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Molecular Features Associated with Polyamine Modulation of NMDA Receptors

Anita H. Lewin,*,† Guobin Sun,† Louise Fudala,† Hernan Navarro,† Li-Ming Zhou,‡ Piotr Popik,‡ Alexander Faynsteyn,‡ and Phil Skolnick‡,§

Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina 27709, and National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

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The effect of 1,3-diamines on basal and spermine-stimulated [^{3}H]MK-801 binding was investigated. Systematic variations in the molecular parameters revealed that, in general, lipophilic 1,3-diamines inhibited and hydrophilic 1,3-diamines enhanced [^{3}H]MK-801 binding in the nominal absence of glutamate and glycine. Furthermore, 1,3-diamines which were highly monoprotonated at physiologic pH were more effective in modulating basal binding (at 100 μM 1,3-diamine) than analogues which were mostly diprotonated or unprotonated. Finally, the internuclear distance between the amino nitrogens and the extent of modulation of basal [^{3}H]MK-801 binding were correlated. Similar, but more modest, effects were seen for spermine-enhanced [^{3}H]MK-801 binding. These results are consistent with the existence of two polyamine binding sites associated with the NMDA receptor complex. One of the sites appears to preferentially recognize lipophilic substances while the other favors hydrophilic materials. Both sites appear to recognize polyamines with at least one charged (protonated) amino group and one uncharged amino group. The distance between amino groups is a determining factor as well.

Introduction

The role of polyamines in modulating NMDA receptor activity and function has been under intensive investigation since the observation that endogenous polyamines such as spermine and spermidine enhance [^{3}H]MK-801 binding to the NMDA-coupled calcium ion channel.[1,2] Neurochemical and electrophysiological studies indicate that endogenous and synthetic polyamines produce multiple effects at NMDA receptors. For example, concentration dependency studies have demonstrated that the effects of many polyamines on the binding of use-dependent channel blockers such as MK-801 are biphasic; at low concentrations (1−20 μM) of spermine a robust enhancement of basal binding (in the nominal absence of glutamate and glycine) is observed while at higher concentrations this enhancement is reversed.[3] In addition, polyamines which decrease basal [^{3}H]MK-801 binding, and compounds capable of inhibiting the stimulation of [^{3}H]MK-801 binding by spermine, have also been reported.[3] Polyamines have been defined as agonists if they enhance basal MK-801 binding and as antagonists if they decrease spermine-stimulated MK-801 binding. Compounds which inhibit basal MK-801 binding may either be true inverse agonists or may produce a use-dependent block of the ion channel.[3] Numerous examples of the various functional classes of polyamines have been described, and it has been noted that diamines with chain length C2−C3 are partial agonists; diamines with chain length C4−C7 act as selective antagonists, inhibiting spermine-promoted enhancement of MK-801 binding. Longer chain length diamines (C8−C12) behave as inverse agonists, decreasing basal MK-801 binding.[4] The authors of this study postulated that full agonism required interaction at three amine interaction points of which two are 5 Å apart and the third is 5−6 Å distant from either one or both of the other two points. A "concerted action by two molecules" was proposed to account for the partial agonist effect of the short chain length diamines, and antagonism was attributed to an interaction with two of the three sites.[4] A fourth amine interaction point, some 12 Å away, was proposed to account for the inverse agonist action observed for long chain diamines.[4] In addition to these in vitro effects, NMDA antagonists that appear to act at polyamine-associated sites have been found to reduce ischaemic brain injury.[5]

Most representations of the NMDA receptor complex now include at least two polyamine binding sites,[3,6] and some studies with recombinant receptors indicate strict subunit requirements for these sites.[3,7,8]

To determine the molecular characteristics associated with polyamine modulation of NMDA receptors, we have undertaken a systematic study of 1,3-diamines. Our results shed light on the role of lipophilicity, basicity of the amino groups, and stereochemistry.

Results

Chemistry. The N-alkylated 1,3-diaminopropanes **11**−**13** were synthesized by reaction of 1,3-dibromopropane (**27**) with allylamine (**28**), diallylamine (**29**), and piperidine (**30**), respectively (Scheme 1). The 4-aminopiperidine analogues **20** and **17** were prepared by reductive amination[9] of 1-benzyl-4-piperidone (**31**) and 2,2,6,6-tetramethyl-4-piperidone (**32**), respectively, with β-ethanolamine (**33**) (Scheme 2); debenzylation[10] of commercially available 4-amino-1-benzylpiperidine (**22**) (Scheme 3) provided **14**. The conformationally re-

* Please address correspondence to: Anita H. Lewin, Ph.D., Medicinal Chemistry Building, Room 283, Research Triangle Institute, Post Office Box 12194, Research Triangle Park, NC 27709. Phone: (919) 541-6691. Fax: (919) 541-8868, E-mail: ahl@rti.org.

† Research Triangle Institute.

‡ National Institutes of Health.

§ Current address: Neuroscience Discovery, Lilly Research Laboratories, Indianapolis, IN 46285.

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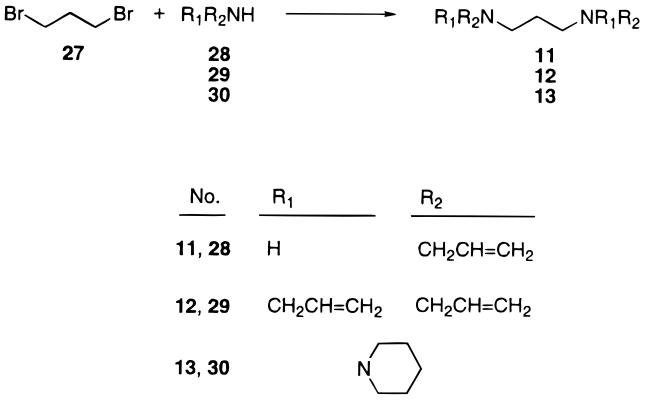
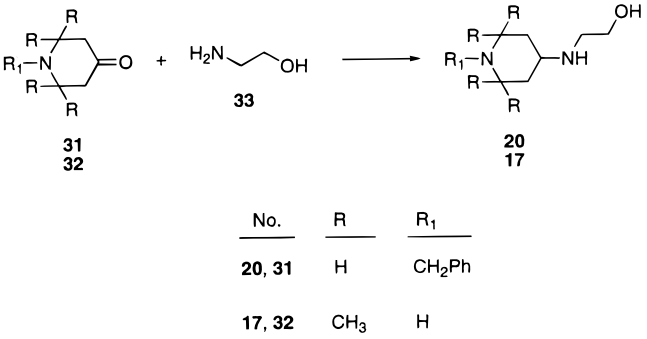
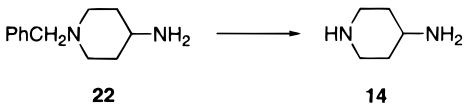
Scheme 1



Scheme 2



Scheme 3

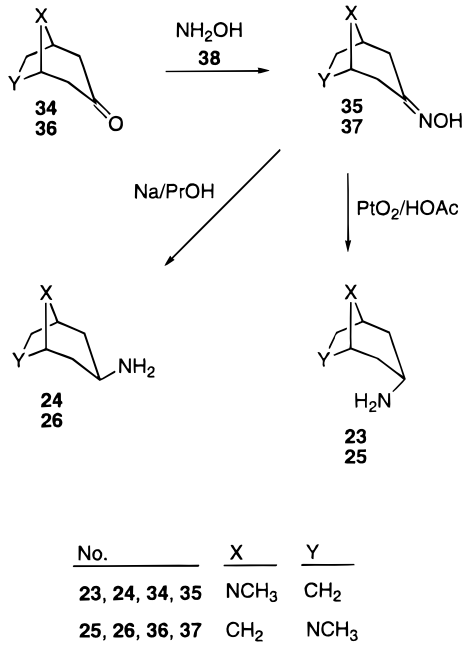


stricted 3-aminotropanes **23** and **24** were prepared from 3-tropinone oxime (**35**).[11] The α-isomer **23** was obtained by hydrogenation over Adams catalyst in acetic acid,[12] and the *β*-isomer **24** was prepared by reduction with sodium in 1-propanol (Scheme 4). The 6-aza analogues **25** and **26** were prepared using methods similar to those used in the preparation of the 3-aminotropanes. Thus, treatment of 6-methyl-6-azabicyclo[3.2.1]octan-3-one (**36**)[13] with hydroxylamine hydrochloride (**38**), as in the preparation of 3-tropinone oxime (**35**), provided the oxime (**37**) which was reduced with Adams catalyst in acetic acid or with sodium in 1-propanol to give the α-isomer **25** or the *β*-isomer **26**, respectively (Scheme 4). The remaining diamines were commercially available.

Solutions (0.01 M) of each of the 26 diamines (or of diamine dihydrochloride salts) in this study were titrated with 0.1 M HCl (or 0.1 M NaOH, for the salts), and the data were used to determine the p*K* values of the diamines. The most basic amine was 3α-aminotropane (**24**) with $pK_1 = 13.4$ and the least basic was *N*-benzyl-4-(*N*-(2-hydroxyethyl)amino)piperidine (**20**) with $pK_2 = 6.67$. This amine also possessed the highest percentage of monoprotonated amine at pH 7.4 (84%); the amines which were the least monoprotonated at pH 7.4 (5%) were 1,3-diaminopropane (**4**), *N*-methyl-1,3-diaminopropane (**5**) and *N*,*N*′-dimethyl-1,3-diaminopropane (**6**). These results are shown in Tables 1−3.

The lipophilicities of each of the 1,3-diamines in this study were calculated using the ClogP program (Tables

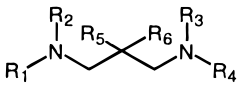
Scheme 4



1−3). The most hydrophilic compound in this set was 1,3-diamino-2-propanol (**1**) with ClogP = −2.05, and the most lipophilic was *N*-(3-propylpiperidino)piperidine (**13**) with ClogP = 2.64.

Pharmacology. The regulatory effects of the diamines examined in this study were evaluated using [^{3}H]MK-801 binding since, under nonequilibrium conditions, this assay has been shown to be a sensitive and specific probe for effects at NMDA receptors.[1,14−16] Specifically, enhancement or inhibition of [^{3}H]MK-801 binding identifies substances that facilitate or reduce activity at the NMDA-gated calcium ion channel.[1,17−19] In overall agreement with the literature,[1,20] we found that in the nominal absence of glutamate and glycine (basal conditions) [^{3}H]MK-801 binding was enhanced by the addition of spermine. At an optimum concentration of 25 μM spermine, basal binding was increased between 350 and 1300%; basal binding decreased at higher concentrations of spermine. The diamines **1**−**26** were all tested for their effect on basal and on spermine-stimulated [^{3}H]MK-801 binding in the nominal absence of glutamate and glycine. To normalize the results for the variability in the maximal percent enhancement by spermine which results from the low level of basal [^{3}H]-MK-801 binding in this preparation, the effects of these polyamines are expressed as a percentage of maximum increase in [^{3}H]MK-801 binding produced by 20 μM spermine (Tables 1 and 2). The diamines **1**−**7, 9, 14, 15, 25** and **26** enhanced basal [^{3}H]MK-801 binding at 100 μM. The largest enhancements at 100 μM were observed for 1,3-diaminoacetone (**2**), 4-(dimethylamino)-piperidine (**15**), and 1,3-diamino-2-propanol (**1**). Both 1,3-diaminoacetone (**2**) and the analogous alcohol 1,3-diamino-2-propanol (**1**) increased spermine-enhanced binding by ∼25%. The remaining diamines were inverse agonists; one, 4-amino-*N*-benzylpiperidine (**22**), completely inhibited basal [^{3}H]MK-801 binding at 100 μM. Several were antagonists of spermine-promoted [^{3}H]MK-801 binding, decreasing these values by as much as 43%.

Table 1. Extent of Monoprotonation, Lipophilicity, and Effectiveness To Modulate [^{3}H]MK-801 Binding of 100 μM of 1,3-Diamines of Structure A

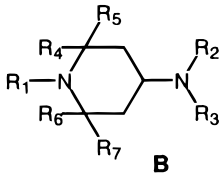


A

									% [^{3}H]MK-801					
no.	R_1	R_2	R_3	R_4	R_5	R_6	%+[a]	ClogP	Bsl[b]	% Sp Max[c]	Sp St[d]	pK_1	pK_2	ΔpK
1	H	H	H	H	OH	H	15	−2.05		35 ± 6	118 ± 9	9.68	8.14	1.54
2	H	H	H	H	O		70	−1.82		72 ± 7	136 ± 5	8.32	6.89	1.43
3	$(CH_2)_2OH$	H	H	H	H	H	14	−1.82		16 ± 9	102 ± 5	10.28	8.19	2.09
4	H	H	H	H	H	H	5	−1.49		22 ± 8	98 ± 8	10.55	8.69	1.86
5	CH_3	H	H	H	H	H	5	−1.35		15 ± 4	99 ± 7	10.67	8.66	2.01
6	CH_3	H	H	CH_3	H	H	5	−1.20		16 ± 1	113 ± 8	10.70	8.68	2.02
7	CH_3	H	H	CH_3	CH_3	H	11	−0.80		11 ± 6	105 ± 6	10.55	8.29	2.26
8	H	H	H	H	CH_3	CH_3	27	−0.69		5 ± 3	114 ± 15	10.18	7.81	2.37
9	CH_3	CH_3	CH_3	CH_3	H	H	33	−0.56		22 ± 3	103 ± 3	9.81	7.77	2.04
10	CH_3	CH_3	H	H	CH_3	CH_3	65	−0.22	87 ± 7		102 ± 14	10.28	7.13	3.15
11	$CH_2CH{=}CH_2$	H	H	$CH_2CH{=}CH_2$	H	H	14	−0.05	45 ± 10		79 ± 7	10.04	8.18	1.86
12	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	H	H	71	1.75	22 ± 13		69 ± 5	8.80	6.96	1.84
13	$(CH_2)_5$			$(CH_2)_5$	H	H	10	2.64	74 ± 12		99 ± 10	10.39	8.33	2.06

[a] Percent of the 1,3-diamine which is monoprotonated at pH 7.4 [b] Percent [^{3}H]MK-801 bound in the nominal absence of glycine and glutamate assuming 100% in the absence of added diamine. [c] Percentage of maximal enhancement by spermine (100%). [d] Percent [^{3}H]MK-801 bound in the presence of 20 μM spermine assuming 100% in the absence of added diamine.

Table 2. Extent of Monoprotonation, Lipophilicity, and Effectiveness to Modulate [^{3}H]MK-801 Binding of 100 μM of 1,3-Diamines of Structure B

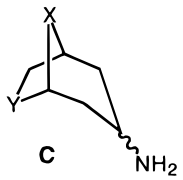


B

										% [^{3}H]MK-801					
no.	R_1	R_2	R_3	R_4	R_5	R_6	R_7	%+[a]	ClogP	Bsl[b]	% Sp Max[c]	Sp St[d]	pK_1	pK_2	ΔpK
14	H	H	H	H	H	H	H	17	−1.43		12 ± 4	108 ± 5	10.63	8.10	2.53
15	H	CH_3	CH_3	H	H	H	H	33	−0.72		44 ± 11	103 ± 2	10.66	7.71	2.95
16	CH_3	CH_3	H	H	H	H	H	53	−0.44		2 ± 1	104 ± 6	10.09	7.34	2.75
17	H	$(CH_2)_2OH$	H	CH_3	CH_3	CH_3	CH_3	50	0.32	80 ± 11		84 ± 6	10.52	7.42	3.10
18	H	$(CH_2)_5H$			H	H	H	21	0.543	24 ± 12		57 ± 15	10.73	7.97	2.76
19	H	H	H	CH_3	CH_3	CH_3	CH_3	24	0.64	36 ± 8		65 ± 8	10.63	7.90	2.73
20	$PhCH_2$	$(CH_2)_2OH$	H	H	H	H	H	84	1.08	21 ± 9		106 ± 10	9.24	6.67	2.57
21	H	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	40	1.36	71 ± 5		72 ± 6	10.56	7.67	2.89
22	$PhCH_2$	H	H	H	H	H	H	69	1.40	2 ± 1		81 ± 11	9.79	7.05	2.74

[a] Percent of the 1,3-diamine which is monoprotonated at pH 7.4. [b] Percent [^{3}H]MK-801 bound in the nominal absence of glycine and glutamate assuming 100% in the absence of added diamine. [c] Percentage of maximal enhancement by spermine (100%). [d] Percent [^{3}H]MK-801 bound in the presence of 20 μM spermine assuming 100% in the absence of added diamine.

Table 3. Extent of Monoprotonation, Lipophilicity, and Effectiveness to Modulate [^{3}H]MK-801 Binding by 100 μM of 1,3-Diamines of Structure C



C

						% [^{3}H]MK-801		
no.	X	Y	config at C-3	%+[a]	ClogP	Bsl[b]	% Sp Max[c]	Sp St[d]
23	NCH_3	CH_2	α	41	−0.27	85 ± 6		103 ± 3
24	NCH_3	CH_2	*β*	19	−0.27	80 ± 8		99 ± 3
25	CH_2	NCH_3	α	54	−0.27		8 ± 4	108 ± 7
26	CH_2	NCH_3	*β*	9	−0.27		13 ± 5	104 ± 3

[a] Percent of the 1,3-diamine which is monoprtonated at pH 7.4. [b] Percent [^{3}H]MK-801 bound in the nominal absence of glycine and glutamate assuming 100% in the absence of added diamine. [c] Percentage of maximal enhancement by spermine (100%). [d] Percent [^{3}H]MK-801 bound in the presence of 20 μM spermine assuming 100% in the absence of added diamine.

Discussion

The regulatory effects manifested by endogenous polyamines at NMDA receptors may, in principle, be controlled by a large number of factors. Since endogenous polyamines contain three or more amino functionalities, these factors may include (1) the number of

amino groups, (2) the number of carbon atoms separating the amino groups, (3) the distance between the amino groups, and (4) the steric congestion around the amino groups. The basicity of individual amino groups, as well as general molecular properties such as steric bulk, lipophilicity, and polarity, may also be contributing factors. As a starting point for this investigation, we eliminated some of these variables by electing to study diamines, since it has been reported that the modulatory effects of polyamines at NMDA receptors are also manifested by diamines. Limiting the number of amino groups involved simplifies the system, and maintaining constant the number of carbons separating them reduces conformational heterogeneity and variation in inductive effects between amino groups.

The basicity of aliphatic amines is primarily a function of the substituents on nitrogen. Thus, increased electron donation to nitrogen by the addition of an electron-donating group enhances basicity. For example, the pK values of primary amines such as methylamine, ethylamine, and butylamine are in the range 10.7−10.8, while the pK values of secondary amines such as diethylamine and piperidine are 11.1. Conversely, the addition of electron-withdrawing groups such as benzyl and allyl lowers the pK value. Thus, the pK value of the primary amine benzylamine is 9.3, and the pK values of the secondary amines benzylmethylamine and allylmethylamine are 9.6 and 10.1, respectively. Similar effects are seen in the pK_1 values of 1,3-diamines. For example, the pK_1 value of the secondary amine (*N*,*N*′-dimethylamino)propane (**6**) is 0.15 units higher than that of the primary amine 1,3-diaminopropane (**4**) while that of the *N*,*N*′-diallyl analogue **11** is 0.5 units lower. The observed pK_1 of 1,3-diaminopropane (**4**), 10.6 (Table 1), demonstrates that a 3-aminopropyl group only slightly lowers the basicity. However, a protonated 3-aminopropyl group has a large effect on basicity, lowering the pK value by about 2 units to 8.69 (pK_2). Examination of the difference in pK values (ΔpK) in the acyclic 1,3-diamines (Table 1) shows the effect to be smallest (1.43) in diaminoacetone (**2**) and largest (3.15) in 2,2,*N*,*N*-tetramethylpropane (**10**). Since **10** is likely to exist in a gauche conformation which minimizes steric congestion between the methyl groups, the distance between the amino groups in **10** will be smaller than in 1,3-diamines such as **4**, which will exist preferentially in a fully extended conformation. Therefore, it appears that the magnitude of ΔpK is a general indication of the distance between the amino groups. This is consistent with the larger effect (Δp$K = \sim$2.75), on average, which is seen in the piperidine analogues (Table 2) in which a fully extended conformation is not possible.

The parent open chain primary diamine, 1,3-diaminopropane (**4**), as well as the open chain secondary diamine analogues *N*-methyl-1,3-diaminopropane (**5**) and *N*,*N*′-dimethyl-1,3-diaminopropane (**6**), and the tertiary open chain diamine analogue, *N*,*N*,*N*′,*N*′-tetramethyl-1,3-diaminopropane (**9**), were found to enhance basal [^{3}H]MK-801 binding. Our results indicate that, in the nominal absence of both glutamate and glycine, there is no clear-cut relationship between the number of substituents on amino nitrogens of 1,3-diamines and their ability to modulate [^{3}H]MK-801 binding. On the other hand, the nature of the substituents appears to influence the extent to which these polyamines modulate [^{3}H]MK-801 binding. Thus, while replacement of primary amino groups by tertiary dimethylamino groups has no effect on efficacy, replacement by diallylamino or piperidino groups affords inverse agonists/antagonists. For example, whereas 1,3-diaminopropane (**4**) and its *N*,*N*,*N*′,*N*′-tetramethyl analogue **9** produce a similar enhancement of [^{3}H]MK-801 binding, the tertiary *N*,*N*,*N*′,*N*′-tetraallyl analogue **12** and *N*-(3-propylpiperidino)piperidine (**13**) either reduce or have no effect on ligand binding. Similarly, replacement of the dimethylamino group of the agonist 4-(dimethylamino)piperidine (**15**) by a piperidino group, to give 4-piperidinopiperidine (**18**), results in an inverse agonist/antagonist. A benzyl group also seems to impart inverse agonist/antagonist activity to inactive or agonist diamines, as in the case of the inverse agonist/antagonist 1-benzyl-4-aminopiperidine (**22**) relative to the agonist 4-aminopiperidine (**14**). Changes in the carbon backbone also have an effect on efficacy; these are particularly striking in comparing the open chain 1,3-diaminopropanes in Table 1 to the cyclic 4-aminopiperidine analogues in Table 2.

Since both the lipophilicity and the basicity of amines can be affected by substituents on either nitrogen or carbon or both, the observed substituent effects to modulate [^{3}H]MK-801 binding could be related to these parameters. To examine the possible correlation with lipophilicity, the 1,3-diamines in Tables 1 and 2 were sorted by the calculated logP (ClogP). Inspection of the sorted tables indicates that, in general, hydrophilic 1,3-diamines (ClogP $<$ 0) enhance basal [^{3}H]MK-801 binding while lipophilic 1,3-diamines (ClogP $>$ 0) decrease basal [^{3}H]MK-801 binding. Within these groupings, however, the magnitude of the effect at 100 μM diamine appears to correlate with the percentage of the monoprotonated species at pH 7.4 as calculated from the pK values. Thus, the significantly different enhancement of basal [^{3}H]MK-801 binding observed for the equally hydrophilic diamines 1,3-diaminoacetone (**2**) and *N*-(2-hydroxyethyl)-1,3-diaminopropane (**3**) may be explained by the observation that **2** is 70% monoprotonated at pH 7.4, while **3** is only 14% monoprotonated under these conditions. A similar rationale applies to **9**, which although less hydrophilic than **4** is 33% monoprotonated at pH 7.4, while **4** is only 5% monoprotonated at pH 7.4 and therefore exerts an effect similar to that of **4** on basal [^{3}H]MK-801 binding (at 100 μM). Another striking example is a comparison of *N*,*N*′,-2-trimethyl-1,3-diaminopropane (**7**) and 4-(dimethylamino)piperidine (**15**), which are both somewhat hydrophilic with ClogP ~ -0.8. Indeed, while both enhance basal [^{3}H]MK-801 binding, **15** is 4 times as effective in enhancing basal [^{3}H]MK-801 binding as **7**, consistent with its 3-fold greater monoprotonation at pH 7.4 (33%) relative to **7** (11%). Thus it appears that while the effectiveness of a 100 μM concentration of 1,3-diamines in modulating basal [^{3}H]MK-801 binding is a function of the concentration of the monoprotonated amine at physiological pH, the intrinsic efficacy (i.e., agonist vs inverse agonist) is controlled by lipophilicity. There are, however, notable exceptions.

On the basis of the above analysis, 1,3-diamino-2,2-dimethylpropane (**8**) and *N*,*N*,*N*′,*N*′-tetramethyl-1,3-diaminopropane (**9**) should have similar effects on basal [^{3}H]MK-801 binding due the similarities in their hydrophilicities and extent of monoprotonation at pH 7.4. In fact, **8** has virtually no effect on [^{3}H]MK-801 binding in the range of 1−100 μM while **9** enhances basal [^{3}H]-MK-801 binding by 22% at 100 μM (an enhancement of 60% of the maximal stimulation obtained with spermine is obtained at 12.5 μM **9**). This apparent discrepancy might be due to the different distances between the amino groups in these highly conformationally mobile molecules. An experimentally determined physical parameter which should be related to the distance between the amino nitrogens is the difference between their p*K* values (Δp*K*). Since the amino groups are separated by chains with an equal number of saturated aliphatic carbon atoms in the two compounds, the effect of protonation of one amino group on the basicity of the remaining amino group should be related to the distance between the nitrogens. For the 1,3-diamines **8** and **9** Δp*K* is 2.37 and 2.04, respectively, suggesting that, since the effect is smaller in **9**, the distance between amino groups is larger in this compound and this longer distance may be in the range recognized by the polyamine binding site responsible for the effective enhancement of basal [^{3}H]MK-801 binding.

Because the 1,3-diamines in Tables 1 and 2 are all conformationally mobile and heterogeneous, assessment of the distance between the amino groups would be difficult. Therefore, to explore the relationship of the distance between amino nitrogens and the effect of 1,3-diamines on basal and spermine-stimulated [^{3}H]MK-801 binding, 1,3-diamino derivatives of conformationally constrained [3.2.1]bicyclooctane were synthesized and evaluated for their modulation of [^{3}H]MK-801 binding (Table 3). The four isomers represent different conformations of 1,3-diaminopropane: 3β-aminotropane (**24**) and 3β-amino-6-aza[3.2.1]bicyclooctane (**26**) represent fairly extended conformations; 3α-aminotropane (**23**) represents a double-gauche (semifolded) conformation, while 3α-amino-6-aza[3.2.1]bicyclooctane (**25**) represents a double gauche conformation with eclipsing of the amino functionalities. The internuclear distances measured on models of these compounds indicate that the interatomic distance between the amino nitrogens is greatest in **26**, similar in **23** and **24**, and smallest in **25**. The experimentally determined p*K* values of the diamines **23**−**26** support the rank order of internuclear distances between the amino nitrogens. Thus Δp*K* for **25** is the largest, consistent with an extremely small N−N distance, and Δp*K* for **26** is the smallest, with Δp*K* for **23** exceeding that of **24**. In addition, the differences in basicity result in considerable variations in the percentage of the monoprotonated species at pH 7.4. The least monoprotonated at pH 7.4 is 3β-amino-6-aza[3.2.1]-bicyclooctane (**26**). Its α-isomer **25** is the most highly (54%) monoprotonated at the same pH; similarly the α-isomer (**23**) of 3-aminotropane is more highly (41%) monoprotonated at pH 7.4 than the β-isomer **24** (19%) at pH 7.4 (Table 3). Thus, in the absence of other effects, the rank order of effectiveness of these 1,3-diamines to modulate basal [^{3}H]MK-801 binding might be expected to be **25** > **23** > **24** > **26**. The ClogP (−0.27) value for these four diamines indicates that they are neither highly hydrophilic nor highly lipophilic and, therefore, does not provide information regarding their efficacy to modulate basal [^{3}H]MK-801 binding. For example, the diamine **10** (ClogP = −0.22) inhibits basal [^{3}H]MK-801 binding by 23%, but the diamine **16** (ClogP = −0.44) is inactive. For the aminotropanes **23**−**26**, the results (Table 3) show that, at 100 μM, there is no correlation between the extent of monoprotonation and the effectiveness to modulate [^{3}H]MK-801 binding. Thus, **26** and **25**, which are the least and the most monoprotonated at pH 7.4, respectively, are essentially without effect, while the isomer **23**, which is twice as monoprotonated as **24** at pH 7.4, is as effective in inhibiting [^{3}H]MK-801 binding as **24**. These observed differences in the effects of the four isomers on basal [^{3}H]MK-801 binding are further indication of the geometric constraints of the polyamine recognition site(s).

Our results strongly indicate that the effects of 1,3-diamines on the binding of [^{3}H]MK-801 are manifested through at least two binding sites. Both sites prefer 1,3-diamines with one protonated, or charged, amino group and one neutral amino group. One of these sites recognizes hydrophilic residues and is responsible for enhancing [^{3}H]MK-801 binding, while the other site binds lipophilic substances and inhibits the binding of [^{3}H]MK-801. It follows that weakly hydrophilic or lipophilic 1,3-diamines may exhibit inconsistent behavior. In the absence of other factors, e.g. stereochemistry, such agents may bind with similar affinity to both sites with the consequent apparent lack of activity associated with mutual cancellation of enhancing and inhibitory effects. This may be the case for *N*-methyl-4-(methylamino)piperidine (**16**) and for the bicyclic diamine **25**. There are, however, exceptions: the weakly hydrophilic (ClogP = −0.05) *N*,*N*′-diallyl-1,3-diaminopropane (**11**) is only 14% monoprotonated at pH 7.4 and yet inhibits both basal and spermine-promoted [^{3}H]MK-801 binding. Our working hypothesis is that this is due to the fact that in the 1,3-diamine **11** the interatomic distance between the amino nitrogens is close to optimal for binding to the lipophilic site. Experiments to examine the stereochemical requirements of the lipophilic site are underway.

Whether our results and our working hypothesis are applicable to other diamines and polyamines remains to be determined. In a general sense, our working hypothesis is consistent with published observations. For example, examination of a series of terminally substituted polyamines has led Bergeron[21] to conclude that increased bulk on the nitrogen atoms of the terminal amino groups leads to enhanced antagonism. Our hypothesis is also consistent with these observations because ClogP values for this series of polyamines demonstrate increasing lipophilicity as well. It is also possible, while speculative, that bulky N-substituents decrease the basicity of the terminal amino functionalities (by steric hindrance to protonation), thereby leading to the desirably high concentration of partially protonated polyamines. Similarly, our hypothesis accounts for the high potency of aminoglycosides to enhance basal [^{3}H]MK-801 binding.[22] These compounds are highly hydrophilic and possess 1,3-diamine groupings which, based on our experience with 1,3-diamines,

are likely to provide the preferred partially protonated species at pH 7.4.

Our working hypothesis also serves to explain other literature results. Thus, spermine and spermidine are highly hydrophilic compounds with ClogP values of −2.04 and −1.65, respectively; based on the published p*K* values (10.97, 10.27, 9.04, and 8.03 for spermine and 11.16, 10.06, and 8.51 for spermidine)[23] there would be between 8 and 20% of species with one unprotonated amino group at physiological pH. Furthermore, as flexible molecules, both would be able to adapt the internuclear distances between the relevant amino nitrogen atoms (i.e., a protonated amino nitrogen and an unprotonated amino nitrogen) to meet the requirements of the recognition site. We envision spermine binding to the hydrophilic (stimulatory) site more potently than to the lipophilic (inhibitory) site. Therefore, the hydrophilic site is occupied at 1−20 μM spermine, resulting in enhanced [^{3}H]MK-801 binding. At high spermine concentrations, even the lipophilic site becomes occupied, leading to auto-inhibition of spermine-promoted [^{3}H]MK-801 binding.

Conclusions

The results of our investigation of the effects of systematic variations in the basicity, lipophilicity, and stereochemistry of 1,3-diamines to modulate NMDA receptors suggest the involvement of two binding sites. Both sites appear to recognize the monoprotonated form of 1,3-diamines. The site associated with enhancing [^{3}H]MK-801 binding, however, favors hydrophilic 1,3-diamines while the site whose occupation results in decreased [^{3}H]MK-801 binding favors lipophilic 1,3-diamines. The recognition site responsible for enhancement of [^{3}H]MK-801 binding appears to be stereochemically sensitive, apparently preferring a specific internuclear distance between the charged (protonated) amino nitrogen and the neutral amino nitrogen.

Experimental Section

General Methods. Melting points were measured on an Electrothermal melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as internal standard for $CDCl_3$ solutions and the sodium salt of 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid as internal standard for D_2O solutions. Chemical shifts are in ppm; coupling constants, *J*, are reported in hertz. Titrations were carried out on a Mettler DL40GP Memo Titrator. Elemental analyses were performed by Atlantic Microlab of Norcross, GA.

***N,N′*-Diallyl-1,3-diaminopropane (11) Dihydrochloride.** To a cold (0 °C) mixture of 1,3-dibromopropane (4.04 g, 20 mmol) and H_2O (1.1 mL) was added allylamine (**28**) (5.71 g, 100 mmol). The mixture was slowly allowed to warm to room temperature and then heated to reflux overnight. The resulting solution was cooled, diluted with a small portion of H_2O, and saturated with solid KOH. The mixture was then extracted with EtOAc (3 × 40 mL), dried over Na_2SO_4, concentrated, and distilled under vacuum. The distillate was dissolved in a small amount of *i*-PrOH and treated with HCl in *i*-PrOH. The white precipitate was filtered and dried under vacuum to give 1.68 g (37%) of *N,N′*-diallyl-1,3-diaminopropane (**11**) dihydrochloride as a white solid, mp 255−265 °C (dec). ^{1}H NMR (D_2O): 2.06−2.19 (m, 2H), 3.17 (5, 4H, *J* = 7.9), 3.71 (d, 4H, *J* = 6.7), 5.49−5.57 (m, 4H), 5.84−6.01 (m, 2H).

***N,N,N′,N′*-Tetraallyl-1,3-diaminopropane (12) Dihydrochloride.** A mixture of 1,3-dibromopropane (**27**) (2.02 g, 10 mmol), diallylamine (**29**) (2.14 g, 22 mmol), and K_2CO_3 (4.14 g, 30 mmol) in dry THF (25 mL) was refluxed for 2 days. The precipitate was filtered and washed with THF. The filtrate was concentrated and distilled to give a colorless oil (bp 135−140 °C/24 Torr), which was dissolved in Et_2O and treated with HCl in *i*-PrOH. The solution was placed in the freezer for 2 days, affording 0.98 g (32%) of *N,N,N′,N′*-tetraallyl-1,3-diaminopropane (**12**) dihydrochloride as colorless crystals, mp 105.8−106.5 °C. ^{1}H NMR ($CDCl_3$): 2.64 (pentet, 2H, *J* = 7.5), 3.24−3.32 (m, 4H), 3.65−3.76 (m, 8H), 5.56−5.62 (m, 8H), 6.07−6.24 (m, 4H).

***N*-(3-Propylpiperidino)piperidine (13).**[24] A mixture of 1,3-dibromopropane (**27**) (2.02 g, 10 mmol), piperidine (**30**) (1.87 g, 22 mmol), and K_2CO_3 (4.14 g, 30 mmol) in dry THF (25 mL) was refluxed for 2 days. The precipitate was filtered and washed with THF. The filtrate was concentrated. Vacuum distillation of the residue gave 1.51 g (72%) of *N*-(3-propylpiperidino)piperidine **(13)** as a colorless oil, bp 116−118 °C/0.3 Torr. ^{1}H NMR ($CDCl_3$): 1.41−1.76 (m, 14 H), 2.25−2.36 (m, 12 H).

4-Aminopiperidine (14) Dihydrochloride. A mixture of 4-amino-1-benzylpiperidine (**22**) (2.5 g, 12.8 mmol), HCO_2H (4 g), and Pd/C (10%, 1.8 g) in MeOH (80 mL) was stirred under N_2 at room temperature overnight. The catalyst was removed by filtration and washed with MeOH. The filtrate was concentrated and dried under vacuum, giving a light yellow solid. This crude product (diformate) was dissolved in H_2O (20 mL), basified with solid NaOH to pH > 13, extracted with CH_2Cl_2 (5 × 20 mL), and dried over Na_2SO_4. After removal of the drying agent, the solution was treated with HCl in Et_2O. The precipitate was filtered and dissolved in MeOH (80 mL) to which CH_2Cl_2 was added dropwise. The precipitate was filtered and dried in vacuo to afford 1.0 g (46%) of 4-aminopiperidine (**14**) dihydrochloride as a white solid, mp >300 °C. ^{1}H NMR (D_2O): 1.80−1.98 (m, 2H), 2.27−2.36 (m, 2H), 3.08−3.21 (m, 2H), 3.51−3.64 (m, 3H).

1-Benzyl-4-(2-ethanolamino)piperidine (20) Dihydrochloride. To a solution of ethanolamine (**33**) (4.88 g, 80 mmol) in MeOH (40 mL) was added 1-benzyl-4-piperidone (**31**) (3.78 g, 20 mmol) followed by $NaBH_3CN$ (4 g). The pH of the solution was adjusted to ∼6 with HCl in MeOH, and the solution was stirred at room temperature under N_2 for 3 days. The reaction mixture was treated with concentrated HCl until gas evolution ceased. The precipitate was removed by filtration, and the filtrate was concentrated. The residue was dissolved in H_2O (10 mL) and extracted with CH_2Cl_2. The aqueous phase was basified with solid NaOH and extracted with CH_2Cl_2. After drying over Na_2SO_4, the CH_2Cl_2 solution was concentrated and dried under vacuum. The crude product was dissolved in Et_2O and treated with ethereal HCl. The solid obtained was crystallized from MeOH by vapor diffusion with Et_2O to give 2.82 g (46%) of 1-benzyl-4-(2-ethanolamino)-piperidine (**20**) dihydrochloride as white crystals, mp 250−256 °C dec. ^{1}H NMR (D_2O): 1.86−2.04 (m, 2H), 2.43 (br, 2H), 3.11−3.21 (m, 2H), 3.23−3.28 (m, 2H), 3.50−3.69 (m, 3H), 3.84−3.88 (m, 2H), 4.35 (s, 2H), 7.50−7.57 (m, 5H).

4-(2-Ethanolamino)-2,2,6,6-tetramethylpiperidine (21). To a solution of ethanolamine (**33**) (4.88 g, 80 mmol) in MeOH (40 mL) was added 2,2,6,6-tetramethyl-4-piperidone (**39**) monohydrate (3.46 g, 20 mmol) followed by $NaBH_3CN$ (4 g). The pH of the solution was adjusted to ∼6 with HCl in MeOH, and the solution was stirred at room temperature under N_2 for 3 days. The reaction mixture was treated with concentrated HCl until gas evolution ceased. The precipitate was removed by filtration, and the filtrate was concentrated. The residue was dissolved in H_2O (10 mL) and extracted with CH_2Cl_2. The aqueous phase was basified with solid NaOH and extracted with CH_2Cl_2. After being dried over Na_2SO_4, the combined extract was concentrated and dried in vacuo to give a light yellow solid, which was crystallized from Et_2O to afford 2.44 g (61%) of 4-(2-ethanolamino)-2,2,6,6-tetramethylpiperidine (**21**) as white crystals, mp 98.8−99.8 °C. ^{1}H NMR ($CDCl_3$): 0.85 (dd, 2H, *J* = 12.0), 1.13 (s, 6H), 1.20 (s, 6H), 1.87 (dd, 2H, *J* = 12.7, 3.6), 2.80 (5, 2H, *J* = 5.2), 2.91 (tt, 1H, *J* = 3.6, 11.7), 3.65 (t, 2H, *J* = 5.2).

3α-Aminotropane (23) Dihydrochloride. Tropinone oxime (**35**) (0.47 g, 3.05 mmol) in HOAc (30 mL) was hydrogenated at 45 psi over PtO_2 (50 mg) at room temperature overnight. The solvent was evaporated after removal of the catalyst by filtration. The residue was dissolved in H_2O (6 mL) and basified with solid NaOH to pH > 12. The mixture was extracted with CH_2Cl_2 (4 × 15 mL) and dried over Na_2SO_4. The extract was treated with ethereal HCl and concentrated to give a white solid, which was crystallized from MeOH by vapor diffusion with Et_2O to afford 0.44 g (67%) of 3α-aminotropane (**23**) dihydrochloride as white crystals (mp >300 °C). ^{1}H NMR (D_2O): 2.10−2.27 (m, 4H), 2.50−2.70 (m, 4H), 2.83 (s, 3H), 3.77 (t, 1H, J = 7.4), 4.01 (br, 2H). ^{13}C NMR (D_2O): 64.12, 43.03, 41.43, 35.33, 25.89.

3β-Aminotropane (24) Dihydrochloride. To a solution of tropinone oxime (**35**) (3.5 g, 22.7 mmol) in n-PrOH (75 mL) was added Na (5.23 g, 227 mmol) over 10 min. The mixture was refluxed for 1.5 h. After cooling, H_2O (100 mL) was added, and the mixture was extracted with CH_2Cl_2 (4 × 70 mL). The combined extract was extracted with aqueous HCl (2 N, 70 mL, 30 mL), and after basification with solid KOH, the aqueous phase was extracted with CH_2Cl_2 (4 × 70 mL). The free base was obtained by drying of the organic phase over Na_2SO_4, concentration, and distillation under reduced pressure (bp 108−109 °C/23 Torr; lit.[12] bp 104−106 °C/22 Torr) to give 2.23 g (70%) of 3β-aminotropane (**24**) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.16 (br, 2H), 1.33−1.58 (m, 4H), 1.67−1.76 (m, 2H), 1.95−2.02 (m, 2H), 2.28 (s, 3H), 2.83−2.97 (m, 1H), 3.10−3.15 (m, 2H). ^{13}C NMR ($CDCl_3$): 60.45, 42.22, 41.64, 39.17, 26.06.

Alternatively, the solution was treated with ethereal HCl and concentrated to give a white solid, which was crystallized from MeOH by vapor diffusion with Et_2O to afford 3.81 g (78%) of 3β-aminotropane (**24**) dihydrochloride as white crystals (mp >300 °C). ^{1}H NMR (D_2O): 2.03−2.42 (m, 8H), 2.81 (s, 3H), 3.71−3.85 (m, 1H), 4.06 (br, 2H). ^{13}C NMR (D_2O): 65.62, 43.78, 41.25, 36.52, 26.28.

3α-Amino-6-methyl-6-azabicyclo[3.2.1]octane (25) Dihydrochloride. This compound (mp >300 °C) was prepared as described for 3α-aminotropane (**23**) dihydrochloride starting from 6-methyl-6-azabicyclo[3.2.1]octan-3-one oxime (**37**). ^{1}H NMR (D_2O): 1.57 (dd, 1H, J = 1.07, 14.3), 1.85−2.14 (m, 3H), 2.56−2.76 (m, 2H), 2.82−2.92 (m, 1H), 2.95 (s, 3H), 3.23−3.37 (m, 1H), 3.47−3.62 (m, 2H), 4.04 (dd, 1H, J = 4.6, 7.8). ^{13}C NMR (D_2O): 66.21, 65.86, 44.81 (br), 43.07, 35.57, 33.99, 33.20 (br), 29.72.

3β-Amino-6-methyl-6-azabicyclo[3.2.1]octane (26) Dihydrochloride. This compound (mp >300 °C) was prepared as described for 3β-aminotropane (**24**) dihydrochloride starting from 6-methyl-6-azabicyclo[3.2.1]octan-3-one oxime (**37**). It was crystallized from MeOH. ^{1}H NMR (D_2O): 1.71−1.96 (m, 3H), 2.18−2.36 (m, 2H), 2.48−2.62 (m, 1H), 2.95 (s, 3H), 2.89−3.02 (m, 1H), 3.28−3.39 (m, 1H), 3.55−3.78 (m, 2H), 4.02 (br, 1H). ^{13}C NMR (D_2O): 67.48, 62.32, 46.44, 45.24 (br), 36.94, 36.24, 35.52, 34.07.

Tropinone Oxime (35).[11] A mixture of tropinone (**34**) (7.5 g, 54 mmol), H_2NOH (**38**)·HCl (5.44 g, 78 mmol), and pyridine (8.1 mL) in EtOH (110 mL) was refluxed for 2 h. After cooling, the mixture was concentrated, and the residue was dissolved in NaOH solution (2.5 M, 40 mL) and extracted with EtOAc (3 × 70 mL). After drying over K_2CO_3, the solution was concentrated and dried in vacuo to give 7.67 g (92%) of tropinone oxime as a white crystalline solid, which was used in the next reactions without purification. ^{1}H NMR ($CDCl_3$): 1.42−1.69 (m, 2H), 2.02 (br, 2H), 2.14 (d, 1H, J = 14.9), 2.25 (dd, 1H, J = 3.5, 15.6), 2.38 (s, 3H), 2.62 (dd, 1H, J = 2.8, 15.0), 3.00 (d, 1H, J = 15.4), 3.31 (br, 2H), 10.73 (br, 1H). ^{13}C NMR ($CDCl_3$): 155.01, 60.66, 59.92, 39.03, 36.99, 30.97, 27.20, 26.29.

6-Methyl-6-azabicyclo[3.2.1]octan-3-one Oxime (37). This compound was prepared as described for tropinone oxime (**35**) starting from 6-methyl-6-azabicyclo[3.2.1]octan-3-one (**36**).[13] A diastereomeric mixture was obtained and used for the next reactions without purification. ^{13}C NMR ($CDCl_3$): 156.37, 155.88, 59.64, 59.43, 59.30, 58.49, 40.01, 39.95, 38.60, 36.54, 36.46, 35.10, 35.06, 34.34, 32.33, 28.52.

Determination of pK_a's of Amine Compounds. Free amines and amine hydrochloride salts were titrated with standard HCl solution and NaOH solution, respectively, using and autotitrator at 22 °C. The pK_a values were calculated using TableCurve 2D Automated Curve Fitting Software. Equations 1 and 2 were used for amines and amine dihydrochloride salts, respectively.

$$V_{HCl} = V_B^0\left\{\frac{C^0_B(K_2[H^+]^2 + 2[H^+]^3)}{K_1K_2 + K_2[H^+] + [H^+]^2} + [H^+]^2 - Kw\right\}\Big/ C^0_{HCl}[H^+] + Kw - [H^+]^2 \quad (1)$$

C^0_B: original concentration of diamine solution

V^0_B: original volume of diamine solution

C^0_{HCl}: original concentration of standard HCl solution

V_{HCl}: volume of standard HCl solution added

$$V_{NaOH} = V_B^0\left\{\frac{C^0_B(2K_1K_2[H^+] + K_2[H^+]^2)}{K_1K_2 + K_2[H^+] + [H^+]^2} + [H^+]^2 + Kw\right\}\Big/ C^0_{NaOH}[H^+] - Kw + [H^+] - Kw + [H^+]^2 \quad (2)$$

C^0_B: original concentration of diamine dihydrochloride solution

V^0_B: original volume of diamine dihydrochloride solution

C^0_{NaOH}: original concentration of standard NaOH solution

V_{NaOH}: volume of standard NaOH solution added

Membrane Preparation. Membrane preparations from the forebrain of adult, male Sprague−Dawley rats (175−300 g, Taconic Farms, Germantown, NY) followed "buffy" coat method. Briefly, rats were decapitated, and the forebrains were removed (minus cerebellum and brainstem) and disrupted with a Polytron (30s, setting 6) in 10 volumes (w/v) of 5 mM HEPES/4.5 mM Tris buffer (pH 7.6) containing 0.32 M sucrose. Unless otherwise stated, all procedures were carried out at 4 °C. The homogenate was diluted to 50 volumes with this buffer and centrifuged at 1000*g* for 10 min. The supernatant was decanted and recentrifuged at 20000*g* for 20 min. The resulting pellet was resuspended in 50 volumes of 5 mM HEPES/4.5 mM Tris buffer (assay buffer) and centrifuged at 8000*g* for 20 min. The supernatant and outer "buffy" pellet coat was centrifuged at 20000*g* for 20 min and the remaining pellet core discarded. The resulting supernatant was discarded, the pellet was resuspended in 50 volumes of assay buffer containing 1 mM EDTA, and the suspension was recentrifuged. This resuspension/centrifugation procedure was repeated four times, with the last cycle being performed using assay buffer without EDTA. The resulting pellet was resuspended in 5 volumes of assay buffer and quickly frozen over dry ice (−70 °C). On the day of the assay, the tissue was thawed, diluted 10-fold with assay buffer, and centrifuged twice at 20000*g* for 20 min. The pellet was resuspended in 50 volumes of assay buffer.

[^{3}H]MK-801 Binding Assay. Binding assays were performed in a total volume of 0.5 mL containing 0.15 mL (∼50 of μg protein) of rat brain membranes, 0.05 mL of [^{3}H]MK-801 (final concentration 4−4.5 nM), and test compounds or buffer. Assays were incubated at room temperature for 2 h and terminated by rapid filtration under partial vacuum (Brandel cell harvester, Gaithersburg, MD) over glass fiber filters presoaked in 0.03% polyethylenimine. The filtration was followed by a 10 mL wash with ice cold assay buffer. Nonspecific binding was determined using 100 μM PCP and represented 60−80% of the total binding in the absence of

modulatory agents. In the presence of modulatory agents (20 μM spermine), however, nonspecific binding represented 10−20% of the total binding. Radioactivity retained in the filter was measured in "CytoScint" scintillation liquid using a Beckman LS 6500 liquid scintillation counter.

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