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Competitive Inhibition of Magnesium-Induced [^{3}H]*N*-(1-[Thienyl] cyclohexyl)piperidine Binding by Arcaine: Evidence for a Shared Spermidine-Magnesium Binding Site

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

The polyamine competitive antagonist arcaine (1,4-diguanidinobutane) produced complete inhibition of basal [^{3}H]*N*-(1-[thienyl] cyclohexyl)piperidine ([^{3}H]TCP) binding, with an IC_{50} value of 4.52 ± 0.93 μM. Arcaine (5 and 10 μM) produced a decrease in the affinity without a significant change in the receptor density of [^{3}H]TCP binding under equilibrium conditions. In addition, arcaine did not alter either *N*-methyl-D-aspartate-specific [^{3}H] glutamate or strychnine-insensitive [^{3}H]glycine binding. Furthermore, increasing concentrations of arcaine produced parallel rightward shifts in the concentration-response curves for both spermidine- and magnesium-induced [^{3}H]TCP binding, suggesting that arcaine is a competitive inhibitor of both agonists. Similar rightward shifts were observed for barium- and strontium-induced [^{3}H]TCP binding. In contrast, arcaine decreased the efficacy of glutamate- and glycine-induced [^{3}H]TCP binding without changing their EC_{50} values, indicating a noncompetitive type of inhibition. These results imply that spermidine and certain divalent cations including magnesium share the same mechanism for enhancing [^{3}H]TCP binding, whereas glutamate and glycine have different sites of action. This is further supported by the additive effect of spermidine when tested in the presence of maximal concentrations of glutamate and glycine. On the other hand, spermidine and magnesium were not additive and, in fact, magnesium was able to block the effects of spermidine under certain conditions. The possibility that magnesium is a partial agonist at the polyamine site is discussed.

The NMDA receptor channel complex is thought to be regulated by several inhibitory and stimulatory effectors. These include PCP acting at a site inside the channel (1), divalent cations such as Mg^{2+} (2) and Zn^{2+} (3), glycine acting at a strychnine-insensitive binding site (4), and the polyamines spermine and spermidine (5). The presence of this novel polyamine binding site is supported by work showing a strict structural requirement for the full activation of this site (6, 7).

In [^{3}H]TCP and [^{3}H]MK 801 binding experiments, both the inorganic divalent cation Mg^{2+} and the organic divalent cation spermidine produced bell-shaped dose-response curves, with Mg^{2+} being less efficacious (8, 9). This biphasic effect is indicative of two independent processes. We have previously provided evidence that the inhibitory effects of Mg^{2+} could be related to those produced by putrescine (7). In this study we have investigated the possibility that magnesium and spermidine share the same mechanism of action. This was facilitated by the discovery of arcaine, a competitive polyamine antagonist (10). We report that arcaine competitively antagonized both spermidine- and magnesium-induced [^{3}H]TCP binding. Conversely, arcaine inhibited glutamate- and glycine-induced [^{3}H] TCP binding in a noncompetitive manner. These results indicate that Mg^{2+}, but not glutamate or glycine, acts at the spermidine site. Other evidence supporting this hypothesis also will be presented.

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Experimental Procedures

Materials. [^{3}H]TCP (47.8 Ci/mmol), L-[^{3}H]glutamate (54.7 Ci/mmol), and [^{3}H]glycine (53.3 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Spermidine and arcaine were purchased from Sigma Chemical Company (St. Louis, MO). L-Glutamate and glycine were purchased from Aldrich Chemical Company (Milwaukee, WI).

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

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; TCP, *N*-(1-[thienyl]cyclohexyl)piperidine; MK 801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine; HEPES, 4-(2-hydroxyphenyl)-1-piperazineethanesulfonic acid).

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).

Binding assays. [^{3}H]TCP was incubated at 2.5 nM with approximately 200 μg of protein/ml of 10 mM HEPES (pH 7.4), in the presence or absence of the test drug dissolved in buffer, 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%. Equilibrium experiments were conducted as previously described (7), using seven concentrations of [^{3}H]TCP ranging from 2.5 to 162.5 nM, and incubated for 17 hr in the presence and absence of the test drug. TCP (3 μM) was used to define nonspecific binding. Specific binding of 2.5 nM [^{3}H]TCP defined in this manner was identical to that defined with 30 μM PCP.

NMDA-specific [^{3}H]glutamate binding and strychnine-insensitive [^{3}H]glycine binding assays were done as previously described (11).

Statistical analysis. All experiments were replicated three to seven times. IC_{50} values were estimated by an iterative nonlinear regression program (13), as adapted for the IBM PC (14). Equilibrium K_D and B_{max} values for saturation isotherms were estimated by the same iterative nonlinear regression program (13, 14), using the untransformed data. However, for ease of visual interpretation, these data are presented in an Eadie-Hofstee format. 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). The dissociation constant for a partial agonist, K_p, was calculated according to the equation $K_p = K_A(A_p - A_i) \cdot P_i/(A_p + K_A) \cdot A_i$, where K_A is the dissociation constant for the full agonist and A_p is the concentration of full agonist that produces a response equal to the maximum produced by the partial agonist (16). P_i and A_i are any pair of concentrations of P and A that produce equal effects (here chosen to be 300% of control). Results were analyzed by Student's *t* test or one-way analysis of variance, with Tukey's *post hoc* tests where appropriate. Values of $p < 0.05$ were considered significantly different from control.

Results

Arcaine produced complete inhibition of basal [^{3}H]TCP binding, with an $IC_{50} = 4.52 \pm 0.93$ μM and a Hill coefficient of 0.85 ± 0.1 (Fig. 1). This effect is presumably due to antagonism of the residual endogenous spermidine agonists found in the membranes even after extensive washing and lysing, suggesting the possibility that polyamine receptor occupation may be required for full activation of the NMDA response in this system. We have previously reported that spermidine increased the affinity of TCP for its binding site by increasing and decreasing the on- and off-rates, respectively (7). Fig. 2 shows that, at equilibrium (17-hr incubation), arcaine decreased the affinity of [^{3}H]TCP for its binding site without significantly changing the receptor density [control (six experiments): $K_D = 30.8 \pm 2.3$ nM, $B_{max} = 3.8 \pm 0.3$ pmol/mg of protein; 5 μM arcaine (seven experiments): $K_D = 55.6 \pm 7.5$ nM, $B_{max} = 4.4 \pm 0.3$ pmol/mg of protein; 10 μM arcaine (three experiments): $K_D = 81.9 \pm 7.4$ nM, $B_{max} = 3.8 \pm 0.9$ pmol/mg of protein].

Arcaine did not alter either NMDA-specific [^{3}H]glutamate or strychnine-insensitive [^{3}H]glycine binding, as shown in Fig. 3. This argues against a direct interaction of arcaine at either the NMDA or the glycine recognition sites. Reynolds (10) has shown that spermidine decreased the potency of arcaine displacement of [^{3}H]MK 801 binding. We have confirmed this finding by showing that increasing concentrations of arcaine produced a parallel rightward shift in the EC_{50} for spermidine-induced [^{3}H]TCP binding (Fig. 4 and Table 1). We also utilized this approach to compare the effects of arcaine against mag-

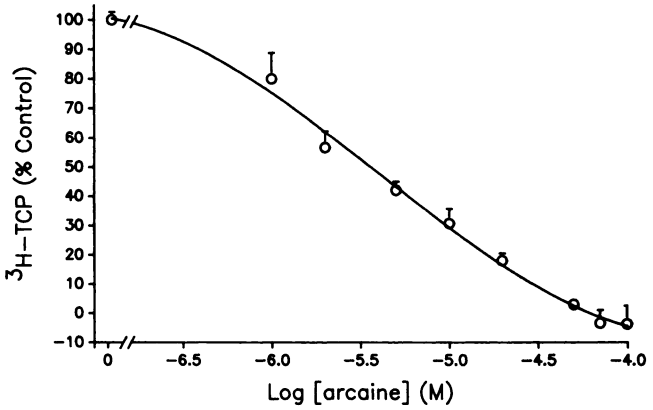

Fig. 1. Displacement of basal [^{3}H]TCP binding by arcaine. Under these conditions (2.5 nM [^{3}H]TCP, 0.1–0.2 mg of protein/ml, 2-hr incubation), total binding was about 2000 dpm, with about 750 dpm being nonspecific. Each *point* represents the mean ± standard error of three or four experiments performed in triplicate.

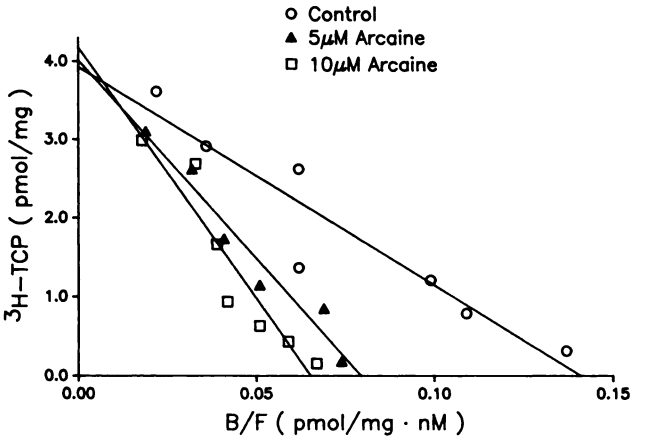

Fig. 2. Representative Eadie-Hofstee analysis of [^{3}H]TCP binding at equilibrium (17-hr incubation) in the absence and presence of 5 or 10 μM arcaine. Differences in K_D and B_{max} were evaluated by analysis of variance followed by Tukey's *post hoc* tests. (See results for means and standard errors for each condition studied).

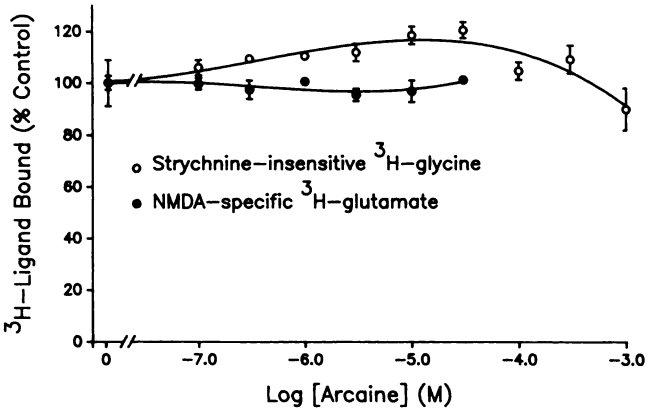

Fig. 3. Effect of arcaine on NMDA-specific [^{3}H]glutamate binding and strychnine-insensitive [^{3}H]glycine binding. Each *point* represents the mean ± standard error of three experiments performed in triplicate.

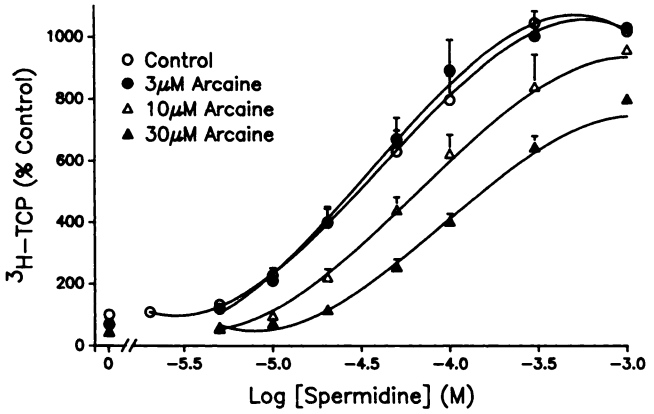

Fig. 4. Effect of increasing concentrations of spermidine on [^{3}H]TCP binding in the presence and absence of 3, 10, or 30 μM arcaine. Each *point* represents the mean ± standard error of four to seven experiments performed in triplicate.

nesium-, glutamate-, and glycine-induced [^{3}H]TCP binding. As observed when tested against spermidine, 30 μM arcaine produced a similar rightward parallel shift in the concentration-response curve for magnesium-induced [^{3}H]TCP binding (Fig. 5 and Table 1). Assuming magnesium is a partial agonist (see below), its dissociation constant was calculated to be 115 μM, a value slightly below its EC_{50} value (Table 1). These data, taken together, suggest that the stimulatory effects of magnesium and spermidine are being mediated via the same binding site. In contrast, arcaine decreased the maximal effect but not the potency of both glutamate- and glycine-induced [^{3}H]TCP binding (Figs. 6 and 7 and Table 1). This strongly supports the hypothesis that arcaine does not interact directly at the NMDA or the glycine recognition sites.

In addition to magnesium, several other divalent cations have been shown to affect either [^{3}H]TCP or [^{3}H]MK 801 binding in a biphasic manner (8, 17). Therefore, in order to strengthen the argument that spermidine and magnesium act at the same site to stimulate [^{3}H]TCP binding, we determined the effect of 30 μM arcaine on the stimulatory actions of barium and strontium, two cations with presumably the same site of action as magnesium. Arcaine produced a similar parallel rightward shift in the concentration-response curve for both cations (Fig. 8).

As can be seen in Figs. 4, 5, and 8, arcaine, in addition to producing a parallel rightward shift in the concentration-response curve for spermidine, magnesium, barium, and strontium, also appears to significantly diminish the maximal response to these agonists. This would be classically interpreted as mixed inhibition. An alternative interpretation is that this is the expected inhibition pattern for an inhibitor acting only on the ascending limb of a bell-shaped dose-response curve. Assuming that the dissociation constants of spermidine acting at the stimulatory and inhibitory sites differed by a factor of about 500[1] and that arcaine acts only at the stimulatory site with an approximate K_i of 5 μM (Fig. 2), we constructed the complete theoretical dose-response curve in the presence and absence of arcaine (Fig. 9). For comparison, we reproduced the experimental data for spermidine in the presence and absence of arcaine, shown in Fig. 4. In both the experimental and theoretical curves, arcaine, in addition to producing a rightward shift, also appears to diminish the maximal response. This occurs despite the fact that the theoretical binding equation describes arcaine as a classic competitive inhibitor (see legend to Fig. 9).

We also tested the effect of spermidine in the presence of maximally effective concentrations of either magnesium or glutamate and glycine. The data in Fig. 10 demonstrate that spermidine does not enhance [^{3}H]TCP binding above that induced by a saturating concentration of $MgCl_2$ (3 mM). On the other hand, spermidine produced an additive effect with saturating concentrations of both glutamate and glycine. This supports the notion that spermidine and magnesium act at the same binding site, whereas glutamate and glycine act at unique sites to enhance [^{3}H]TCP binding.

Finally, we addressed the hypothesis inferred from the data in Figs. 4 and 5, that magnesium may be a partial agonist at the spermidine site. Also shown in Fig. 10 are concentration-response curves for spermidine in the absence and presence of magnesium at several concentrations. All magnesium concentrations were stimulatory alone, producing a maximal stimulation of about 50% of that produced by spermidine. $MgCl_2$ at 100 μM did not alter the maximal effect of spermidine on [^{3}H]TCP binding. However, at 3 mM magnesium, spermidine was unable to stimulate binding above that caused by magnesium alone; 300 μM magnesium produced an intermediate effect.

Discussion

In well washed rat cortical membranes, arcaine produced a complete inhibition of [^{3}H]TCP binding (Fig. 1). The Hill coefficient for inhibition was not significantly different from unity, suggesting that arcaine acts at a single site. Presumably, the inhibition of [^{3}H]TCP binding is due to inhibition of the effect of residual spermidine or magnesium in the membrane preparation. In equilibrium saturation experiments, arcaine produced a decrease in the affinity of [^{3}H]TCP for its site without affecting the receptor density. This might indicate a

[1] Sacaan and Johnson, unpublished data.

TABLE 1

Effect of arcaine on spermidine; $MgCl_2$-, L-glutamate- and glycine-induced [^{3}H] TCP binding

Drug	EC_{50} for enhancing [^{3}H]TCP binding			
	0[a]	3 μM[a]	10 μM[a]	30 μM[a]
	μM			
Spermidine	26.3 ± 3.4	34.1 ± 6.7	47.9 ± 3.9	104 ± 16.6[b]
$MgCl_2$	252 ± 26	269 ± 64	311 ± 63	1212 ± 343[b]
L-Glutamate	0.23 ± 0.07	0.19 ± 0.09	0.13 ± 0.04	ND[c]
Glycine	0.12 ± 0.02	0.14 ± 0.04	0.15 ± 0.02	ND

[a] Concentration of arcaine.
[b] Significantly different from control, $p < 0.05$, by analysis of variance and Tukey *post hoc* tests.
[c] ND, not determined.

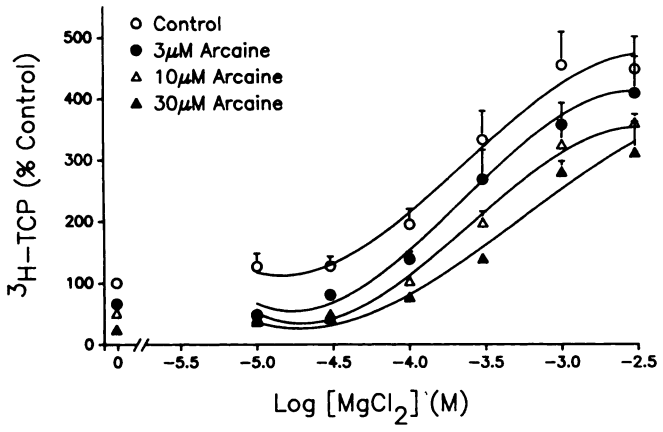

Fig. 5. Effect of increasing concentrations of $MgCl_2$ on [^{3}H]TCP binding in the presence and absence of 3, 10, or 30 μM arcaine. Each *point* represents the mean ± standard error of four to seven experiments performed in triplicate.

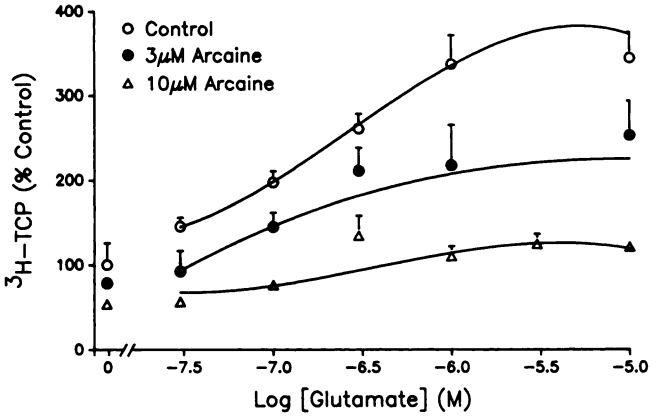

Fig. 6. Effect of increasing concentrations of glutamate on [^{3}H]TCP binding in the presence and absence of 3 or 10 μM arcaine. Each *point* represents the mean ± standard error of three experiments performed in triplicate.

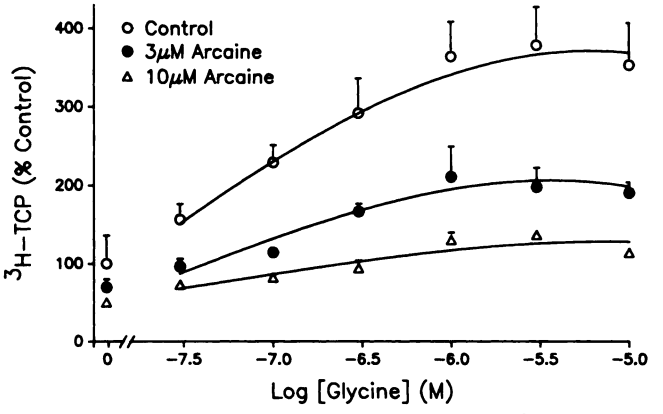

Fig. 7. Effect of increasing concentrations of glycine on [^{3}H]TCP binding in the presence and absence of 3 or 10 μM arcaine. Each *point* represents the mean ± standard error of three experiments performed in triplicate.

direct effect of arcaine at the PCP site. However, inasmuch as arcaine appears to be a competitive spermidine antagonist (Ref. 10 and Fig. 4), it is postulated that, because spermidine increases [^{3}H]TCP binding affinity (7), antagonism of this effect by arcaine would result in an apparent decrease in the affinity of [^{3}H]TCP binding. Moreover, it is unlikely that arcaine acts directly at the PCP/MK 801 recognition site, because spermi-

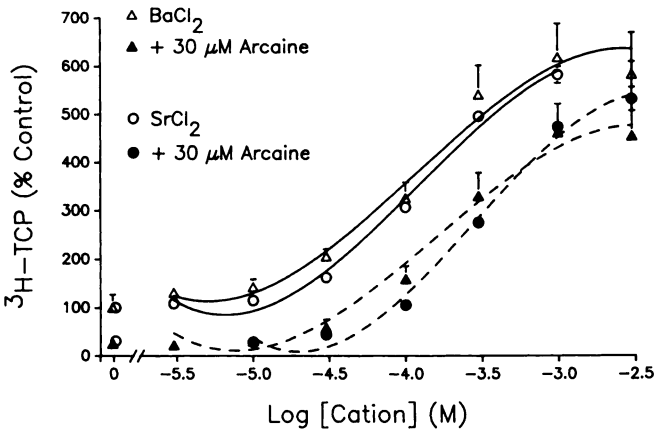

Fig. 8. Inhibition of barium- and strontium-induced [^{3}H]TCP binding by arcaine. Each data *point* is the mean ± standard error of three independent determinations, each in triplicate.

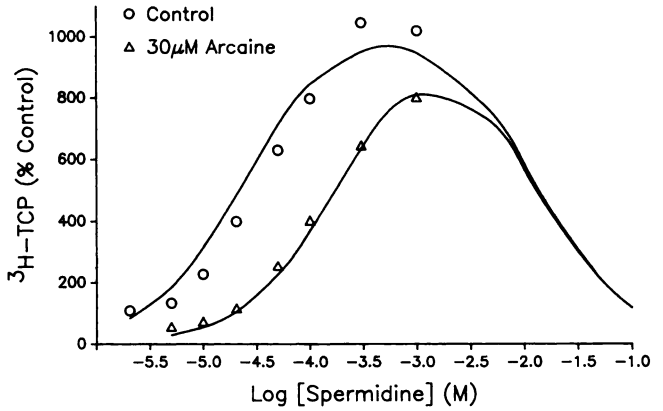

Fig. 9. Comparison of the experimental (*open symbols*) and theoretical (*solid lines*) effects of arcaine on spermidine-induced [^{3}H]TCP binding. The theoretical data are derived from the assumptions that spermidine has a bell-shaped dose-response curve (6)[1] and that the effect of arcaine is competitive and is selective against the mechanism underlying only the ascending limb of the curve. The theoretical control curve was drawn using the equation $B = B_{max}[S/(K_A + S) - S/(K_B + S)]$, whereas that in the presence of arcaine was $B_i = B_{max}\,[S/(K_A\,(1 + i/K_i) + S) - S/(K_B + S)]$; in both, B_{max} was arbitrarily set to 1100% of control. Other parameter estimates were based on data from this laboratory, i.e., K_A for spermidine stimulation = 26 μM, K_B for spermidine inhibition = 13 mM, K_i for arcaine = 4.5 μM, and i = 30 μM (the concentration of arcaine).

dine, which increases the affinity of TCP for its site (7), decreases arcaine's potency for displacing [^{3}H]MK 801 binding (10).

The finding that arcaine produced a complete inhibition of [^{3}H]TCP binding suggests an obligatory role for polyamines in the full expression of NMDA responses. This is supported by the following observations. First, inasmuch as arcaine has no activity at either the NMDA or the glycine recognition sites (Fig. 3), the most reasonable hypothesis is that arcaine acts solely at the polyamine site to decrease NMDA channel activation. Second, arcaine produced complete inhibition of NMDA-evoked [^{3}H]norepinephrine release from the rat hippocampus in a manner that was reversed upon the addition of spermidine.[2]

The mechanism by which magnesium and other divalent

[2] A. I. Sacaan, and K. M. Johnson. Spermidine reverses arcaine's inhibition of *N*-methyl-D-aspartate-induced hippocampal [^{3}H]NE release. Submitted for publication.

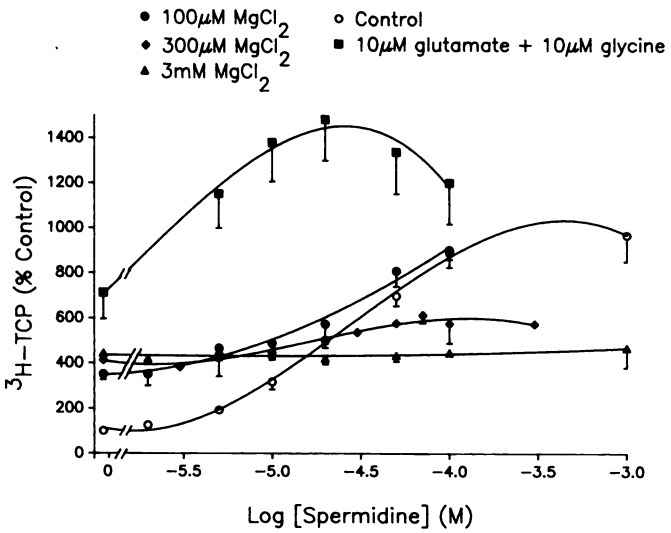

Fig. 10. Effect of increasing concentrations of spermidine in the presence and absence of 0.1, 0.3, or 3 mM $MgCl_2$ or 10 μM glutamate plus 10 μM glycine. Each *point* represents the mean ± standard error of three to seven experiments performed in triplicate.

cations interact with the NMDA ionophore is quite complex. Physiological concentrations of $MgCl_2$ block NMDA-mediated responses in a voltage-dependent manner (2). In binding studies, magnesium ions inhibit [^{3}H]TCP binding in the unwashed crude synaptosomal preparation (18), whereas in the well washed membrane preparation low concentrations of Mg^{2+} stimulate and higher concentrations inhibit [^{3}H]TCP binding (8). To date, the exact mechanism(s) and site(s) of action of magnesium are still debatable. In this study we have provided evidence that the stimulatory effects of several divalent cations could be mediated by an action at the stimulatory spermidine site. Arcaine produced a rightward parallel shift in the EC_{50} values for spermidine-, barium-, strontium-, and magnesium-induced [^{3}H]TCP binding (Figs. 4, 5, and 8). This suggests a competitive type of inhibition against each of these agonists. Alternatively, if spermidine (a cation at physiological pH) and the inorganic cations are acting at two different yet allosterically coupled binding sites, then a change in the affinity of the spermidine site (which would theoretically be induced by arcaine) would also result in an apparent affinity change for the magnesium site. However, the ability of arcaine to completely inhibit [^{3}H]TCP binding in the presence of spermidine (data not shown) argues for a classic competitive mechanism, rather than an allosteric or partially competitive mechanism.

It is also apparent in Figs. 4, 5, and 8 that there is a trend towards a decrease in the maximum response with increasing concentrations of arcaine; however, it is thought that this effect arises from the superimposition of a low affinity inhibitory action of spermidine or magnesium onto the high affinity site mediating enhancement of binding, as previously suggested (10). This interpretation is completely supported by the theoretical curves shown in Fig. 9. A shared spermidine-magnesium binding site is also supported by the work of Schoemaker *et al.* (19), who have shown that $MgCl_2$ displaces the binding of [^{3}H] ifenprodil, a ligand that putatively labels the spermidine site.

The uniqueness of the site of action for spermidine and magnesium stimulation is also supported by the lack of additivity between these two agonists (Fig. 10). Spermidine failed to enhance [^{3}H]TCP binding above that induced by a saturating concentration of magnesium. However, spermidine was able to enhance [^{3}H]TCP binding above that induced by saturating concentrations of glutamate and glycine (Fig. 10). Further, when magnesium is added to a maximally effective concentration of spermidine (100 μM), inhibition rather than stimulation is observed (Fig. 10). This phenomenon is similar to that observed when magnesium was incubated in the presence of NMDA and glycine (8). One possible explanation is that activation of the ionophore by either spermidine or NMDA and glycine alters the conformation of the inhibitory magnesium site within the channel in such a way that its binding affinity is greatly increased and only inhibition, even at low concentrations, is observed. In fact, in a crude synaptosomal membrane preparation, in which the concentrations of endogenous glutamate, glycine, and spermidine are relatively high, the IC_{50} value for magnesium inhibition of [^{3}H]TCP binding is 150 μM,[1] about 2-fold less than its EC_{50} value for stimulation in a well washed preparation (Table 1).

The reason why spermidine is unable to stimulate [^{3}H]TCP binding in the presence of a saturating concentration of magnesium (3 mM) may also be related to the possibility that magnesium is a partial agonist at the spermidine site. That is, even though the stimulation produced by magnesium is less than half that observed in the presence of spermidine (Figs. 4 and 5), the percentage of receptors occupied may be very high, as is the case for many agonists with low intrinsic activity. This would effectively prevent the effect of 100 μM spermidine, as shown in Fig. 8. On the other hand, when the magnesium concentration is dropped to 300 μM the magnitude of inhibition is reduced and it is virtually absent when the concentration of magnesium is reduced below that of its dissociation constant. However, this behavior is not what would be expected of a classical agonist/partial agonist interaction; that is, one should observe, as with any competitive inhibitor, a surmountable inhibition. We postulate that this is not observed for two reasons. First, as with arcaine, magnesium tends to shift the concentration-response curve into a region where spermidine is inhibitory and, thus, unable to surmount the inhibition. Second, with increasing activation of the ionophore with increasing spermidine concentrations, the inhibitory site becomes more dominant because of its increased binding affinity and prevents the potential increase in [^{3}H]TCP binding expected from spermidine. Thus, the biphasic effects of both spermidine and the putative partial agonist magnesium, along with their complex nonclassic interactions, make it impossible to prove that magnesium is a partial agonist at the spermidine site. Nevertheless, the considerable evidence presented here that both agonists act at the same site, together with the lesser intrinsic activity of magnesium, strongly suggests that magnesium is indeed a partial agonist.

The physiological relevance of these findings at this time is not clear. Based on experiments in brain slices,[2] we proposed the the polyamine receptor is localized on the intracellular surface of the neuronal membrane. Given that the free intracellular concentration of Mg^{2+} is on the order of 0.3 mM (20, 21), it is possible that this concentration is high enough to block the intracellular effects of spermidine and spermine on the NMDA ionophore, via its action as a partial agonist. Thus, the response characteristics of the NMDA receptor complex to extracellular glutamate, glycine, and magnesium may very well be dependent on the relative intracellular concentrations of

magnesium and the various polyamines, including putrescine (7). Further, inasmuch as intracellular magnesium ion concentration varies inversely with extracellular calcium concentration (20, 21), the ability of magnesium to act as a spermidine antagonist could also serve to dampen an NMDA response dependent on a decrease in the ratio of extracellular to intracellular calcium.

References

1. Anis, N. A., S. C. Berry, N. R. Burton, and D. Lodge. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by *N*-methyl-aspartate. *Br. J. Pharmacol.* **79**:565–575 (1983).
2. Mayer, M. L., G. L. Westbrook, and P. B. Guthrie. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* (*Lond.*) **309**:261–263 (1984).
3. Westbrook, G. L., and M. L. Mayer. Micromolar concentrations of Zn^{+2} antagonize NMDA and GABA responses of hippocampal neurons. *Nature* (*Lond.*) **328**:640–643 (1987).
4. Johnson, J. W., and P. Ascher. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* (*Lond.*) **325**:529–531.
5. Ransom, R. W., and N. L. Stec. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J. Neurochem.* **51**:830–836 (1988).
6. Williams, K., C. Romano, and P. B. Molinoff. Effects of polyamines on the binding of [^{3}H]MK 801 to the *N*-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. *Mol. Pharmacol.* **36**:575–581 (1989).
7. Sacaan, A. I., and K. M. Johnson. Characterization of the stimulatory and inhibitory effects of polyamines on [^{3}H]TCP binding to the NMDA receptor ionophore complex. *Mol. Pharmacol.* **37**:572–577 (1990).
8. Johnson, K. M., L. D. Snell, A. I. Sacaan, and S. M. 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).
9. Reynolds, I. J., and R. J. Miller. Ifenprodil is a novel type of N-methyl-D-aspartate receptor antagonist: interaction with polyamines. *Mol. Pharmacol.* **36**:758–765 (1989).
10. Reynolds, I. J. Arcaine is a competitive antagonist of the polyamine site on the NMDA receptor. *Eur. J. Pharmacol.* **177**:215–216 (1990).
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. Tallarida, R. J., and L. S. Jacob. *The Dose Response Relation in Pharmacology*. Springer-Verlag New York Inc., New York (1979).
17. 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).
18. Vignon, J., J. P. Vincent, J. N. Bidard, J. M. Kamenka, P. Geneste, and M. Lazdunski. Biochemical properties of the brain phencyclidine receptor. *Eur. J. Pharmacol.* **81**:531–542 (1982).
19. Schoemaker, H., J. Allen, and S. Z. Langer. Binding of [^{3}H]ifenprodil, a novel NMDA antagonist, to a polyamine-sensitive site in the rat cerebral cortex. *Eur. J. Pharmacol.* **176**:249–250 (1990).
20. Heinonen, E., and K. E. O. Akerman. Intracellular free magnesium in synaptosomes measured with entrapped eriochrome blue. *Biochim. Biophys. Acta* **898**:331–337 (1987).
21. Brooks, K. J., and H. S. Bachelard. Changes in intracellular free magnesium during hypoglycemia and hypoxia in cerebral tissue as calculated from ^{31}P-nuclear magnetic resonance spectra. *J. Neurochem.* **53**:331–334 (1989).

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