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Effects of Guanine Nucleotides on *N*-Methyl-D-Aspartate Receptor-Ligand Interactions

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SUMMARY

Guanine nucleotides have been examined as to their effects on subclass-specific excitatory amino acid receptor-ligand interactions. Guanine nucleotides selectively inhibit L-[^{3}H]glutamate binding to the *N*-methyl-D-aspartate (NMDA) recognition site while showing a lesser effect on [^{3}H]kainate, [^{3}H]α-amino-3-hydroxy-5-methylisoxazole-4-propionate and sodium-dependent L-[^{3}H]glutamate binding. Of the series of guanine nucleotides tested in the inhibition of NMDA-specific L-[^{3}H]glutamate binding, GTP, GDP, 5′-guanylylimidodiphosphate and 5′-guanylylmethylenediphosphate were significantly more potent than GMP, cyclic GMP and guanosine. Scatchard analysis indicates that the GTP inhibition (IC_{50} = 28 μM) of this NMDA-specific L-[^{3}H]glutamate binding results from a decrease in the affinity of L-glutamate for the NMDA receptor whereas no alteration in the number of binding sites is observed. A kinetic analysis indicates that this decrease in affinity may be attributed to a decrease in association rate whereas no change in dissociation rate is observed. GTP (25 μM) lowers the affinities of both NMDA agonists (NMDA, L-glutamate, L-aspartate, and L-homocysteate) and antagonists (D-2-amino-5-phosphonovalerate, D-2-amino-7-phosphonoheptanoate, and D-2-aminoadipate). Pretreatment of the synaptic plasma membranes with either pertussis or cholera toxin had no significant effect on the GTP inhibition of NMDA-specific L-[^{3}H]glutamate binding. The data suggest that guanine nucleotides can negatively modulate the NMDA receptor; however, the mechanism of this modulation is unclear.

The mechanism through which the excitatory amino acid receptors are coupled to their physiological effector(s) remains unclear. The quisqualate (AA2) and kainate (AA3) subclasses of excitatory amino acid receptors have been shown to activate nonselective cationic channels with permeability to sodium and potassium ions (1, 2), whereas NMDA receptor (AA1) agonists activate a functionally distinct channel with a permeability to calcium in addition to sodium and potassium (2). Furthermore, excitatory amino acid agonists including NMDA have been shown to induce accumulation of cyclic GMP in cerebellar neurons (3) and to stimulate phosphoinositide turnover in both hippocampal slices (4) and cultured striatal neurons (5). Excitatory amino acids also induce phosphoinositide turnover in *Xenopus* oocytes injected with rat brain mRNA in an IAP-sensitive manner (6). These studies indicate that excitatory amino acid receptors may be involved in the mediation of a number of complex cellular responses. The mechanism by which this signal is transduced from the receptor to the effector, however, remains to be elucidated.

In many systems, receptors have been shown to be coupled to their effectors through regulatory protein(s) that bind to and are modulated by guanine nucleotides (GTP-binding proteins). The two prominent second messenger systems coupled through GTP-binding proteins are cyclic AMP production by adenylate cyclase (7–9) and phosphoinositide hydrolysis, catalyzed by phospholipase C, to form diacylglycerol and inositol triphosphate (9, 10). Receptors may be coupled to adenylate cyclase through either a stimulatory (G_s) or an inhibitory (G_i) GTP-binding protein. Although not as well characterized, there is evidence that receptors that activate phospholipase C may be coupled through a distinct GTP-binding protein (10). Additionally, GTP-binding proteins (G_o, G_k) may be the transducers associated with receptor-mediated regulation of ionic channels (11, 12). Acetylcholine receptors have been reported to directly regulate atrial muscarinic potassium channels through a GTP-binding protein (12). In a similar manner, calcium channels in dorsal root ganglia appear to be directly linked to norepinephrine and γ-aminobutyric acid receptors and are activated without the need of a soluble second messenger (13).

Guanine nucleotides have been shown to regulate the binding of ligands to receptors that are coupled through a GTP-binding protein to their effectors. For example, guanine nucleotides inhibit ligand binding to D-2 dopamine (14), substance P (15), δ-, κ- and μ-opioid (16), β-adrenergic (17), muscarinic acetylcholine (18), and serotonin (19) receptors. In addition, there have also been studies describing the effects of guanine nucleo-

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AP5, 2-amino-5-phosphonovalerate; AP7, 2-amino-7-phosphonoheptanoate; AMPA, (*R, S*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate; Gpp (NH)p, 5′-guanylylimidodiphosphate; Gpp(CH_2)p, 5′-guanylylmethylenediphosphate; IAP, islet activating protein, pertussis toxin; SPM, synaptic plasma membranes.

tides on the binding of L-[^{3}H]glutamate (20) and on the binding of the acidic amino acid ligand D, L-[^{3}H]2-amino-4-phosphonobutyrate (21). However, the pharmacological profile of these latter binding sites does not correspond to any of the electrophysiologically defined excitatory amino acid receptor subclasses and their physiological relevance is unclear (22, 23). Recently, guanine nucleotides have been shown to inhibit both NMDA-sensitive L-[^{3}H]glutamate (24) and [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (25) binding to rat brain membranes, but the nature of this inhibition was not addressed in these studies.

In the present study, we have analyzed the effects of guanine nucleotides on excitatory amino acid receptors using receptor binding assays with ligands and conditions that have been shown to specifically label the NMDA receptor/AA1 recognition site (L-[^{3}H]glutamate) (26); the quisqualate receptor/AA2 recognition site ([^{3}H]AMPA) (27, 28); the kainate receptor/AA3 recognition site ([^{3}H]kainate) (28, 29), and the sodium-dependent L-[^{3}H]glutamate site (28, 30), which has characteristics of the sodium-dependent glutamate uptake system. Furthermore, we have examined the inhibition of NMDA-specific L-[^{3}H]glutamate binding by guanine nucleotides for properties associated with involvement of a GTP-binding protein in the coupling of the NMDA receptor to its physiological effector mechanism.

Experimental Procedures

Materials

Radioactive ligands (L-[^{3}H]glutamate, 40-50 Ci/mmol; [^{3}H]kainate, 60.0 Ci/mmol; and [^{3}H]AMPA, 27.1 Ci/mmol) and [α-^{32}P]NAD were purchased from New England Nuclear (Boston, MA). L-Homocysteate, GTP, GDP, GMP, cyclic GMP, guanosine, Triton X-100, D-2-aminoadipate, kainate, cholera toxin, and IAP were purchased from Sigma Chemical Company (St. Louis, MO); NMDA, D-AP5, and D-AP7 were from Tocris Neuramin (Essex, England); L-aspartate and L-glutamate from Pierce Chemical Company (Rockford, Il). AMPA was from Research Biochemicals Inc. (Atlantic Beach. NY); Gpp(NH)p and Gpp(CH_2)p were from Boehringer Mannheim (Indianapolis, IN). All other chemicals were reagent grade.

Methods

Membrane preparation. SPM were prepared as previously described (26). The SPM were stored at a concentration of 10–15 mg/ml in 0.32 M sucrose, 0.5 mM EDTA, 1 mM $MgSO_4$, 5 mM Tris/SO_4, pH 7.4., under liquid nitrogen. The identity and purity of the subcellular fractions were confirmed by both electron microscopy and marker enzymes. Protein concentrations were determined by using a modification of the method of Lowry (31).

Receptor binding assays. The SPM were treated identically for the [^{3}H]AMPA, [^{3}H]kainate, and sodium-dependent L-[^{3}H]glutamate binding assays. The SPM were thawed at room temperature, diluted 20-fold with 50 mM Tris/acetate, pH 7.4, incubated at 37° for 30 min and centrifuged at 100,000 × g for 15 min. The dilution, incubation, and centrifugation was repeated a total of three times.

Before use in the NMDA-specific L-[^{3}H]glutamate binding assay, the SPM were thawed, diluted 20-fold with 50 mM Tris/acetate, pH 7.4, containing 0.04% (v/v) Triton X-100, incubated for 30 min at 37°, and centrifuged as described above. The Triton X-100-treated membranes were washed with 50 mM Tris/acetate, pH 7.4, and centrifuged at 100,000 × g for 15 min a total of four times. Triton X-100 treatment of the SPM resulted in a higher affinity and more consistency in this L-[^{3}H]glutamate binding assay. For this reason the K_d for glutamate and the K_i values for other compounds are lower than previously reported (26); however, the pharmacological profile of this binding site was unaltered. Additionally, Triton X-100 treatment of the SPM had no effect on guanine nucleotide inhibition of L-[^{3}H]glutamate binding. The relative potencies of the guanine nucleotides tested were comparable in assays using both control and Triton X-100-treated SPM (data not shown).

The basic procedure for all four of the receptor subclass binding assays was similar (26, 28). This general method involved adding the radioligand (12.5 nM L-[^{3}H]glutamate, 0.5 nM [^{3}H]kainate, or 10 nM [^{3}H]AMPA) to the appropriate concentration of the test compound and initiating the assay by the addition of ice-cold SPM (0.2–0.45 mg). The binding assays were performed in 1.5-ml centrifuge tubes with the total volume adjusted to 1.0 ml. Additions of drugs were made in 50 mM Tris/acetate, pH 7.4, and incubations were carried out at 0–4°. The incubation time for the NMDA and the AMPA binding assays was 10 min, for the kainate binding assay 60 min, and for the sodium-dependent glutamate binding assay 15 min. The AMPA binding assay contained 100 mM KSCN and the sodium-dependent glutamate binding assay contained 150 mM sodium acetate in addition to the previously described reagents. Equilibrium was achieved in all assays both in the presence and absence of guanine nucleotides and the following constants were calculated for the radiolabeled ligand binding in SPM in the absence of nucleotides in the respective assays: NMDA-displaceable L-[^{3}H]glutamate (K_d = 0.05 μM, B_{max} = 5.8 pmol/mg), [^{3}H]kainate (K_d = 5 nM, B_{max} = 0.24 pmol/mg), [^{3}H]AMPA (K_d = 0.2 μM, B_{max} = 15.0 pmol/mg), and sodium-dependent L-[^{3}H]glutamate (K_d = 1.6 μM, B_{max} = 53.7 pmol/mg).

To terminate the incubation, the samples were centrifuged for 15 min at 12,000 × g and 4° in a Beckman Microfuge 12. The supernatant was aspirated and the pelleted membranes were dissolved in Beckman BTS-450 tissue solubilizer for a minimum of 6 hr at room temperature. Beckman MP scintillation cocktail containing 7 ml/liter acetic acid was then added and the samples were counted on a Beckman LS 5800 or 3801 liquid scintillation counter with automatic corrections for quenching and counting efficiency.

Nonspecific binding was defined as the residual binding in the presence of either excess L-glutamate (0.1 mM in the [^{3}H]AMPA and sodium-dependent L-[^{3}H]glutamate assay), kainate (0.01 mM), or NMDA (0.5 mM) and was 15–25% of the total binding in the NMDA binding assay, 19–27% in the AMPA binding assay, 20–30% in the kainate binding assay, and 10–15% in the sodium-dependent glutamate binding assay. Radioligand binding to the SPM was analyzed using Scatchard and Hill transformations and the K_i values of the compounds were determined using logit-log transformations. Calculations and regression analyses were performed using templates developed for Lotus 1,2,3 as previously described (32). The pharmacological profile of compounds interacting at these recognition sites corresponds with previously published reports of ligand binding and functional analysis (22, 24–30).

A filtration protocol was used for experiments in which the association and dissociation rates for NMDA-specific L-[^{3}H]glutamate were determined. For the determination of the association rate, L-[^{3}H]glutamate was incubated with SPM in the absence or presence of GTP (50 μM) in 50 mM Tris/acetate, pH 7.4, and aliquots were removed at specified times. Dissociation analysis was performed after addition of 500 μM NMDA and 50 μM GTP to a tube containing L-[^{3}H]glutamate and SPM, which had been preincubated for 10 min at 2° and samples were removed at various times. The samples from both the association and dissociation experiments were vacuum-filtered through glass fiber filters and washed three times with ice-cold 50 mM Tris/acetate, pH 7.4. The total washing time was less than 5 sec. The filters were then treated with solubilizer and counted as described above. There was no statistical difference between the K_d and B_{max} values determined using either the filtration or centrifugation protocols.

To analyze the effects of toxins, SPM (30 mg, at a concentration of 2 mg/ml) were preincubated with either cholera toxin (3 mg) or IAP (0.25 mg) for 30 min at 37° in 50 mM Tris/1 mM NAD/1 mM EDTA/5 mM dithiothreitol/1 mM ATP/10 mM thymidine/2.5 mM $MgCl_2$, pH

7.4. The SPM were then washed three times in 50 mM Tris/acetate, pH 7.4, and L-[^{3}H]glutamate binding was carried out as described above.

Results

The ability of guanine nucleotides to modulate the binding of ligands in excitatory amino acid subclass-specific binding assays is summarized in Table 1. Varying degrees of inhibition were observed in all four binding assays by the series of nucleotides. [^{3}H]Kainate, [^{3}H]AMPA, and sodium-dependent L-[^{3}H]glutamate binding were decreased up to 37% by 100 μM concentrations of the nucleotides tested. The guanine nucleotides inhibited NMDA-specific L-[^{3}H]glutamate binding more potently than binding to any of the other three sites. GTP (77% inhibition of NMDA-specific glutamate binding), Gpp(NH)p (70%) Gpp(CH_2)p (67%), and GDP (66%) were the most potent, whereas GMP (34%), cyclic GMP (22%), and guanosine (5%) exhibited significantly less activity. ATP (24%) was less active than GTP, providing evidence for guanine moity specificity.

The profile of the guanine nucleotide inhibition of NMDA-specific L-[^{3}H]glutamate binding is consistent with other binding studies in which the receptor was shown to be coupled to a GTP-binding protein (15, 17–19, 33). We chose to further investigate this interaction in an effort to determine whether the inhibition by guanine nucleotides exhibited other properties expected of a receptor coupled to a GTP-binding protein.

The GTP inhibition of NMDA-specific L-[^{3}H]glutamate binding is concentration dependent (Fig. 1) and has an IC_{50} of 28 μM. A Scatchard analysis of L-[^{3}H]glutamate binding to the NMDA recognition site in the presence of 25 μM GTP is compared with control binding in Fig. 2. From these results it is clear that the decrease in binding observed in the presence of GTP is due to a decrease in affinity of L-[^{3}H]glutamate for the NMDA receptor. The K_d for L-glutamate in the absence of GTP is 0.051 ± 0.005 μM whereas the K_d in the presence of 25 μM GTP is increased to 0.120 ± 0.03 μM. The number of NMDA receptors is not significantly altered in the presence of GTP, with a B_{max} = 6.2 ± 0.4 pmol/mg compared with 5.8 ± 0.5 pmol/mg in control membranes.

The effect of GTP on the kinetics of L-[^{3}H]glutamate binding was examined in an effort to further characterize this inhibition. The association of L-[^{3}H]glutamate was decreased in the presence of GTP (Fig. 3) with the association rate constant in the control binding k_{+1} = 22.2 μM^{-1} min^{-1} and in the presence of 50 μM GTP, k_{+1} = 2.5 μM^{-1} min^{-1}. A concentration of 50 μM GTP had no significant effect on the dissociation rate of L-[^{3}H]glutamate (k_{-1} = 0.366 min^{-1} in control, k_{-1} = 0.338 min^{-1} in the presence of GTP) (Fig. 4).

TABLE 1

Effects of nucleotides on excitatory amino acid subclass specific binding

The results are expressed as per cent inhibition of specific binding for each receptor binding assay (performed as described in Experimental Procedures) in the presence of 100 μM of various nucleotides. Each value represents the mean ± standard error of at least three experiments performed in triplicate.

Nucleotide	NMDA-specific L-[^{3}H] glutamate	[^{3}H]AMPA	[^{3}H]Kainate	Na-dependent L-[^{3}H]glutamate
		% inhibition		
GTP	77 ± 3	21 ± 6	23 ± 5	34 ± 9
GDP	66 ± 5	35 ± 2	14 ± 1	25 ± 8
GMP	34 ± 9	20 ± 3	4 ± 1	15 ± 4
cyclic GMP	22 ± 2	18 ± 3	18 ± 6	−5 ± 1
Guanosine	5 ± 2	29 ± 3	37 ± 1	−8 ± 7
Gpp (NH)p	70 ± 3	23 ± 3	13 ± 5	29 ± 8
Gpp (CH_2)p	67 ± 2	20 ± 4	26 ± 1	13 ± 9

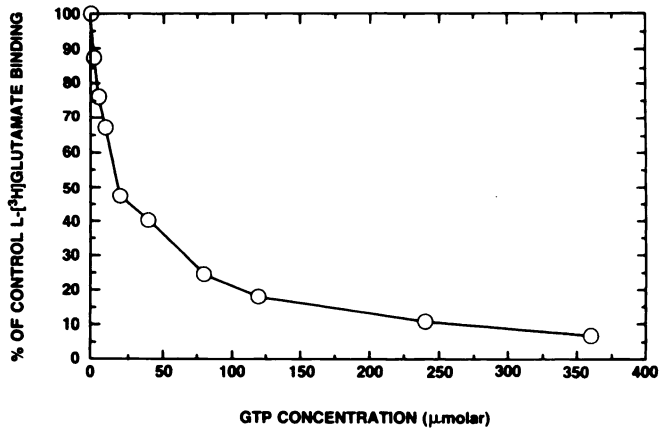

Fig. 1. GTP inhibition of NMDA-specific L-[^{3}H]glutamate binding. Various concentrations of GTP, 2–360 μM, were incubated with 12.5 nM L-[^{3}H]glutamate for 10 min at 2°. The results are expressed as per cent inhibition of specific L-[^{3}H]glutamate bound, with nonspecific binding measured in the presence of 500 μM NMDA. The results are the mean of three separate experiments each performed in triplicate (SE for each point was less than 15% of the mean value).

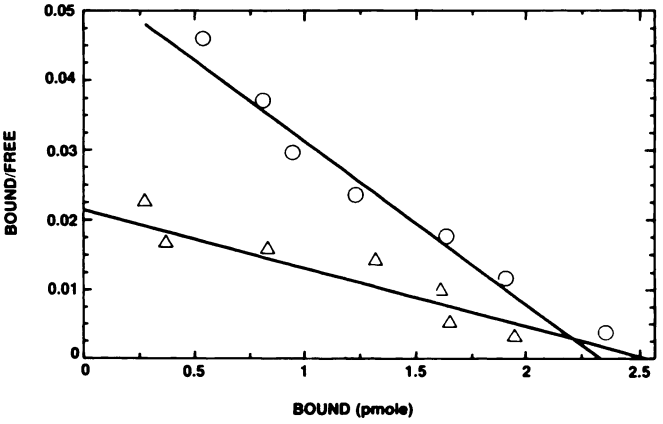

Fig. 2. Analysis of GTP inhibition of NMDA-specific L-[^{3}H]glutamate binding. Scatchard analysis of L-[^{3}H]-glutamate binding in 50 mM Tris/acetate, using 0.3–0.4 mg of SPM, pH 7.4, at 2° for 10 min in the absence (○) and presence (△) of 25 μM GTP. The data result from the transformation of a saturation curve with nonspecific binding defined with 0.1 mM glutamate. These results are from a single representative experiment (from a total of five experiments each performed in triplicate).

The effect of GTP on the affinity of a series of NMDA receptor agonists and antagonists is shown in Table 2. The affinity of both agonists and antagonists is lowered by 25 μM GTP. However, the affinity of the agonists (L-glutamate, L-aspartate, NMDA, and L-homocysteate) was, in general, decreased to a greater extent than that of the antagonists (D-AP5, D-AP7, and D-2-aminoadipate).

We have examined the effects of both cholera toxin and IAP on the ability of GTP to alter the affinity of L-[^{3}H]glutamate for the NMDA receptor. As shown in Table 3, neither IAP nor cholera toxin altered the effects of GTP on NMDA-specific binding. In control experiments, under similar conditions an IAP- and cholera toxin-dependent incorporation of radioactivity from [α-^{32}P]NAD into SPM polypeptides (M_r = 40,000–50,000) was observed using sodium dodecyl sulfate polyacryl-

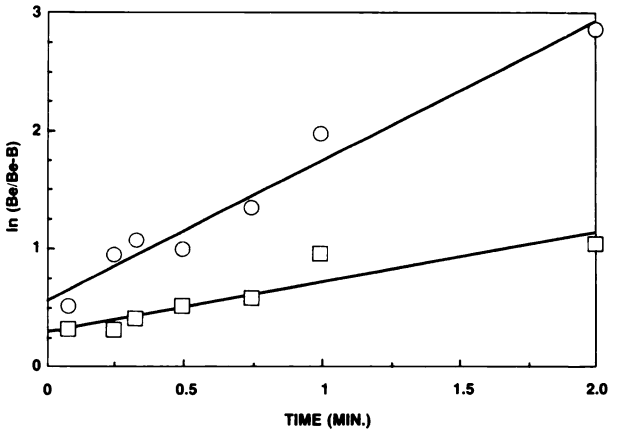

Fig. 3. Effect of GTP on the association rate of NMDA-specific L-[^{3}H] glutamate binding. SPM were incubated in the absence (○) or presence [□] of 50 μM GTP. At various times after the addition of 12.5 nM L-[^{3}H] glutamate aliquots were removed and filtered as described in Experimental Procedures. The results are the mean of three separate experiments each performed in triplicate (SE for each point was less than 15% of the mean value).

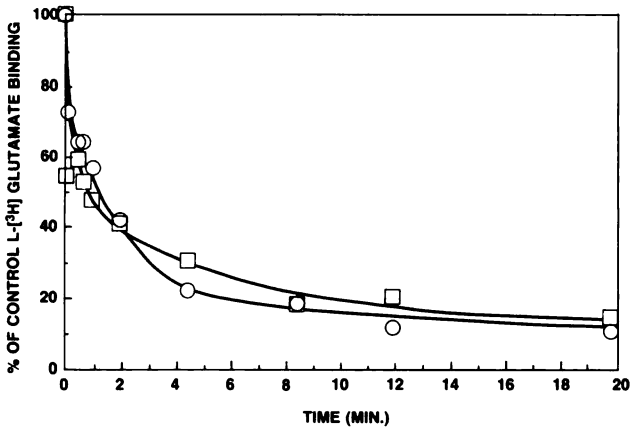

Fig. 4. Effect of GTP on the dissociation of NMDA-specific L-[^{3}H]glutamate binding. SPM were incubated with 12.5 nM L-[^{3}H]glutamate at 2° in the absence of GTP. After a 10-min incubation, NMDA was added to a final concentration of 0.5 mM either alone (○) or with 50 μM GTP (□) and aliquots were removed at various times and filtered as described in Experimental Procedures. The results are the mean of three separate experiments each performed in triplicate (SE for each point was less than 15% of the mean value).

amide gel electrophoresis and autoradiographic analysis, providing evidence of toxin activity with these SPM substrates (data not shown).

Discussion

Excitatory amino acid receptors have been shown to be the major mediators of excitatory neurotransmission in the mammalian central nervous system. There is evidence from electrophysiological studies that these receptors are linked directly to cationic channels and thereby induce membrane depolarization without induction of a soluble second messenger (1, 2). There are also studies in which excitatory amino acid agonists increase cyclic GMP levels (3) and alter phosphoinositide turnover (4–6). Presently, it is not clear whether the signal from the activation of excitatory amino acid receptors is transduced through a GTP-binding protein to mediate any of these responses.

Although the series of nucleotides tested in this study inhibited binding to all four recognition sites, binding to the NMDA receptor was decreased to the greatest extent. In studies of receptors known to be coupled to their effector by a GTP-binding protein, GTP, its nonhydrolyzable analogs (Gpp(NH)p and Gpp(CH_2)p) and GDP are the most effective in inhibiting receptor ligand binding (15, 17–19, 33). Similarly, potencies of the various guanine nucleotides (GTP, Gpp(NH)p, Gpp(CH_2)p, GDP > GMP, cyclic GMP, and guanosine) in inhibiting binding to the NMDA recognition site has the rank order characteristic of an interaction with a GTP-binding protein.

Consistent with action through a GTP-binding protein, the IC_{50} for inhibition of NMDA-specific L-[^{3}H]glutamate binding by GTP (IC_{50} = 28 μM) is similar to values reported for GTP analogs in studies with other receptors (14–21, 33). Furthermore, this nucleotide-induced inhibition occurs by decreasing the affinity of the receptor without changing the number of binding sites. The decrease in affinity is seen with both NMDA agonists and antagonists although agonists are affected to a greater degree. The effect on antagonist binding is in apparent contrast to the characteristic GTP-binding protein effects on receptor ligand binding in which the decrease in ligand affinity is agonist specific (17, 29, 33). The possibility that the NMDA receptor antagonists used in this study have some agonist character cannot be ruled out, however, no evidence exists that supports this possibility (34).

TABLE 2

Effects of GTP on the binding affinities of NMDA receptor agonists and antagonists

Various concentrations of compounds were incubated in the presence and absence of 25 μM GTP under conditions specified for the NMDA-specific L-[^{3}H]glutamate binding assay (see Experimental Procedures). Logit-log analysis was used for the determination of the K_i values (assuming a single noninteractive class of binding sites (26) and the K_d for glutamate of 0.05 μM in the absence of GTP and 0.12 μM in the presence of 25 μM GTP). The K_i values are assessed as means ± standard errors from at least three experiments, each performed in triplicate. Statistical comparisons with control values were done using a two-tailed Student's *t* test.

	K_i		% of Control
	Control	+25 μM GTP	
	μM		
Agonists			
L-Glutamate	0.049 ± .002	0.123 ± .012[a]	+253
NMDA	3.25 ± 0.21	5.37 ± 0.68[b]	+165
L-Aspartate	0.72 ± 0.05	1.51 ± 0.31[b]	+209
L-Homocysteate	0.35 ± 0.02	0.64 ± 0.04[a]	+182
Antagonists			
D-AP7	2.05 ± 0.39	3.55 ± 0.37[b]	+171
D-AP5	0.24 ± 0.03	0.36 ± 0.07[c]	+155
D-2-Aminoadipate	2.92 ± 0.22	4.11 ± 0.39[b]	+144

[a] $p < 0.005$.
[b] $p < 0.05$.
[c] $p < 0.1$.

TABLE 3

Effects of toxins on GTP modulation of NMDA-specific L-[^{3}H] glutamate binding

Membranes were pretreated with either buffer, IAP, or cholera toxin as described in Experimental Procedures. The K_d and B_{max} values were calculated from Scatchard transformations. Results are expressed as mean ± standard error values from at least three experiments each performed in triplicate.

Membrane Treatment	−GTP		+25 μM GTP	
	K_d	B_{max}	K_d	B_{max}
	μM	pmol/mg	μM	pmol/mg
Control	0.051 ± .009	5.8 ± 0.7	0.116 ± .020	5.5 ± 0.2
IAP	0.062 ± .017	5.8 ± 0.7	0.123 ± .024	5.9 ± 0.9
Cholera	0.052 ± .011	6.3 ± 0.6	0.107 ± .003	6.4 ± 0.9

Another apparent departure from the characteristic GTP-binding protein effect was in the kinetic analysis of NMDA-specific L-[^{3}H]glutamate binding. In this study, GTP was shown to decrease NMDA receptor affinity primarily by alteration of the association rate. Other studies have reported that guanine nucleotides acting through a GTP-binding protein alter receptor affinity by increasing the dissociation rate (15, 19, 35).

Cholera toxin has been shown to catalyze the ADP-ribosylation of G_s resulting in persistent activation of the coupled second messenger system and a decrease in the ability of ligand-bound receptor to regulate adenylate cyclase (9). In a similar manner IAP can ADP-ribosylate the GTP-binding protein G_i resulting in an uncoupling of the receptors from G_i and a reduction in the effect of guanine nucleotides on the coupled receptor's affinity for ligands (36, 37) [other GTP-binding proteins have also been shown to be sensitive to IAP (38, 39)]. We have found that neither IAP nor cholera toxin alter the modulatory effect of GTP on the NMDA receptor under conditions that were shown to promote ADP-ribosylation. This lack of activity indicates that, if GTP is modulating NMDA receptor affinity through a GTP-binding protein, then this protein may be similar to those that have been shown to be resistant to ADP-ribosylation by both of these toxins (40, 41).

The results reported in this study clearly demonstrate that guanine nucleotides selectively inhibit L-[^{3}H]glutamate binding to the NMDA recognition site in rat brain SPM. The mechanism of this inhibition, however, remains unclear. Although the inhibition with guanine nucleotides has some characteristics expected of typical GTP-binding protein modulation, several inconsistencies are observed when compared with studies on receptors known to be coupled to their second messenger through a GTP-binding protein. There are alternative explanations for the observed guanine nucleotide effects on ligand binding to the NMDA receptor that cannot be ruled out by the present study. The NMDA receptor could contain a guanine nucleotide regulatory site unrelated to signal transduction. Alternatively, these guanine nucleotides could be acting directly at the NMDA recognition site. Further studies on the NMDA receptor complex and its effector mechanisms are necessary to discern these or other mechanisms of guanine nucleotide action. Irrespective of the mechanism of action, the results presented herein indicate that the endogenous guanine nucleotides GTP and GDP may modulate NMDA receptor-mediated excitatory neurotransmission in the mammalian central nervous system. Additionally, these results indicate additional complexity of the NMDA receptor, which has recently been shown to be functionally coupled to a phencyclidine receptor (42) and positively modulated by neutral amino acids such as glycine (43).

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