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Polyamines and the NMDA receptor: Modifying intrinsic activities with aromatic substituents

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Abstract—Thirty-four spermidine (SPD) and spermine (SPM) derivatives with aromatic substituents were synthesized and tested as inhibitors of specific binding of the NMDA channel blocker [^{3}H]MK-801 to membranes prepared from rat hippocampus and cerebral cortex. SPD and SPM derivatives with aromatic substituents at the primary amino groups were the most potent inhibitors (IC_{50} 3.9–4.7 μM). These compounds most likely act directly at the NMDA ion channel, since 30 μM SPM had no pronounced influence on their inhibiting activities. SPD derivatives with aromatic substituents at the secondary amino group were either inactive or highly SPM-sensitive inhibitors (IC_{50} 10–82 μM), depending on the size of the substituent. Our results support the hypothesis that an aromatic interaction site near the center of polyamine derivatives contributes to polyamine inverse agonism.

Endogenous polyamines including spermidine (SPD) and spermine (SPM) influence the activities of several ion channels, including inward rectifier K^+ channels and ion channels associated with AMPA and NMDA receptors.[1] The NMDA receptor complex has been implicated in learning and memory, and may also occupy a central role in various conditions leading to neuronal degeneration. In addition to the binding site for the main agonist glutamate, the NMDA receptor complex contains binding sites for the co-agonist glycine, a channel blocking site, and modulatory sites for Ifenprodil and Zn^{2+}, and is influenced by oxidizing agents and by protons.[2] Also polyamines exert several different effects at the NMDA receptor. SPD and SPM increase the opening frequency of the ion channel associated with the prevalent NMDA receptor splice variant by relief from inhibition by protons at physiological pH. Compounds inhibiting the NMDA receptor via the same mechanism are described as polyamine inverse agonists.[3] SPD and SPM themselves act as weak NMDA channel blockers.[4] This channel blocking potency is increased by incorporation of aromatic substituents as, for example, in N^1-dansyl-SPM[5] and certain *N*-benzyl-substituted SPD and SPM derivatives.[6]

For this study, 34 derivatives of SPD or SPM with aromatic substituents appended to the amino groups (as shown in Scheme 1) were synthesized. We describe that, depending on the specific substitution pattern, SPD and SPM derivatives with aromatic residues not only act as NMDA channel blockers, but also as agonists and as inverse agonists at the polyamine-regulatory site of the NMDA receptor complex. These results support the hypothesis that an aromatic interaction site near the center of polyamine derivatives contributes to polyamine inverse agonism.[7] Some of these results have been presented as an abstract.[8]

We have previously described the syntheses of several of the polyamine derivatives used in this study.[9,10] Note that the N-atoms in SPD are numbered by convention as N^1-$(CH_2)_3$-N^4-$(CH_2)_4$-N^8 and those of SPM as N^1-$(CH_2)_3$-N^4-$(CH_2)_4$-N^8-$(CH_2)_3$-N^{12}. The ^{1}H and ^{13}C NMR spectra of all new compounds were consistent with their proposed structures. Compound **1d** was prepared by reacting N^1,N^8-bis(trifluoroacetyl)-SPD[11] with 5-chlorodibenzosuberane in the presence of triethylamine and CH_3CN, followed by removal of the trifluoroacetyl groups by refluxing in NH_4OH and CH_3OH. For preparation of **5b**, N^1,N^{12}-bis(trifluoroacetyl)-SPM[11]

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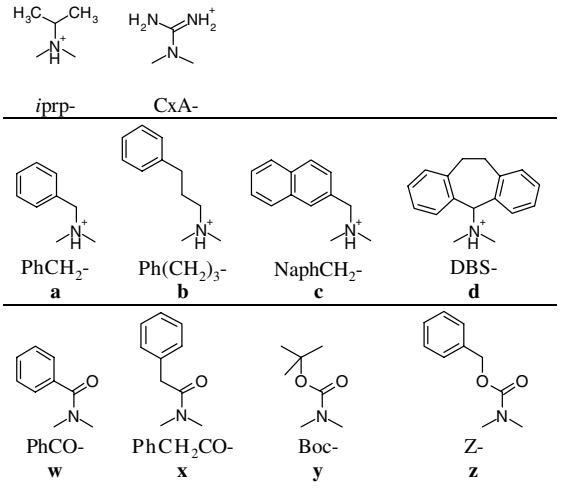


Scheme 1. Abbreviations for N-substituents used in the tables: *i*prp-, isopropyl-; CxA-, carboxamidino-; $PhCH_2$-, benzyl-; PhCO-, benzoyl-; Boc-, *t*-butoxycarbonyl-; $PhCH_2CO$-, phenylaceto-; Z-, benzyloxycarbonyl-; $Ph(CH_2)_3$-, 3-phenylpropyl-; $NaphCH_2$-, 2-naphthylmethyl-; DBS-, dibenzosuberyl-. Bold lowercase letters refer to the compound numbering code. Note that the N shown is a component of the polyamine backbone and is positively charged at physiological pH for substituents **a**–**d** and neutral for substituents **w**–**z**.

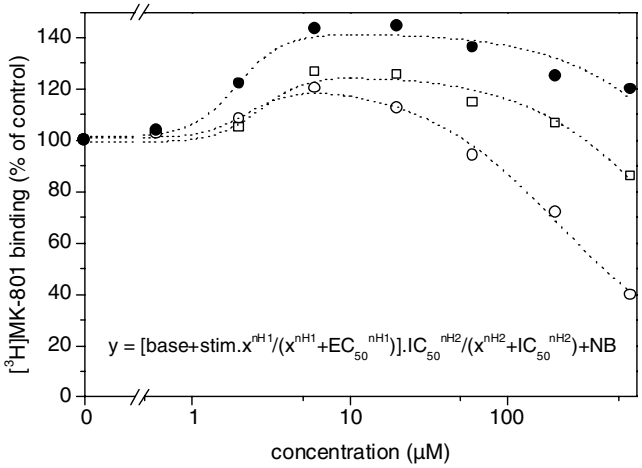


Figure 1. NMDA receptor stimulation by low concentrations and inhibition by high concentrations of N^4-benzylspermine (**5a**, □), N^4-(3-phenylpropyl)spermine (**5b**, ○) (Table 2), and spermine (●). Iterative curve fitting (to the formula indicated) resulted in the following EC_{50} values (% stimulation in parentheses): **5a**, 2.7 μM (28%); **5b**, 2.0 μM (26%); spermine, 2.2 μM (44%). Data pooled from two to three experiments.

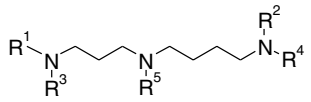
was refluxed with 1-bromo-3-phenylpropane in the presence of triethylamine and CH_3CN to give a mixture of N^4,N^8-bis(3-phenylpropyl)-N^1,N^{12}-bis(trifluoroacetyl)-SPM and N^4-(3-phenylpropyl)-N^1,N^{12}-bis(trifluoroacetyl)-SPM, which were purified. Then the trifluoroacetyl groups were removed from N^4-(3-phenylpropyl)-N^1,N^{12}-bis(trifluoroacetyl)-SPM by refluxing in NH_4OH and CH_3OH to give compound **5b**. Compound **5c** was prepared in the same manner as **5b**, except that N^1,N^{12}-bis(trifluoroacetyl)-SPM was refluxed with 2-(bromomethyl)naphthalene in the presence of triethylamine and CH_3CN. Compounds **7b** and **8b** were prepared from **6c**, by reaction with either 1.0 or 2.2 equiv of the *N*,*N*′-bis-(*t*-butoxycarbonyl) derivative of 1H-pyrazole-1-carboxamidine in THF,[12] respectively; followed by the removal of the *t*-butoxycarbonyl groups with trifluoroacetic acid in CH_3OH. **3c** was obtained from **1y** by reductive amination with 4.8 equiv of 2-naphthaldehyde using sodium triacetoxyborohydride in 1,2-dichloroethane;[13] followed by removal of the *t*-butoxycarbonyl group with trifluoroacetic acid in CH_2Cl_2. The same strategy was used to prepare **11c** from **6y**, and **11b** from **6y** (with 3-phenylpropanal).

In vitro pharmacological testing has been performed as described.[7,14] Data were computer-fitted to $B = B_0 * IC_{50}^{nH}/(x^{nH} + IC_{50}^{nH}) + NB$ (B, amount of radioligand bound; x, concentration of test compound; B_0, specific binding at $x = 0$; nH, Hill coefficient; NB, nonspecific binding). Stimulation at low and inhibition at high concentrations was fitted to an inhibition function superimposed to a stimulation function (given in Fig. 1).[15]

Nineteen of the polyamine derivatives investigated were potent inhibitors of [^{3}H]MK-801 binding, with IC_{50} values below 20 μM. Substitution at the secondary amino group of SPD yielded compounds with widely differing properties (R^5 in Table 1). N^4-(2-naphthylmethyl)- (**1c**) and N^4-(benzyloxycarbonyl)-SPD (**1z**) exhibited properties typical of inverse polyamine agonists, with inhibition of [^{3}H]MK-801 binding strongly attenuated by 30 μM SPM (by factors 13 and 67, respectively). SPM attenuation factors >50 have recently been described for aromatic diamines with an additional uncharged nitrogen.[16] Compounds **1a** and **1b**, with smaller substituents at N^4, were inactive. Slight structural changes in the N^4 substituent had major consequences. Compound **1d**, with dibenzosuberyl (DBS-) at N^4, had a similar IC_{50} as naphthylmethyl-substituted **1c**, but was much less sensitive to SPM. The N^4-benzoyl and benzyloxycarbonyl derivatives **1w** and **1z** are chemically cognate (neither are protonated at N^4 at pH 7), however, **1z** was a more effective inhibitor and extremely sensitive to SPM. A Boc-substituent at N^4 of SPD resulted in the inactive **1y**, either for steric reasons, or because this substituent was not aromatic. Alkylation at both primary amino groups in SPD (which at pH 7 preserve the protonated nitrogens) yielded the potent inhibitors N^1,N^8-bis(benzyl)- (**2a**) and N^1,N^8-bis(2-naphthylmethyl)-SPD (**2c**), with moderate to poor SPM-sensitivities. Acylation, leading to the loss of both terminal charges, resulted in inactive N^1,N^8-bis(benzoyl)-SPD (**2w**) and in the weakly active N^1,N^8-bis(benzyloxycarbonyl)-SPD (**2z**), the latter exhibiting high SPM-sensitivity like its N^4 homologue **1z**.

Substitution of just one of the secondary amino groups in SPM resulted in inactive (**5a**) or weak inhibitors (**5b**, **5c**; Table 2) with poor SPM-sensitivity. However, for **5a** and **5b** some agonistic activity was maintained: both stimulated specific [^{3}H]MK-801 binding with EC_{50} values similar to SPM itself, but with somewhat lower intrinsic activities (reaching lower levels of stimulation,

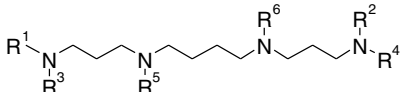
Table 1. Inhibition of [^{3}H]MK-801 binding by derivatives[a] of spermidine and influence of spermine



Compound	R^1	R^3	R^5	R^2	R^4	IC_{50} (μM) ±SD[b]	Attenuation[c] by 30 μM spermine[b]
1a			$PhCH_2$-			>300	—
1b			$Ph(CH_2)_3$-			>300	—
1c			$NaphCH_2$-			9.8 ± 5.5 (4)	13.1 ± 5.6 (3)
1d			DBS-			14.2 ± 4.4 (4)	5.1, 4.4 (2)
1w			PhCO-			82 ± 12 (3)	>10
1y			Boc-			>300	—
1z			Z-			17.6 ± 9.3 (5)	67 ± 25 (3)
2a	$PhCH_2$-			$PhCH_2$-		6.2 ± 1.9 (5)	5.9 ± 1.1 (4)
2c	$NaphCH_2$-			$NaphCH_2$-		4.37 ± 1.16 (4)	2.2, 2.0 (2)
2w	PhCO-			PhCO-		>300	—
2z	Z-			Z-		56 ± 22 (3)	>10
3c	$NaphCH_2$-	$NaphCH_2$-		$NaphCH_2$-	$NaphCH_2$-	14.3, 12.4 (2)	1.66, 2.46 (2)
4b	$Ph(CH_2)_3$-		$Ph(CH_2)_3$-	$Ph(CH_2)_3$-		9.5 ± 5.6 (4)	2.03, 1.60 (2)

[a] Key for substituents in Scheme 1; where no substituent is indicated, the substituent was H.
[b] Numbers of repeat experiments are given in parentheses.
[c] Ratio (IC_{50} with SPM)/(IC_{50} without SPM).

Table 2. Inhibition of [^{3}H]MK-801 binding by derivatives[a] of spermine and influence of spermine



Compound	R^1	R^3	R^5	R^6	R^2	R^4	IC_{50} (μM) ±SD[b]	Attenuation[c] by 30 μM spermine[b]
5a			$PhCH_2$-				>300[d]	—
5b			$Ph(CH_2)_3$-				182 ± 40 (3)[e]	0.93, 0.94 (2)
5c			$NaphCH_2$-				58 ± 15 (3)	1.45, 0.59 (2)
6a			$PhCH_2$-	$PhCH_2$-			38 ± 18 (3)	4.3, 2.5 (2)
6b			$Ph(CH_2)_3$-	$Ph(CH_2)_3$-			26.7 ± 3.1 (3)	2.6 ± 0.3 (3)
6c			$NaphCH_2$-	$NaphCH_2$-			9.1 ± 2.8 (4)	2.20 ± 0.2 (3)
6y			Boc-	Boc-			66, 77 (2)	5.3, 5.6 (2)
6z			Z-	Z-			17.2 ± 6.6 (5)	4.7 ± 1.0 (3)
7b	CxA-		$Ph(CH_2)_3$-	$Ph(CH_2)_3$-			13.0 ± 2.9 (7)	1.5 ± 0.2 (3)
8b	CxA-		$Ph(CH_2)_3$-	$Ph(CH_2)_3$-	CxA-		11.2, 8.0 (2)	0.63, 0.94 (2)
9b	$Ph(CH_2)_3$-						25.6 ± 11.0 (4)	1.43, 1.01 (2)
10a	$PhCH_2$-				$PhCH_2$-		6.12 ± 1.81 (3)	2.27, 2.10 (2)
10b	$Ph(CH_2)_3$-				$Ph(CH_2)_3$-		7.4 ± 1.9 (6)	0.92 ± 0.17 (5)
10c	$NaphCH_2$-				$NaphCH_2$-		4.7 ± 2.2 (4)	2.84, 1.77 (2)
10x	$PhCH_2CO$-				$PhCH_2CO$-		103 ± 35 (3)	>10
11b	$Ph(CH_2)_3$-	$Ph(CH_2)_3$-			$Ph(CH_2)_3$-	$Ph(CH_2)_3$-	3.9 ± 1.1 (3)	1.58 ± 0.65 (3)
11c	$NaphCH_2$-	$NaphCH_2$-			$NaphCH_2$-	$NaphCH_2$-	8.7 ± 3.6 (3)	1.32 ± 0.33 (3)
12b	$Ph(CH_2)_3$-		$Ph(CH_2)_3$-	$Ph(CH_2)_3$-			13.1 ± 2.8 (4)	1.44, 1.29 (2)
12c	*i*prp-		$NaphCH_2$-	$NaphCH_2$-			14.0, 10.0 (2)	1.96, 1.20 (2)
13b	$Ph(CH_2)_3$-		$Ph(CH_2)_3$-	$Ph(CH_2)_3$-	$Ph(CH_2)_3$-		8.4 ± 3.8 (5)	1.49 ± 0.18 (3)
13c	*i*prp-		$NaphCH_2$-	$NaphCH_2$-	*i*prp-		44.6, 38.5 (2)	0.48, 0.66 (2)

[a] As in Table 1.
[b] As in Table 1.
[c] As in Table 1.
[d] Stimulation at lower concentrations (EC_{50} 2.7 μM, Fig. 1).
[e] Stimulation at lower concentrations (EC_{50} 1.8 μM, Fig. 1).

Fig. 1). Partial stimulation of [^{3}H]MK-801 binding has also been described for other SPM derivatives.[17,18] Substitution at both secondary amino groups of SPM yielded potent inhibitors, with SPM-sensitivities below those of the respective SPD analogues (Table 2). Only **6y** and **6z**, with N^4 and N^8 being uncharged at pH 7, were slightly influenced by SPM (factor 5).

The most potent inhibitors of [^{3}H]MK-801 binding in this study were N^1,N^8-bis(2-naphthylmethyl)-SPD (**2c**)

with an IC_{50} value of 4.4 μM (Table 1), N^1,N^{12}-bis-(2-naphthylmethyl)-SPM (**10c**) (IC_{50} 4.7 μM), and N^1,N^1,N^{12},N^{12}-tetrakis(3-phenylpropyl)-SPM (**11b**) (IC_{50} 3.9 μM) (Table 2), all of which are substituted at the distal, primary amino groups. However, these compounds appeared to act directly at the ion channel, since their potencies were not markedly influenced by 30 μM SPM (attenuation factors 1–2.3). In this respect, they behaved similarly to the benzyl-substituted SPD and SPM derivatives **2a** and **10a**, described as NMDA channel blockers by Igarashi et al.[6] This type of behavior is also displayed by N^1-dansyl-SPM.[5] The only compounds substituted at the primary amino groups with pronounced SPM-sensitivities were the weak inhibitors **2z** (Table 1) and **10x** (Table 2), with SPM-factors >10. Both of these compounds have terminal N's that are uncharged at physiological pH.

Several SPM derivatives with additional substituents were investigated. Adding isopropyl groups to one or both of the primary amino groups of the fairly potent channel blocker **6c** resulted in the equivalent **12c** and the much less potent **13c** (Table 2). Replacing the primary amino groups of moderately potent channel blocker **6b** with the more basic guanidino groups resulted in more potent inhibitors (**7b**, **8b**), but their sensitivity to SPM was even less than that of **6b**.

One trivial explanation for the observed attenuating effect of 30 μM SPM on the inhibition of [^{3}H]MK-801 binding by various compounds would be a slightly alkaline microenvironment at the receptor created by SPM (macroscopically, the pH was kept at 7.0). To test this possibility, we investigated the influence of a pH change from 6.4 to 8.2 on the inhibiting effects of 16 compounds. We found that, in fact, the potencies of most compounds were weakened by increasing the pH by 1.8 units (by factors up to 6.4), however there was no significant correlation with the attenuation by SPM (Fig. 2). Thus, SPM's attenuating influence was not due to its basicity, but involves a more specific molecular interaction.

Several compounds inhibiting the NMDA receptor complex in this study also exhibit trypanocidal activity at micromolar (**10b**) or sub-micromolar concentrations (**4b**, **6b**, **12b**, and **13b**).[9] If in the future, related compounds were used in high concentrations as drugs to combat diseases caused by trypanosomes or leishmania, side effects related to inhibition of NMDA receptors should not be excluded. Interestingly, analogues of pentamidine (an aromatic diamidine currently used in the treatment of African trypanosomiasis and leishmaniasis) display both trypanocidal activities[19] and inverse polyamine agonism at the NMDA receptor.[20]

Our results provide further evidence in support of the hypothesis that an aromatic moiety near the center of polyamine derivatives contributes to polyamine inverse agonism.[7,21] Substitution of the primary amino groups of SPD and SPM resulted in potent NMDA channel blockers (e.g., compounds **2c**, **10c**, and **11b**) with low

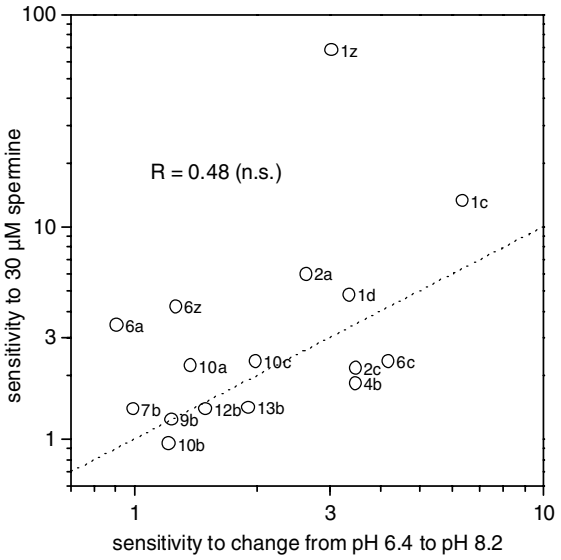


Figure 2. For 16 compounds, the sensitivity of their inhibiting potency to spermine is plotted against sensitivity to alkalization. Dotted line indicates identity.

SPM-sensitivities; these compounds most likely act directly at the NMDA receptor associated ion channel. However, SPD derivatives with moieties at the central N^4 position (**1c** and **1z**) exhibited pronounced sensitivity to SPM in their inhibition, suggesting their interaction with a polyamine-sensitive site of the NMDA receptor. The clinical application of most channel blocking NMDA antagonists is hampered by severe psychotic side effects. Addressing the polyamine regulatory site might offer a promising alternative strategy for drug development.

Acknowledgments

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14. In brief, the NMDA channel ligand [^{3}H]MK-801 (5.0 nM) was incubated in 10 mM Tris–acetate buffer for 3 h in the presence of glutamate and glycine (10 μM each) with membranes prepared from rat hippocampus or cerebral cortex. Bound ligand was separated by filtration. For nonspecific binding, the channel-opening co-agonists glutamate and glycine were replaced by their respective antagonists D-2-amino-5-phosphonopentanoic acid (100 μM) and 5,7-dichlorokynurenic acid (10 μM, both from Tocris Bioscience).
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