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Characterization of the Stimulatory and Inhibitory Effects of Polyamines on [^{3}H]*N*-(1-[thienyl]cyclohexyl) piperidine Binding to the *N*-Methyl-D-aspartate Receptor Ionophore Complex

AIDA I. SACAAN and KENNETH M. JOHNSON

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550

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SUMMARY

Spermidine and spermine, as well as several other structurally related compounds, were tested in a [^{3}H]*N*-(1-[thienyl]cyclohexyl) piperidine ([^{3}H]TCP) binding assay to determine the structural requirements of polyamines for activation of the *N*-methyl-D-aspartate-operated ion channel. Under nonequilibrium conditions, the polyamines enhanced [^{3}H]TCP binding approximately 9-fold, with EC_{50} values ranging from 0.8 to 60 μM. The order of potency in enhancing [^{3}H]TCP binding was *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine > *N*,*N*′-bis-(3-aminopropyl)-ethylenediamine > spermine > spermidine > *N*,*N*′-bis-(2-aminoethyl)-1,3-propanediamine. 1,3-Diaminopropane produced a partial agonistic effect, whereas putrescine, cadaverine, and 1,7-diaminoheptane were without effect at concentrations up to 1 mM. Eadie-Hofstee analysis of spermidine-induced [^{3}H]TCP binding at equilibrium revealed a 3-fold increase in the affinity without a significant change in receptor density. This was further supported by kinetic data that showed that spermidine produced an increase in the association rate and a decrease in the dissociation rate of [^{3}H]TCP binding to its site. Putrescine, cadaverine, and 1,3-diaminopropane antagonized the effects of spermidine by an apparently noncompetitive mechanism. Magnesium ions mimicked the effects of putrescine, suggesting the possibility that the inhibitory effects of Mg^{2+} and putrescine are mechanistically related.

Extensive work over the past few years has provided a better understanding of the regulation and function of excitatory amino acid receptors, particularly the NMDA subtype. The NMDA receptor is linked to a nonselective cation channel (1, 2), the activation of which is dependent on glycine (3). This channel contains the binding site for the dissociative anesthetic PCP, which blocks NMDA-mediated responses in a noncompetitive (4) and voltage-dependent manner (5). Magnesium ions at physiological concentrations also have been shown to noncompetitively and voltage-dependently antagonize NMDA-mediated responses (6, 7).

Recently, data have been presented suggesting that polyamines may also play a role in the regulation of the NMDA receptor-ionophore complex (8). The polyamines are a group of naturally occurring compounds that include spermidine, spermine, and their precursor putrescine. These compounds are found in virtually every cell, and their concentration in the brain is reportedly in the micromolar range (9). Ransom and Stec (8) reported that the polyamines spermine and spermidine, but not putrescine, enhanced [^{3}H]MK 801 binding to the PCP binding site. These authors proposed that a novel binding site, distinct from either the NMDA or glycine recognition sites, mediated the effects of the polyamines. In addition, it has been reported that NMDA produced a transient induction of ODC activity (10), the rate-limiting enzyme in the polyamine synthetic pathway. Moreover, these authors reported that the irreversible ODC inhibitor difluoromethylornithine inhibited NMDA-induced [^{3}H]norepinephrine release and $^{45}Ca^{2+}$ flux from hippocampal synaptosomes, in a manner reversed by the addition of putrescine. Therefore, it is quite possible that the polyamines play an important role in the regulation of NMDA receptor function.

Experimental Procedures

Materials. [^{3}H-TCP (48.9 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Spermine, spermidine, cadaverine, and putrescine were purchased from Sigma Chemical Company (St. Louis, MO). All synthetic polyamine analogs were purchased from Aldrich Chemical Company (Milwaukee, WI).

Membrane preparation. [^{3}H]TCP binding was assessed in a buffy

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; [^{3}H]MK 801, (+)-[^{3}H]5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine; ODC, ornithine decarboxylase; [^{3}H]TCP, [^{3}H]*N*-(1-[thienyl]cyclohexyl) piperidine; GABA, γ-aminobutyric acid; CPP, (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); PCP, phencyclidine.

coat preparation of rat cortical membranes developed in our laboratory as previously described (11). A crude P_2 preparation was resuspended in 20 volumes of ice-cold H_2O and centrifuged for 20 min at 8000 × *g*. After the supernatant and the buffy coat were collected and centrifuged at 48,000 × *g* for 20 min, the pellet was washed two times with ice-cold water and stored at −20° for not more than 2 weeks. On the day of the assay the pellet was thawed, resuspended in 40 volumes of buffer, incubated at 37° for 20 min, and centrifuged for 10 min at 48,000 × *g* three times before resuspension in a buffer suitable for the binding assay. Protein concentration was determined by the method described by Smith *et al.* (12).

[^{3}H]TCP binding assay. [^{3}H]TCP was incubated at 5 nM with approximately 200 μg of protein/ml, in the presence or absence of the test drug dissolved in 10 mM HEPES (pH 7.4), at 25° for 2 hr. The samples were then filtered over GF/C filters on a Brandel cell harvester, followed by washing with 6 ml of ice-cold buffer over a 6-sec period. The filters were presoaked in 0.03% polyethyleneimine (Sigma), to reduce filter binding. Nonspecific binding was determined in the presence of 30 μM PCP. The filters were placed into scintillation vials containing 5 ml of Safety-solve scintillation cocktail (Research Products International Corp., Mount Prospect, IL), and tritium was estimated using a Beckman LS5000TD liquid scintillation counter with an efficiency of 39%. In equilibrium experiments, seven concentrations of [^{3}H]TCP, ranging from 5 to 165 nM, were incubated for 17 hr in the presence or absence of the test drug. TCP (3 μM) was used to define nonspecific binding. Association experiments were initiated by the addition of tissue at different time intervals ranging from 1 min to 17 hr; the reaction was then stopped by filtration. Dissociation experiments were brought to equilibrium (17 hr) and dissociation was initiated by addition of PCP (300 μM, final concentration) at different time intervals ranging from 1 min to 6 hr before filtration.

Statistical analysis. All experiments were replicated 3 to 10 times. IC_{50} values were estimated by an iterative nonlinear regression program (13), as adapted for the IBM PC (14). EC_{50} values were estimated by fitting the data to a sigmoidal logistic equation with the assistance of an iterative curve-fitting program (ALLFIT), according to the method of DeLean *et al.* (15). Results were analyzed by Student's *t* test or one-way analysis of variance, with multiple *t post hoc* tests where appropriate. Values of $p < 0.05$ were considered significantly different from control.

Results

The effect of the naturally occurring polyamines on [^{3}H]TCP binding is shown in Fig. 1. As previously reported with [^{3}H] MK 801 (8), spermine (N-3C-N-4C-N-3C-N) and spermidine

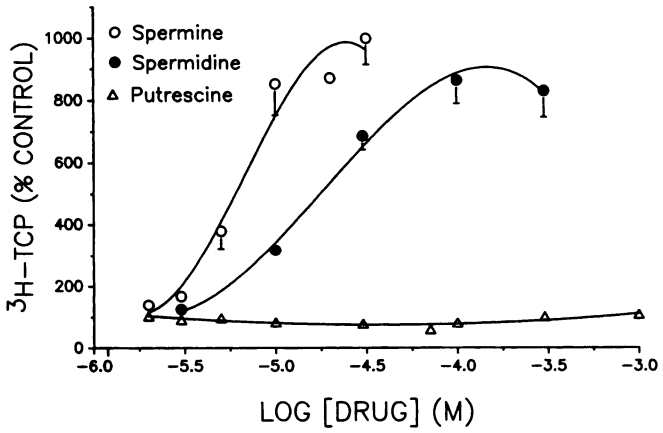

Fig. 1. Effect of increasing concentrations of spermine, spermidine, and putrescine on [^{3}H]TCP binding. Each point is the mean ± standard error of 3 to 10 experiments performed in triplicate. The curves shown here and in Figs. 2 and 5 were drawn using a third-order polynomial equation (Sigmaplot).

(N-3C-N-4C-N) produced a dose-dependent enhancement of [^{3}H]TCP binding, with EC_{50} values of 5.9 ± 0.35 and 15 ± 1.6 μM, respectively (Table 1). The concentration-response curve was significantly steeper than would be expected from simple mass action (Hill coefficients given in Table 1). This stimulation was completely prevented by the NMDA antagonist CPP (30 μM) (data not shown). Putrescine (N-4C-N) at up to 1 mM did not alter [^{3}H]TCP binding (Fig. 1, Table 1).

In order to further elucidate the structural requirements for enhancing [^{3}H]TCP binding, we tested several synthetic polyamine analogs. The results are shown in Fig. 2. Among the compounds tested, *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine (N-3C-N-3C-N-3C-N) was the most potent, followed by *N*,*N*′-bis(3-aminopropyl)-ethylenediamine (N-3C-N-2C-N-3C-N) and *N*,*N*′-bis(2-aminoethyl)-1,3-propanediamine (N-2C-N-3C-N-2C-N). All three compounds were of efficacy similar to that of spermine and spermidine (approximately 9-fold stimulation). The compound 1,3-diaminopropane (N-3C-N) acted like a partial agonist, with 1 mM producing a maximum 3-fold enhancement of [^{3}H]TCP binding above basal level. The other diamine compounds tested did not stimulate binding at concentrations up to 1 mM (Fig. 2, Table 1). We also noticed that the compound 1,8-diaminooctane (N-8C-N) inhibited [^{3}H]TCP binding by 60%, in a concentration-dependent manner (Fig. 2). For comparative purposes, the EC_{50} values and structures of the 10 polyamines tested are given in Table 1.

In order to investigate the mechanism by which the polyamines enhance [^{3}H]TCP binding, we conducted both kinetic and equilibrium saturation experiments in the presence and absence of polyamines. Kinetic experiments (Fig. 3, Table 2) revealed that, under basal conditions, both the association and dissociation were better fit with a two-site model, with about 80% of the sites exhibiting slow kinetics. Spermidine (100 μM) produced a 6-fold increase in the slow pseudo-first-order on-rate (k_{obs}), without significantly altering the fast k_{obs} or the ratio of fast to slow binding components. However, in the presence of spermidine, the dissociation was better fit monoexponentially, with a value not significantly different from the slow off-rate under basal conditions (Table 2). Thus, the net effect of spermidine was an overall increase in the association rate and a decrease in the dissociation rate. This result is supported by the equilibrium saturation experiments shown in Fig. 4. Spermidine (100 μM) produced a 3- to 4-fold increase in the affinity without significantly altering the receptor density (control: K_D = 14.7 ± 2 nM, B_{max} = 4.33 ± 0.36 pmol/mg; 100 μM spermidine: K_D = 4.5 ± 0.1 nM, B_{max} = 4.27 ± 0.22 pmol/mg).

Also shown in Fig. 4 are typical saturation experiments with 300 μM putrescine in the presence and absence of 100 μM spermidine. In accordance with Figs. 2 and 5, putrescine (300 μM) did not significantly alter the K_D or B_{max} of basal [^{3}H]TCP binding (K_D = 16.7 ± 1.0 nM, B_{max} = 3.57 ± 0.11 pmol/mg); however, it inhibited the effect of 100 μM spermidine (100 μM spermidine plus 300 μM putrescine: K_D = 9.3 ± 0.8 nM, B_{max} = 4.12 ± 0.38 pmol/mg).

Some analogs with little or no intrinsic efficacy were tested as potential polyamine antagonists, using spermidine as the prototypic agonist. As shown in Fig. 5, putrescine and cadaverine, which were without any stimulatory effect, as well as the partial agonist 1,3-diaminopropane, inhibited spermidine-induced [^{3}H]TCP binding. Putrescine also inhibited spermine-

TABLE 1
Effect of natural and synthetic polyamines on [^{3}H]TCP binding

Drug	Abbreviation	EC_{50}	n_H	n^a
		μM		
N,N'-bis(3-Aminopropyl)-1,3-propanediamine	N-3C-N-3C-N-3C-N	0.78 ± 0.04	2.98 ± 0.35[b]	3
N,N'-bis(3-Aminopropyl)-ethylenediamine	N-3C-N-2C-N-3C-N	2.3 ± 0.72	1.69 ± 0.03[b]	3
N,N'-bis(3-Aminopropyl)-1,4-butanediamine (spermine)	N-3C-N-4C-N-3C-N	5.9 ± 0.35	2.85 ± 0.46[b]	5
N-(3-Aminopropyl)-1,4-butanediamine (spermidine)	N-3C-N-4C-N	15 ± 1.6	2.02 ± 0.16[b]	10
N,N'-bis(2-aminoethyl)-1,3-propanediamine	N-2C-N-3C-N-2C-N	36 ± 2.5	1.43 ± 0.31	3
1,3-Diaminopropane	N-3C-N	61 ± 14	1.60 ± 0.42	4
1,4-Butanediamine (putrescine)	N-4C-N	>1000[c]	N.D[d]	3
1,5-Pentanediamine (cadaverine)	N-5C-N	>1000[c]	N.D	5
1,7-Heptanediamine	N-7C-N	>1000[c]	N.D	3
1,8-Octanediamine	N-8C-N	*e*	N.D	3

[a] Number of experiments.
[b] Significantly different than 1.0 ($p < 0.05$).
[c] At concentration of up to 1 mM, these compounds had no significant effect.
[d] ND, not determined.
[e] 1,8-Octanediamine produced a maximal inhibition of [^{3}H]TCP binding of 60%, with an IC_{50} = 38.6 ± 10.9 μM.

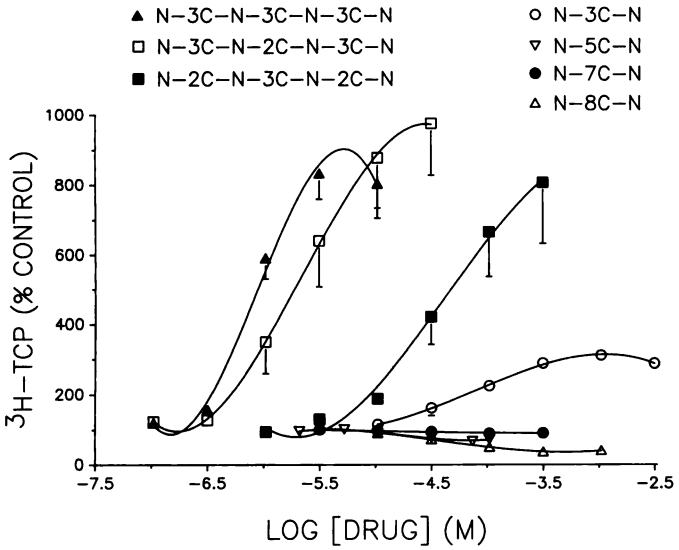

Fig. 2. Effect of polyamine analogs on [^{3}H]TCP binding. Each point is the mean ± standard error of three to five experiments performed in triplicate.

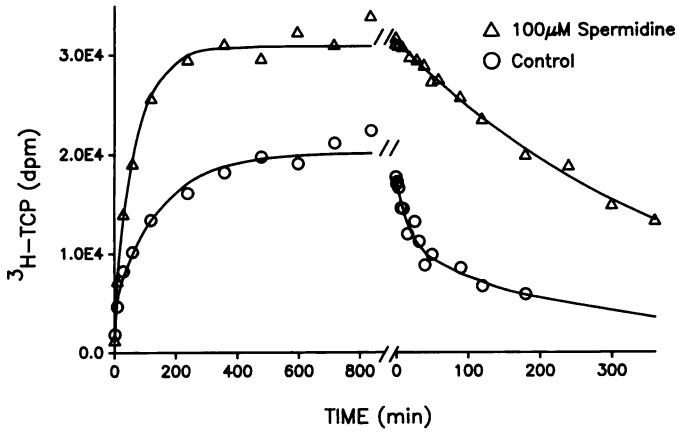

Fig. 3. Representative association and dissociation experiments of [^{3}H] TCP binding in the presence and absence of 100 μM spermidine. These experiments were replicated five to seven times, with identical results.

induced [^{3}H]TCP binding (data not shown). As a control for specificity, we tested GABA, a monoamine similar in size to putrescine. By itself, GABA did not alter [^{3}H]TCP binding and, as shown in Fig. 5D, 300 μM GABA did not inhibit spermidine-induced [^{3}H]TCP binding.

The apparent noncompetitive inhibition exhibited by 300 μM putrescine in Fig. 5 was further investigated over a range of concentrations. Fig. 6 shows that increasing concentrations of putrescine (0.1–1 mM) produced progressive inhibition of the maximal effect of spermidine on [^{3}H]TCP binding. At 1 mM putrescine, the stimulation was almost completely blocked. Finally, because each of the polyamine antagonists is a divalent cation like Mg^{2+}, we tested whether magnesium chloride also could block spermidine-induced [^{3}H]TCP binding. As shown in Fig. 7, increasing concentrations of $MgCl_2$ inhibited spermidine-induced [^{3}H]TCP binding in an apparent noncompetitive manner.

Discussion

In this study, we have confirmed the enhancement of [^{3}H] TCP binding by spermine and spermidine. Furthermore, we have extended that finding to include some synthetic polyamine analogs. The rank order of potency for enhancing [^{3}H]TCP binding was N-3C-N-3C-N-3C-N > N-3C-N-2C-N-3C-N > N-3C-N-4C-N-3C-N > N-3C-N-4C-N > N-2C-N-3C-N-2C-N. From this, it is postulated that the number of methyl groups that separate each of the nitrogen atoms is an important determinant of affinity. Also, at least three amino groups are needed for full efficacy. With the exception of 1,3-diaminopropane, which exhibited a partial agonistic effect, none of the diamines tested produced any stimulatory effect. Therefore, we suggest that, whereas the number of amino groups in this family of compounds may determine their efficacy in enhancing [^{3}H] TCP binding, the distance between these amino groups determines their potency. Further testing with spermidine analogs (three amino groups) and compounds with five amino groups is necessary to establish the validity of this hypothesis.

The steepness of the concentration-response curves is reflected by Hill coefficients greater than unity (Table 1). The reason for this steepness is not clear, but it is commonly interpreted as indicative of a positively cooperative process involving multiple receptor subunits. Although this is a possibility, this steepness could also result from an interaction with

TABLE 2

Kinetics of [^{3}H]TCP binding in the presence and absence of polyamines

Data represent mean ± standard error for five or six association experiments under each condition and six or seven dissociation experiments under each condition.

Treatment	k_{obs}	Amount	k_{off}	Amount
	$\times 10^{-3}$ min^{-1}	% of total	$\times 10^{-3}$ min^{-1}	% of total
Control				
Slow	4.2 ± 1.3	87 ± 3.6	2.0 ± 0.6	74 ± 3.4
Fast	311 ± 65	13 ± 1.6	79 ± 17	26 ± 3.4
Spermidine (100 μM)				
Slow	24 ± 7.7*	79 ± 5.2	2.9 ± 0.7	100*
Fast	497 ± 112	21 ± 4.9		
Spermidine (100 μM) + putrescine (300 μM)				
Slow	27 ± 8.3*	65 ± 8.1*	1.5 ± 0.5	59 ± 3.2*
Fast	322 ± 65	35 ± 8.1*	130 ± 16*	41 ± 3.2*

* Significantly different from control ($p < 0.05$).

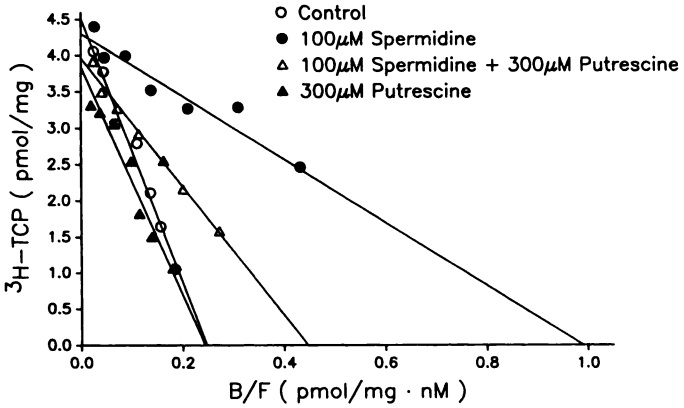

Fig. 4. Representative Eadie-Hofstee analysis of [^{3}H]TCP binding (17-hr incubation) in the absence or presence of 100 μM spermidine, 300 μM putrescine, or 100 μM spermidine plus 300 μM putrescine. Differences in K_D and B_{max} were analyzed by analysis of variance followed by multiple *t post hoc* test.

other positive effector sites in the complex. This possibility is supported by the previous demonstration that the EC_{50} for spermidine-enhanced [^{3}H]MK 801 binding was decreased 3- to 5-fold by the addition of glycine or glutamate, respectively (8). It was also observed that the concentration-response curves for stimulation by spermine and *N,N'*-bis(3-aminopropyl)-1,3-propanediamine are steeper than that for spermidine. This is probably because the former two polyamines, but not the latter, also enhance binding to the strychnine-insensitive glycine receptor (16). This effect (a 3-fold increase in [^{3}H]glycine binding affinity) would increase [^{3}H]TCP binding by increasing the effective interaction between the polyamine site and the glycine site activated by the residual glycine present in membrane preparations of this sort. It is possible that this effect of spermine on glycine binding partially accounts for the 2.5-fold greater potency of spermine relative to spermidine.

Interestingly, the partial agonist 1,3-diaminopropane increased [^{3}H]TCP binding, with a Hill coefficient not different from unity. This suggests that positive cooperativity is not intrinsic to the spermidine site per se but, rather, is dependent on its interaction with other effector sites, as suggested above. The partial agonist properties of 1,3-diaminopropane suggest that the site that mediates the effect of polyamines on [^{3}H]TCP binding contains a pocket, possibly anionic in nature, that is exactly fit by two amino groups separated by three methyl groups. This is supported by the observation that spermidine and each of the spermine analogs tested contained a 1,3-diaminopropane moiety and that diamines with amino groups separated by four, five, seven, or eight methyl groups were without agonistic activity. Interestingly, the butane and pentane analogs were antagonists (the heptane and octane analogs were not tested). However, it is important to recall from Figs. 4 and 6 that these antagonists are not competitive inhibitors, thus making further inference about the structure of this putative receptor impossible.

Both kinetic and equilibrium saturation experiments revealed an increase in the affinity of [^{3}H]TCP binding in the presence of spermidine. This decrease in K_D is interpreted as a true change in the conformation of the PCP binding site. Thus, the mechanism of action of the polyamines is distinct from that previously reported for either glutamate or glycine on the kinetics of [^{3}H]TCP binding (17–19). It has been postulated that the two components of association and dissociation of [^{3}H]MK-801 binding represent diffusion through hydrophobic (slow) and hydrophilic (fast) pathways, where the latter path is the open NMDA channel (19). If this interpretation is applied to our kinetic data, spermidine increases the rate of [^{3}H]TCP binding through the hydrophobic path. Whether this path represents diffusion through the lipid bilayer or through hydrophobic domains of the closed channel is unclear. Nevertheless, it suggests that this path is under physiological (or at least pharmacological) control. At this point, it is unknown whether this effect of spermidine is actually associated with an increase in NMDA-induced current flow. It is conceivable that spermidine would not enhance NMDA function, because, unlike glutamate and glycine, it does not increase the ratio of fast to slow binding components (19). In fact, if the increase in the rate of hydrophobic binding is a reflection of increased diffusion through a path, such as the lipid bilayer, that does not conduct current, then spermidine would be predicted to increase inhibition of NMDA-induced ion flux by either exogenous or endogenous PCP-like ligands.

Spermidine also produced a complete loss of the small (20%) fast component of dissociation. This suggests that the hydrophilic path is also under regulation by spermidine. This could occur as a result of the conformational alterations in the channel induced by changes in the hydrophobic pathway. This model is valid only if the slow and fast binding rates truly reflect diffusion through hydrophobic and hydrophilic path-

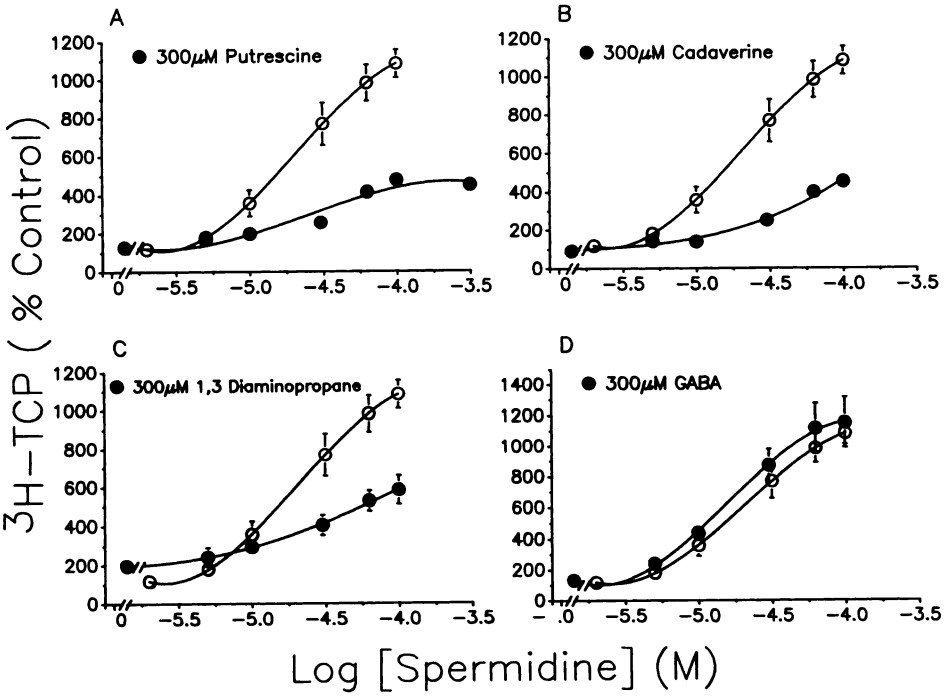

Fig. 5. Effect of putrescine, cadaverine, 1,3-diaminopropane, and GABA on spermidine-induced [^{3}H]TCP binding. Each point is the mean ± standard of error of three experiments performed in triplicate.

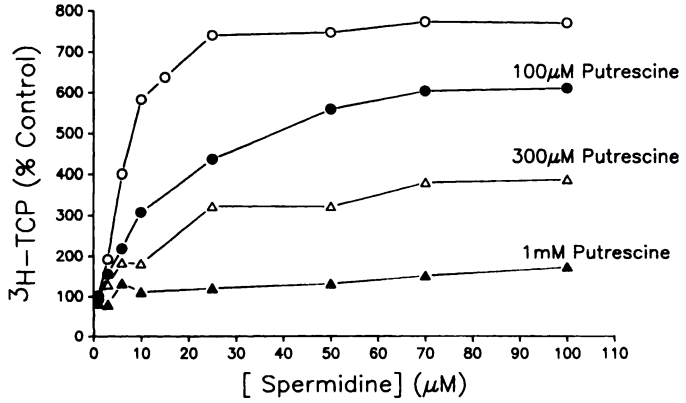

Fig. 6. Representative experiment of the inhibition of spermidine-induced [^{3}H]TCP binding by increasing concentrations of putrescine. The experiment was done in triplicate and was repeated two more times with identical results.

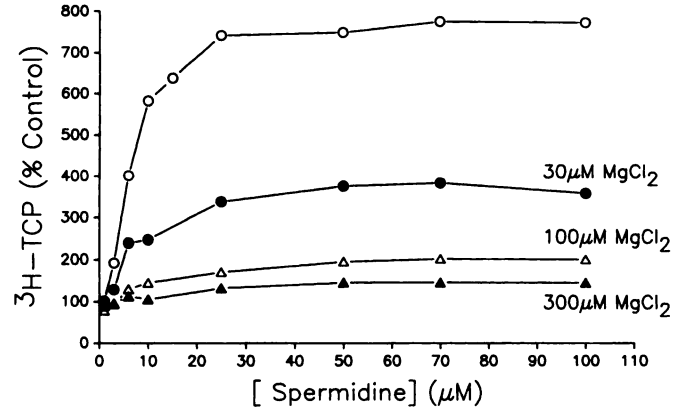

Fig. 7. Representative experiment of the inhibition of spermidine-induced [^{3}H]TCP binding by increasing concentrations of magnesium chloride. The experiment was done in triplicate and was repeated two more times with identical results.

ways, respectively. However, the different binding rates could also reflect either conductance through two different types of channels or two conductance states of the same channel. In both cases, spermidine would be expected to alter current flow mediated upon the activation of the NMDA receptor.

In that spermidine and other polyamines that stimulate [^{3}H]TCP binding are cationic, it is possible that this effect is related to the ability of Mg^{2+} to stimulate binding under certain conditions (20). Even though Mg^{2+} and other divalent cations such as Mn^{2+} and Co^{2+} block the NMDA channel in a voltage-dependent manner, in the nominal absence of glutamate and glycine they stimulate and then inhibit binding in a concentration-dependent manner (20). Using single-site analysis of [^{3}H]MK-801 binding, the stimulation is apparently due to an increase in both association and dissociation rate constants (21). Thus, the stimulatory effect of spermidine can be distinguished from that of Mg^{2+} on kinetic grounds, in that spermidine did not increase [^{3}H]TCP dissociation; in fact, dissociation was slowed by spermidine.

In the presence of glutamate and glycine, Mg^{2+} and other divalent cations do not stimulate binding of either [^{3}H]TCP or [^{3}H]MK-801 (20, 22). Further, the IC_{50} values for inhibition, calculated from the inhibitory portion of the biphasic effect of cations in the nominal absence of glutamate and glycine, are dramatically decreased in their presence (22). Interestingly, spermidine, like glutamate and glycine, converted the stimulatory effects of low Mg^{2+} concentrations (20) into inhibitory effects (Fig. 7). One interpretation of these data is that the stimulatory effects of Mg^{2+} and spermidine are mediated by the same site and that 100 μM spermidine fully occupies this site, thus masking the stimulatory effects of Mg^{2+} and revealing its inhibitory effects. Furthermore, because both putrescine and Mg^{2+} are divalent cations and both noncompetitively inhibited spermidine-induced binding (Fig. 7), it is possible that putrescine and Mg^{2+} share the same site for inhibition. Additional studies are needed to verify these hypotheses.

The fact that the inhibitory effects of putrescine, cadaverine, and 1,3-diaminopropane on spermidine-induced [^{3}H]TCP binding are not competitive suggests that the diamines do not act at the same site at which spermidine and spermine produce their effect. This suggests that the effect of putrescine and the other diamines is not specific for inhibiting polyamine-induced [^{3}H]TCP binding. In fact, putrescine also inhibits glutamate- and glycine-induced [^{3}H]TCP binding.[1] This argues that putrescine and the other diamines may affect the channel in a manner opposite to that of glutamate, glycine, or spermidine. Why the predicted effect of putrescine in the nominal absence of glutamate, glycine, and spermidine is not observed is not yet clear. However, if Mg^{2+} and putrescine share the same inhibitory site, it may be that putrescine, like magnesium and some other divalent cations, becomes a more effective inhibitor when the channel is activated (20, 22).

The finding that putrescine inhibited spermidine-induced [^{3}H]TCP binding (Fig. 5) is potentially quite interesting from a physiological standpoint. First, it suggests that the ratio of spermidine to putrescine could play a role in determining the net effect of these compounds on the modulation of the NMDA receptor channel complex. Second, the finding that NMDA produces a transient increase in ODC activity and, consequently, a rise in the concentration of the different polyamines (10) suggests that activation of the NMDA receptor can regulate the concentration of putrescine and then, depending on the extent of metabolism of putrescine to spermidine and spermine, the relative concentration of polyamines could ultimately lead to either an activation or an inhibition of the NMDA ionophore. Not only is spermidine formed from putrescine, spermidine can be transformed to putrescine by an interconversion pathway (23). This suggests that the activity of this pathway could serve as a mechanism for terminating the action of spermidine.

In summary, we determined that both the number of amino groups and the separation between them play an important role in determining polyamine activation of the NMDA receptor ionophore. 1,3-Diaminopropane appears to be the minimal structure required for activation. Further, it was determined that Mg^{2+} and certain divalent polyamine cations, including putrescine, inhibit spermidine-induced [^{3}H]TCP binding in a noncompetitive fashion. Spermidine produced an increase in the affinity of [^{3}H]TCP binding to its site presumably by increasing and decreasing the association and dissociation rates, respectively. Finally, the divalent character of polyamines may play a role in the activation of the ionophore, but at this time the precise relationship between the mechanism and sites of activation for Mg^{2+} and spermidine is unknown and will require additional work for clarification.

[1] Sacaan, A. I. and K. M. Johnson. Regulation of polyamine inhibition of the NMDA receptor ionophase complex. Manuscript in preparation.

References

1. Mayer, M. L., and G. L. Westbrook. The action of *N*-methyl-D-aspartic acid on mouse spinal neurons in culture. *J. Physiol. (Lond.)* **361**:965–990 (1985).
2. MacDermott, A. B., M. L. Mayer, and G. L. Westbrook. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurons. *Nature (Lond.)* **321**:519–522 (1986).
3. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature (Lond.)* **325**:529–531 (1987).
4. Snell, L. D., and K. M. Johnson. Characterization of the inhibition of excitatory amino acid-induced neurotransmitter release in the rat striatum by phencyclidine-like drugs. *J. Pharmacol. Exp. Ther.* **238**:938–946 (1986).
5. Honey, C. R., Z. Miljkovic, and J. F. MacDonald. Ketamine and phencyclidine cause a voltage-dependent block of responses to L-aspartic acid. *Neurosci. Lett.* **61**:135–139 (1985).
6. Mayer, M. L., G. L. Westbrook, and P. B. Guthrie. Voltage-independent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature (Lond.)* **309**:261–263 (1984).
7. Nowak, L., P. Bregestovski, P. Ascher, A. Herbert, and A. Prochiantz. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature (Lond.)* **307**:462–465 (1984).
8. Ransom, R. W., and N. L. Stec. Cooperative modulation of [^{3}H]-MK801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **51**:830–836 (1988).
9. Shaw, G. G., and A. J. Pateman. The regional distribution of the polyamines spermidine and spermine in brain. *J. Neurochem.* **20**:1225–1230 (1973).
10. Siddiqui, F., Z. Iqbal, and H. Koenig. Polyamine dependence of NMDA receptor-mediated Ca^{+2} fluxes and transmitter release from rat hippocampus. *Soc. Neurosci. Abstr.* **14**:1048 (1988).
11. Jones, S. M., L. D. Snell, and K. M. Johnson. Characterization of the binding of radioligands to the *N*-methyl-D-aspartate, phencyclidine, and glycine receptors in buffy coat membranes. *J. Pharmacol. Methods* **21**:161–168 (1989).
12. Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**:76–85 (1985).
13. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for the characterization of ligand binding systems. *Anal. Biochem.* **107**:220–239 (1980).
14. McPherson, G. A. Analysis of radioligand binding experiments: a collection of computer programs for the IBM PC. *J. Pharmacol. Methods* **14**:213–218 (1985).
15. DeLean, A., P. J. Munson, and D. Rodbard. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves. *Am. J. Physiol.* **235**:E97-E102 (1978).
16. Sacaan A. I., and K. M. Johnson. Spermine enhances binding to the glycine site associated with the *N*-methyl-D-aspartate receptor complex. *Mol. Pharmacol.*, **36**:836–839 (1989).
17. Kloog, Y., R. Haring, and M. Sokolovsky. Kinetic characterization of the phencyclidine-*N*-methyl-D-aspartate receptor interaction: evidence for a steric blockade of the channel. *Biochemistry* **27**:843–848 (1988).
18. Bonhaus, D. W., and J. O. McNamara. *N*-Methyl-D-aspartate receptor regulation of uncompetitive antagonist binding in rat brain membranes: kinetic analysis. *Mol. Pharmacol.* **34**:250–255 (1988).
19. Javitt, D. C., and S. R. Zukin. Biexponential kinetics of [^{3}H]-MK-801 binding: evidence for access to closed and open *N*-methyl-D-aspartate receptor channels. *Mol. Pharmacol.* **35**:387–393 (1989).
20. Johnson, K. M., L. D. Snell, A. I. Sacaan, and S. Jones. Pharmacological regulation of the phencyclidine-binding site associated with the *N*-methyl-D-aspartate receptor-operated ion channel. *Drug Dev. Res.* **17**:281–297 (1989).
21. Reynolds, I. J., and R. J. Miller. Multiple sites for the regulation of the *N*-methyl-D-aspartate receptor. *Mol. Pharmacol.* **33**:581–584 (1988).
22. Reynolds, I. J., and R. J. Miller. [^{3}H]MK-801 binding to the NMDA receptor/ionophore complex is regulated by divalent cations: evidence for multiple regulatory sites. *Eur. J. Pharmacol.* **151**:103–112 (1988).
23. Seiler, N. Inhibition of enzymes oxidizing polyamines, in *Inhibition of Polyamine Metabolism* (P. P. McCann, A. E. Pegg, and A. Sjoerdsma, eds.). Academic Press Inc., Orlando, Florida, 49–77 (1987).

Send reprint requests to: Kenneth M. Johnson, Ph.D., Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.