL), which was basified with 1 N NaOH (12 mL) and 50% NaOH (20 mL) with ice cooling. The solution was extracted with $CHCl_3$ (10 × 50 mL). After $CHCl_3$ extracts were evaporated, the residue was taken up in ethanol (200 mL), acidified with concentrated HCl to a pH of 1, and filtered. Recrystallization from aqueous ethanol provided 1.26 g (83%) of **9** as a white solid: ^{1}H NMR

(D_2O) δ 1.66−2.00 (m, 4 H), 2.02−2.16 (m, 2 H), 2.40 (d, 4 H, J = 4.7), 3.12−3.30 (m, 8 H), 3.51−3.63 (m, 6 H). Anal. ($C_{13}H_{32}Cl_4N_4$) C, H, N.

Tetrakis(mesitylenesulfonyl)-N^1,N^4-bis(4-piperidinyl)-1,4-diaminobutane (8). Sodium hydride (80%, 0.93 g, 31 mmol) was added to **6** (11.24 g, 24.2 mmol) in DMF (145 mL) at 0 °C. The suspension was stirred at room temperature for 45 min. 1,4-Diiodobutane (1.5 mL, 11 mmol) was added, and the reaction mixture was heated at 70 °C for 18 h. Additional 80% NaH (0.35 g, 12 mmol) was added with ice cooling, and then the mixture was stirred for 25 min, followed by addition of more 1,4-diiodobutane (0.6 mL, 4.5 mmol). The reaction mixture was heated as before, cooled, and quenched with absolute EtOH (10 mL). Solvent was removed *in vacuo*, and the residue was treated with water (100 mL) and extracted with $CHCl_3$ (3×). The combined organic extracts were washed with 100 mL of 1% Na_2SO_3, H_2O, and brine, and the solvent was removed. Purification of crude product on a flash silica gel chromatography column, with 30% EtOAc/hexane as eluant, gave 4.59 g (39%) of **8** as a white solid: mp 206−207 °C; ^{1}H NMR δ 1.23−1.34 (m, 4 H), 1.6−1.8 (m, 8 H), 2.30, 2.55, and 2.59 (3 s, 36 H), 2.60−2.76 (m, 2 H), 2.97−3.06 (m, 4 H), 3.4−3.7 (m, 6 H), 6.95 (s, 8 H). Anal. ($C_{50}H_{70}N_4O_8S_4$) C, H, N.

***N*^{1},*N*^{4}-Bis(4-piperidinyl)-1,4-diaminobutane Tetrahydrochloride [PIP(3,4,3)] (10).** HBr in acetic acid (30%, 150 mL) was added to a solution of **8** (7.62 g, 7.75 mmol) and phenol (28.81 g, 0.306 mol) in CH_2Cl_2 (125 mL) at 0 °C. The reaction mixture was stirred for 1 day at room temperature and worked up by the procedure of **4**. Recrystallization from aqueous ethanol provided 1.953 g (63%) of **10** as a white solid: ^{1}H NMR (D_2O) δ 1.72−1.96 (m, 8 H), 2.34−2.45 (m, 4 H), 3.05−3.22 (m, 8 H), 3.45−3.66 (m, 6 H); HRMS calcd for $C_{14}H_{31}N_4$ 255.2549 (free amine, M + H), found 255.2543 (M + H). Anal. ($C_{14}H_{34}Cl_4N_4 \cdot H_2O$) C, H, N.

***N*^{1},*N*^{3}-Bis(4-pyridyl)-1,3-diaminopropane Dihydrochloride [PYR(3,3,3)] (11).** 1,3-Diaminopropane dihydrochloride (3.16 g, 21.5 mmol) and 4-bromopyridine hydrochloride (10.91 g, 56.1 mmol) were heated at 229 °C for 3.5 h. The flask was cooled in ice water, NaOH (1 N, 20 mL) and NaOH (50%, 10 mL) were added with ice cooling, and the mixture was extracted with $CHCl_3$ (150 mL, 2 × 50 mL, 8 × 25 mL). The organic extracts were filtered, and the oil was purified by silica gel flash column chromatography with 1.6% concentrated NH_4OH/CH_3OH as eluant. The free amine was dissolved in EtOH and acidified with concentrated HCl (2 mL). Evaporation of solvents and recrystallization from aqueous ethanol provided 1.12 g (17%) of **11** as a white solid: ^{1}H NMR (D_2O) δ 2.06 (quintet, 2 H, J = 7), 3.49 (t, 4 H, J = 7), 6.80−6.86 (m, 4 H), 7.94−8.01 (m, 4 H). Anal. ($C_{13}H_{18}Cl_2N_4$) C, H, N.

***N*^{1},*N*^{4}-Bis(4-pyridyl)-1,4-diaminobutane Dihydrochloride [PYR(3,4,3)] (12)**. 1,4-Diaminobutane dihydrochloride (4.10 g, 25.4 mmol) and 4-bromopyridine hydrochloride (10.02 g, 51.5 mmol) were heated at 226 °C for 4 h. The flask was cooled, additional 4-bromopyridine hydrochloride (4.11 g, 21.1 mmol) was introduced, and the reactants were heated at 226 °C for 3 h. Isolation and purification by the method of **11** gave 1.46 g (18%) of **12** as a white solid: ^{1}H NMR (D_2O) δ 1.73−1.85 (m, 4 H), 3.35−3.47 (m, 4 H), 6.78−6.85 (m, 4 H), 7.93−8.02 (m, 4 H). Anal. ($C_{14}H_{20}Cl_2N_4$) C, H, N.

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References

(1) Lipton, S. NMDA Receptors - Their Role in CNS Excitatory Neurotransmission. *Trends Neurosci.* **1993**, *16*, 527−532.
(2) Collingridge, G. L.; Bliss, T. V. P. NMDA Receptors - Their Role in Long-term Potentiation. *Trends Neurosci.* **1987**, *10*, 288−293.
(3) Brewer, G. J.; Cotman, C. W. NMDA Receptor Regulation of Neuronal Morphology in Cultured Hippocampal Neurons. *Neurosci. Lett.* **1989**, *99*, 268−273.
(4) Dichter, M. A.; Ayala, G. F. Cellular Mechanisms of Epilepsy: A Status Report. *Science* **1987**, *237*, 157−164.
(5) Choi, D. W. Glutamate Neurotoxicity and Diseases of the Nervous System. *Neuron* **1988**, *1*, 623−634.
(6) Bergeron, R. J.; Weimar, W. R.; Wu, Q.; Austin, J. K., Jr.; McManis, J. S. Impact of Polyamine Analogues on the NMDA Receptor. *J. Med. Chem.* **1995**, *38*, 425−428.
(7) Johnson, J. W.; Ascher, P. Glycine Potentiates the NMDA Response in Cultured Mouse Brain Neurons. *Nature* **1987**, *325*, 529−531.
(8) Reynolds, I. J.; Murphy, S. N.; Miller, R. J. [^{3}H]-Labeled MK-801 Binding to the Excitatory Amino Acid Receptor Complex from Rat Brain is Enhanced by Glycine. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7744−7748.
(9) Peters, S.; Koh, J.; Choi, D. W. Zinc Selectively Blocks the Action of *N*-Methyl-D-aspartate on Cortical Neurons. *Science* **1987**, *236*, 589−593.
(10) Westbrook, G. L.; Mayer, M. L. Micromolar Amounts of Zn^{+2} Antagonize NMDA and GABA Responses on Hippocampal Neurons. *Nature* **1987**, *328*, 640−643.
(11) Mayer, M. L.; Westbrook, G. L.; Guthrie, P. B. Voltage-dependent Block by Mg^{2+} of NMDA Responses in Spinal Cord Neurons. *Nature* **1984**, *309*, 261−263.
(12) Nowak, L.; Bregestovski, P.; Ascher, P.; Herbert, A.; Prochiantz, A. Magnesium Gates Glutamate-activated Channels in Mouse Central Neurons. *Nature* **1984**, *307*, 462−465.
(13) Wong, E. H. F.; Kemp, J. A.; Priestly, T.; Knight, A. R.; Woodruff, G. N.; Iversen, L. L. The Anticonvulsant MK-801 is a Potent *N*-Methyl-D-aspartate Antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 7104−7108.
(14) Huettner, J. E.; Bean, B. P. Block of *N*-Methyl-D-aspartate-activated Current by the Anticonvulsant MK-801: Selective Binding to Open Channels. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1307−1311.
(15) Fagg, G. E. Phencyclidine and Related Drugs Bind to the Activated *N*-Methyl-D-aspartate Receptor-channel Complex in Rat Brain Membranes. *Neurosci. Lett.* **1987**, *6*, 221−227.
(16) Ransom, R. W.; Stec, N. L. Cooperative Modulation of [^{3}H]MK-801 Binding to the *N*-Methyl-D-Aspartate Receptor Ion Channel by Glutamate, Glycine, and Polyamines. *J. Neurochem.* **1988**, *51*, 830−836.
(17) Williams, K.; Romano, C.; Molinoff, P. B. 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. *Mol. Pharmacol.* **1989**, *36*, 575−581.
(18) Williams, K.; Dawson, V. L.; Romano, C.; Dichter, M. A.; Molinoff, P. B. Characterization of Polyamines Having Agonist, Antagonist, and Inverse Agonist Effects at the Polyamine Recognition Site of the NMDA Receptor. *Neuron* **1990**, *5*, 199−208.
(19) Benveniste, M.; Mayer, M. L. Multiple Effects of Spermine on *N*-Methyl-D-aspartic Acid Receptor Responses of Rat Cultured Hippocampal Neurones. *J. Physiol. London* **1993**, *464*, 131−163.
(20) Lynch, D. R.; Lawrence, J. J.; Lenz, S.; Anegawa. N. J.; Dichter, M.; Pritchett, D. B. Pharmacological Characterization of Heterodimeric NMDA Receptors Composed of NR 1a and 2B Subunits: Differences with Receptors Formed from NR 1a and 2A. *J. Neurochem.* **1995**, *64*, 1462−1468.
(21) Bergeron, R. J.; Neims, A. H.; McManis, J. S.; Hawthorne T. R.; Vinson, J. R. T.; Bortell, R.; Ingeno, M. J. Synthetic Polyamine Analogues as Antineoplastics. *J. Med. Chem.* **1988**, *31*, 1183−1190.
(22) Porter, C. W.; Miller, J.; Bergeron, R. J. Aliphatic Chain Length Specificity of the Polyamine Transport System in Ascites L1210 Leukemia Cells. *Cancer Res.* **1984,** *44*, 126−128.
(23) Porter, C. W.; Cavanaugh, P. F., Jr.; Stolowich, N.; Ganis, B.; Kelly, E.; Bergeron, R. J. Biological Properties of N^4- and N^1,N^8-Spermidine Derivatives in Cultured L1210 Leukemia Cells. *Cancer Res.* **1985**, *45*, 2050−2057.
(24) Bergeron, R. J.; Hawthorne, T. R.; Vinson, J. R. T.; Beck, D. E., Jr.; Ingeno, M. J. Role of the Methylene Backbone in the Antiproliferative Activity of Polyamine Analogues on L1210 Cells. *Cancer Res.* **1989**, *49*, 2959−2964.
(25) Porter, C. W.; Pegg, A. E.; Ganis, B.; Madhubala, R.; Bergeron, R. J. Combined Regulation of Ornithine and S-Adenosylmethionine Decarboxylases by Spermine and the Spermine Analogue N^1,N^{12}-Bis(ethyl)spermine. *Biochem. J.* **1990**, *268*, 207−212.
(26) Pegg, A. E.; Madhubala, R.; Kameji, T.; Bergeron, R. J. Control of Ornithine Decarboxylase Activity in α-Difluoromethylornithine-Resistant L1210 Cells by Polyamines and Synthetic Analogues. *J. Biol. Chem.* **1988**, *263*, 11008−11014.
(27) Porter, C. W.; McManis, J.; Casero, R. A.; Bergeron, R. J. Relative Abilities of Bis(ethyl) Derivatives of Putrescine, Spermidine, and Spermine to Regulate Polyamine Biosynthesis and Inhibit L1210 Leukemia Cell Growth. *Cancer Res.* **1987**, *47*, 2821−2825.
(28) Pegg, A. E.; Wechter, R.; Pakala, R.; Bergeron, R. J. Effect of N^1, N^{12}-Bis(ethyl)spermine and Related Compounds on Growth and Polyamine Acetylation, Content and Excretion in Human Colon Tumor Cell. *J. Biol. Chem.* **1989**, *264*, 11744−11749.

(29) Casero, R. A., Jr.; Celano, P.; Ervin, S. J.; Porter, C. W.; Bergeron, R. J.; Libby, P. R. Differential Induction of Spermidine/Spermine N^1-Acetyltransferase in Human Lung Cancer Cells by the Bis(ethyl)polyamine Analogues. *Cancer Res.* **1989**, *49*, 3829−3833.

(30) Libby, P. R.; Henderson, M.; Bergeron, R. J.; Porter, C. W. Major Increases in Spermidine/Spermine-N^1-Acetyltransferase Activity by Spermine Analogues and Their Relationship to Polyamine Depletion and Growth Inhibition in L1210 Cells. *Cancer Res.* **1989**, *49*, 6226−6231.

(31) Libby, P. R.; Bergeron, R. J.; Porter, C. W. Structure-Function Correlations of Polyamine Analog-Induced Increases in Spermidine/Spermine Acetyltransferase Activity. *Biochem. Pharmacol.* **1989**, *38*, 1435−1442.

(32) Porter, C. W.; Ganis, B.; Libby, P. R.; Bergeron, R. J. Correlations Between Polyamine Analog-Induced Increases in Spermidine/Spermine N^1-Acetyltransferase Activity, Polyamine Pool Depletion, and Growth Inhibition in Human Melanoma Cell Lines. *Cancer Res.* **1991**, *51*, 3715−3720.

(33) Fogel-Petrovic, M.; Shappell, N. W.; Bergeron, R. J.; Porter, C. W. Polyamine and Polyamine Analog Regulation of Spermidine/Spermine N^1-Acetyltransferase in MALME-3M Human Melanoma Cells. *J. Biol. Chem.* **1993**, *268*, 19118−19125.

(34) Shappell, N. W.; Fogel-Petrovic, M. F.; Porter, C. W. Regulation of Spermidine/spermine N^1-Acetyltransferase by Intracellular Polyamine Pools-Evidence for a Functional Role in Polyamine Homeostasis. *FEBS Lett.* **1993**, *321*, 179−183.

(35) Bergeron, R. J.; Weimar, W. R.; Luchetta, G.; Streiff, R. R.; Wiegand, J.; Perrin, J.; Schreier, K. M.; Porter, C.; Yao, G. W.; Dimova, H. Metabolism and Pharmacokinetics of N^1,N^{11}-Diethylnorspermine. *Drug Metab. Dispos.* **1995**, *23*, 1117−1125.

(36) Bergeron, R. J.; Weimar, W. R.; Luchetta, G.; Sninsky, C. A.; Wiegand, J. Metabolism and Pharmacokinetics of N^1,N^{14}-Diethylhomospermine. *Drug Metab. Dispos.* **1995**, *23*, 334−343.

(37) Bergeron, R. J.; Yao, G. W.; Yao, H.; Weimar, W. R.; Sninsky, C. A.; Raisler, B.; Feng, Y.; Wu, Q.; Gao, F. Metabolically Programmed Polyamine Analogue Antidiarrheals. *J. Med. Chem.* **1996**, *39*, 2461−2471.

(38) Bergeron, R. J.; McManis, J. S.; Liu, C. Z.; Feng, Y.; Weimar, W. R.; Luchetta, G. R.; Wu, Q.; Ortiz-Ocasio, J.; Vinson, J. R. T.; Kramer, D.; Porter, C. Antiproliferative Properties of Polyamine Analogues: A Structure-Activity Study. *J. Med. Chem.* **1994**, *37*, 3464−3476.

(39) Samejima, K.; Takeda, Y.; Kawase, M.; Okada, M.; Kyogoku, Y. Synthesis of ^{15}N-Enriched Polyamines. *Chem. Pharm. Bull.* **1984**, *32*, 3428−3435.

(40) Bergeron, R. J.; McManis, J. S.; Weimar, W. R.; Schreier, K. M.; Gao, F.; Wu, Q.; Ortiz-Ocasio, J.; Luchetta, G. R.; Porter, C.; Vinson, J. R. T. The Role of Charge in Polyamine Analogue Recognition. *J. Med. Chem.* **1995**, *38*, 2278−2285.

(41) Shirahata, A.; Morohohi, T.; Fukai, M.; Akatsu, S.; Samejima, K. Putrescine or Spermidine Binding Site of Aminopropyltransferases and Competitive Inhibitors. *Biochem. Pharmacol.* **1991**, *41*, 205−212.

(42) Bailey, D. M.; DeGrazia, C. G.; Hoff, S. J.; Schulenberg, P. L.; O'Connor, J. R.; Paris, D. A.; Slee, A. M. Bispyridinamines: A New Class of Topical Antimicrobial Agents as Inhibitors of Dental Plaque. *J. Med. Chem.* **1984**, *27*, 1457−1464.

(43) Bellobono, I. R.; Favini, G. Ultraviolet Absorption and Protonation Equilibria of Amino- and Nitro-substituted Pyridines. *J. Chem. Soc. B* **1971**, 2034−2037.

(44) Igarashi, K.; Williams, K. Antagonist Properties of Polyamines and Bis(ethyl)polyamines at *N*-Methyl-D-aspartate Receptors. *J. Pharmacol. Exp. Ther.* **1995**, *272*, 1101−1109.

(45) Durand, G. M.; Bennett, M. V.; Zukin, R. S. Splice Variants of the *N*-Methyl-D-aspartate Receptor NR1 Identify Domains Involved in Regulation by Polyamines and Protein Kinase C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6731−6735.

(46) Lynch, D. R.; Anegawa, N. J.; Verdoorn, T.; Pritchett, D. B. *N*-Methyl-D-aspartate Receptors: Different Subunit Requirements for Binding of Glutamate Antagonists, Glycine Antagonists, and Channel-blocking Agents. *Mol. Pharmacol.* **1994**, *45*, 540−545.

(47) Williams, K.; Zappia, A. M.; Pritchett, D. B.; Shen, Y. M.; Molinoff, P. B. Sensitivity of the *N*-Methyl-D-aspartate Receptor to Polyamines is Controlled by NR2 Subunits. *Mol. Pharmacol.* **1994**, *45*, 803−809.

(48) Gallagher, M. J.; Huang, H.; Pritchett, D. B.; Lynch, D. R. Interactions Between Ifenprodil and the NR2B Subunit of the *N*-Methyl-D-aspartate Receptor. *J. Biol. Chem.* **1996**, *271*, 9603−9611.

(49) Woodward, J. J.; Cueto, S. Effects of the Putative Polyamine Antagonists Diethylenetriamine and 1,10-Diaminodecane on *N*-Methyl-D-aspartic Acid-stimulated [^{3}H]Norepinephrine Release from Rat Hippocampal Slices. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 563−569.

(50) Subramaniam, S.; Donevan, S. D.; Rogawski, M. A. Hydrophobic Interactions of n-Alkyl Diamines with the *N*-Methyl-D-aspartate Receptor: Voltage-dependent and -independent Blocking Sites. *Mol. Pharmacol.* **1994**, *45*, 117−124.

(51) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

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