Characterization of a Glutamic Acid Neurotransmitter Binding Site on Neuroblastoma Hybrid Cells*

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Glutamate is thought to be a major excitatory neurotransmitter in the central nervous system. To study the glutamate receptor and its regulation under carefully controlled conditions, the specific binding of [3H]glutamate was characterized in washed membranes isolated from a neuroblastoma x retina hybrid cell line, N18-RE-105. [3H]Glutamate bound in a saturable and reversible fashion with an apparent dissociation constant, K_D, of 650 mM and a maximum binding capacity, B_max, of 16 pmol/mg of protein. Pharmacologic characterization of the site indicates that it closely resembles the Na^+-independent binding site for glutamate found on brain membranes and thought to be an excitatory amino acid neurotransmitter receptor. Thus, while kainate, N-methyl-DL-aspartate, and non-amino acid ligands did not displace [3H]glutamate, quisqualate and ibotenate were potent inhibitors of specific binding. Furthermore, this binding site is regulated by ions in a manner which resembles that described in the hippocampus (Baudry, M., and Lynch, G. (1979) Nature (Lond.) 282, 748-750). Calcium (10 mM) increased the number of binding sites 2.6-fold with no change in receptor-ligand affinity. Lanthanum (1 mM) was the only cation added which enhanced (3-fold) the binding of [3H]glutamate. Monovalent cations resulted in a decrease in the number of glutamate binding sites. Incubation of membranes in the presence of chloride ions caused a marked increase in [3H]glutamate binding, an effect which was synergistic with that of calcium incubation. Thus, N18-RE-105 cells possess a binding site for [3H]glutamate pharmacologically similar to an excitatory neurotransmitter binding site in brain and which exhibits regulatory properties resembling those previously described in hippocampal membranes, providing an excellent model for mechanistic studies.

Much evidence suggests that D-glutamic acid, in addition to its role in metabolism and protein structure, acts as one of the major excitatory neurotransmitters in the brain (1, 2). Hayashi was the first to demonstrate that glutamate has potent neuroexcitatory properties when applied to cortical neurons (3). Subsequently, an abundance of electrophysiologic and neurochemical evidence has accumulated to support the candidacy of glutamate as a neurotransmitter for many neuronal pathways throughout the central nervous system (1, 2). More recent electrophysiological studies using specific agonists and selective antagonists have delineated three distinct excitatory amino acid receptors which bind glutamate: the quisqualate-responsive site, the N-methyl-DL-aspartate-responsive site, and the kainate-responsive site (4). The quisqualate-responsive site appears to be the major neurotransmitter receptor for glutamate in the brain and is the central concern of our studies. In addition to the electrophysiological studies referenced above, the quisqualate-responsive site has been identified by direct binding of [3H]glutamate to isolated brain membranes (5-9). Inhibition of [3H]glutamate binding to these membranes by glutamate analogs correlates well with their electrophysiological potency at the quisqualate-responsive site.

Interest in this neurotransmitter binding site has been stimulated recently by data suggesting that its regulation is involved in a simple electrophysiological correlate to learning: long-term potentiation. Repeated high frequency electrical stimulation of certain pathways in the hippocampus results in a long-lasting increase in synaptic efficiency (perhaps via increased depolarization for equivalent electrical stimuli) in postsynaptic cells. This increased postsynaptic excitability is defined as LTP (10). Since there is ample evidence that the hippocampus is involved in learning and memory, it is thought that LTP may represent one possible cellular mechanism involved in these phenomena. A relationship between regulation of the [3H]glutamate receptor and LTP in the hippocampus has been reported by Baudry and Lynch (11-13). They demonstrated, in hippocampal slices, that high frequency electrical stimulation caused an increase in [3H]glutamate binding, while low frequency stimulation, which did not cause LTP, did not alter binding. Since glutamate has been reported to be a major excitatory neurotransmitter in the hippocampus, it is possible that the persistent up-regulation of glutamate receptors could be responsible for LTP. In addition, the formation of LTP in hippocampal slices is dependent on the presence of calcium ions in the external medium and [3H]glutamate binding to membranes from this tissue is sharply increased by calcium in vitro (14). This calcium-induced up-regulation was found on isolated membranes from areas of the brain which demonstrate LTP but not those in which LTP is absent. Thus, a correlation exists

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1 The abbreviations used are: LTP, long-term potentiation; B_max, maximum binding capacity; K_D, apparent dissociation constant.
between LTP and glutamate receptor regulation.

Studies on the molecular mechanisms involved in glutamate receptor regulation are hampered by the cellular heterogeneity of brain tissue. Therefore, we initiated a study to identify a homogenous neuronal cell line which both possessed the pharmacologically relevant glutamate receptor and demonstrated calcium-dependent up-regulation. One such cell line, a neuroblastoma × retina hybrid designated N18-RE-105, was found to possess a high density of such receptors. In this report, the binding of \(^{3}H\)glutamate to membranes isolated from N18-RE-105 cells is characterized in terms of its kinetics, pharmacology, and regulation by mono- and divalent ions. In addition, these binding characteristics are compared to those of hippocampal membranes. In the accompanying report (15), the regulation of this glutamate receptor in intact cells will be described. Together, these two studies demonstrate agonist and ion up-regulation of glutamate receptors in a homogeneous neuronal cell line, providing an excellent system for characterization of the molecular mechanisms which may underlie long-term potentiation and help provide a molecular model for learning or memory.

**EXPERIMENTAL PROCEDURES**

**Materials**

The following materials were obtained from the indicated sources: tissue culture medium (No. 430-2100) and fetal calf serum (virus-screened, mycoplasma-tested) from Grand Island Biological Co. (GIBCO); dibutyl cyclic adenosine 3′,5′-monophosphate, eserine sulfate, thymidine, hypoxanthine, aminopterin, bovine serum albumin, trifluoperazine, a-aminoadipic acid, DL-\(\alpha\)-aminopicinic acid, glutamate diethyl ester, N-methyl-DL-aspartic acid, kainic acid, \(\gamma\)-aminobutyric acid, and quisqualic acid from Sigma; aminophosphonoheptanoic acid from Calbiochem; ibotenate from Regis Chemical Co., Milwaukee, WI; \(\beta\)-\(3\) H glutamic acid (44.1 Ci/mmol), \(\gamma\)-\(3\) H azinoxybutyric acid (36.1 Ci/mmol), tissue solubilizer (Protosol), and liquid scintillation flours from New England Nuclear; tissue culture plasticware (75-cm\(^2\) flasks No. 25116 and 35-mm dishes No. 25000) from Corning Glass Works, Corning, NY. All other reagents were of the highest quality and were obtained from standard sources. Trifluoperazine dihydrochloride was the gracious gift of Smith, Kline and French Laboratories, Philadelphia, PA. Aminophosphonoheptanoic, aminophosphonoheptanoic, and aminophosphonoovaleric acids were the gifts of Dr. J. F. Collins, Department of Chemistry, City of London Polytechnic, London. Neuroblastoma × retina hybrid cell lines were kindly made available by Dr. Marshall Nirenberg and Chinese hamster embryonic neural retina × fibroblast cell lines were kindly provided by Dr. David Trisler, National Institutes of Health, Bethesda, MD.

**Methods**

**Cell Culture**—N18-RE-105 hybrid cells (mouse neuroblastoma clone N18TG-2 × Fisher rat 18-day embryonic neural retina) were grown essentially as described for other neuroblastoma hybrid cell lines (16). Briefly, stock cultures (passage 5-28) were maintained at 37 °C in 75-cm\(^2\) tissue culture flasks in 85% Dulbecco’s Modified Eagle’s medium (supplemented with 10% fetal bovine serum, 44 mM NaHCO\(_3\), thymidine (16 \(\mu\)M), hypoxanthine (100 \(\mu\)M), and aminopterin (1 \(\mu\)M)) and 5% fetal calf serum in a humidified atmosphere of 10% CO\(_2\), 90% air. Cells were subcultured by removing the growth medium and replacing it with 10 ml of (Ca\(^{2+}\)-Mg\(^{2+}\))-free phosphate-buffered saline and cells were collected by centrifugation at 250 \(\times\) g for 5 min. The cells were resuspended in 1.0 ml of Tris-HCl (pH 7.4) and transferred to a scintillation vial, 10 ml of scintillation fluor (with 4-aminobutyric acid), and the cell pellet was resuspended by sonication in these solutions. Routinely, for each binding determination, 100 \(\mu\)l of the membrane suspension was added to a 1.5-ml Eppendorf (polypropylene) microcentrifuge tube containing 100 pmol of \(^{3}H\)glutamate (adjusted to approximately 5,000 cpm/pmol with unlabeled glutamate) and other additives (see below) dissolved in a total volume of 250 \(\mu\)l of Tris-HCl. In contrast, for saturation isotherms, the final concentration of radioligand ranged from 50 nm-1 \(\mu\)M. To measure nonspecific binding, unlabeled L-glutamic acid was added to a final concentration of 0.1 \(\mu\)M. Potential competitors and inhibitors of binding were added at concentrations indicated under “Results.” Binding incubations were conducted for 20 min at 37 °C and then centrifuged for 10 min at 30,000 \(\times\) g (4 °C). The supernatant was aspirated, 1 ml of ice cold buffer was added to the tube without disturbing the pellet and immediately aspirated, and the pellet (with bound radioligand) was dissolved in 0.5 ml of Protocol and subjected to liquid scintillation spectrometry in 10 ml of Ecosfluor (with 0.5% (v/v) acetic acid added).

**Enzyme Assays**—One flask of N18-RE-105 hybrid cells was harvested in 10 ml of (Ca\(^{2+}\)-Mg\(^{2+}\))-free phosphate-buffered saline and cells were collected by centrifugation at 250 \(\times\) g for 5 min. The cells were resuspended in 1.0 ml of Tris-HCl supplemented to 0.02% (v/v) Triton X-100 detergent. The pellet was triturated 5 times and placed in a Potter-Elvehjem glass homogenizer with a Teflon pestle. Cells were homogenized (40 strokes) at 0 °C. Portions (50 \(\mu\)l) of the homogenate were assayed for tyrosine hydroxylase (17), choline acetyltransferase (18), and glutamic acid decarboxylase (19) activities.

**Uptake Studies**—High-affinity \(^{3}H\)glutamate and \(^{3}H\)4-aminobutyric acid transport were studied using N18-RE-105 cells which had firmly attached to the bottom of Costar 24-well plates. The procedure was adapted from the method of Coyle and Emna as described for rat brain synaptosomal preparations (20). Growth medium was aspirated and cells were washed twice in Krebs phosphate buffer. The cells were preincubated for 10 min at 37 °C in 950 \(\mu\)l of the same buffer after which 50 \(\mu\)l of \(^{3}H\)glutamate (specific activity diluted to 0.44 Ci/mmol with unlabeled glutamate) or \(^{3}H\)4-aminobutyric acid (specific activity diluted to 0.56 Ci/mmol with unlabeled 4-aminobutyric acid) were added to give a final concentration of 2 \(\times\) \(10^{-3}\) M. After incubating the cells for 4 min (with glutamate) or 2 min (with 4-aminobutyric acid), the cells were washed twice with ice cold buffer. One ml of 1 M NH\(_4\)OH was then added to each well and the suspension was triturated 10 times (removing all cells from the plate) and transferred to a scintillation vial, 10 ml of scintillation fluor (New England Nuclear Formula 947) and 60 \(\mu\)l of glacial acetic acid were added, and radioactivity was quantified by liquid scintillation spectrometry. Since the high affinity uptake processes are sodium-dependent, control wells were treated as above except that the NaCl in the Krebs buffer was replaced with chloride. Protein was measured by the method of Lowry et al. (21) with bovine serum albumin as standard.

**Statistical Analysis**—All biochemical data were tested for significance using the parametric Student’s two-tailed t-test.

**RESULTS**

**Neuronal Cell Lines with Glutamate Neurotransmitter Binding Sites**—Seven clonal cell lines were screened for specific binding of L-\(^{3}H\)glutamate under conditions that were optimal for labeling these sites in rat brain membranes (Table I). Three cell lines derived from a hybridization of Chinese hamster embryonic neural retina and fibroblasts (A2d, A1a-3, A2a-1) did not exhibit detectable specific binding. The NGV-1 line, a mouse neuroblastoma × rat glioma hybrid with cholinergic characteristics, exhibited modest specific binding. Of three mouse neuroblastoma × rat embryo retina hybrids
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Washed membranes were prepared from the indicated cell lines as described under "Methods." Specific [3H]glutamate binding was measured in the presence of 600 nM radioligand (20-min incubation, 37 °C). Membranes were collected from both control cells and cells grown for 5–7 days in the presence of 1 mM dibutyryl cyclic AMP, an agent used to induce differentiation in neuronal tissue culture cell lines. Nonspecific binding was determined by incubating membranes with the radioligand in the presence of 100 μM unlabeled glutamate and the resulting value was subtracted to generate the specific binding reported. NCE-9a-K, N18-RE-103, and N18-RE-105 are mouse neuroblastoma x embryonic neural retina hybrids, NG-108-15 is a mouse neuroblastoma x rat glioma hybrid, and A2d, A1a-3, and A2a-1 are embryonic Chinese hamster neural retina x fibroblast hybrids.

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*ND, not determined.

Inhibition isotherms for both linear and conformationally restricted excitatory analogs of glutamate were performed (Fig. 3 and Table II). The results of this structure-activity study correlated closely with similar data collected using these compounds at the glutamate binding site of rat cerebellar membranes (Table II). Quisqualic acid was the most potent competitive blocker of [3H]glutamate binding to washed N18-RE-105 membranes closely followed by L-glutamate itself. Of 17 analogs tested, 14 exhibited Kᵢ values comparable to those reported in rat cerebellum, while only three compounds, L-glutamate, L-aspartate, and aminophosphonooxalate exhibited significant discrepancies, with only L-aspartate exhibiting a major shift in rank order of potency (Table II). Analogs which have very low affinities for the cerebellar binding site have equally low affinities for the neuroblastoma binding site.

Regulation of Glutamate Binding by Cations—To characterize the direct effects of cations on glutamate receptors in isolated membranes, N18-RE-105 cells were grown in normal growth medium and their membranes were harvested and split into aliquots and centrifuged. Control pellets were resuspended in buffer (Tris-HCl) alone while the experimental pellets were resuspended in the same buffer supplemented with various concentrations (10 μM–100 mM) of monovalent or divalent cations. Control and experimental membrane suspensions were preincubated for 10 min at 37 °C and then were assayed for [3H]glutamate binding without changing the ions concentrations. A significant increase in binding was measured after treatment with calcium ions (Fig. 4). The binding reached a maximum of 2.6-fold of control when 10 mM CaCl₂ was included but declined back toward control levels when membranes were exposed to higher concentrations. LaCl₃ produced a similar pattern of stimulation, reaching a maximum binding of 3.0-fold of control binding at 1 mM (Fig. 4).

Dose-response curves for monovalent cations and MgSO₄ revealed no enhancement of [3H]glutamate binding at concentrations up to 150 mM. In contrast, these salts caused a decrease in [3H]glutamate binding, similar to results reported...

2 M. Nirenberg, Laboratory of Biochemical Genetics, National Heart, Lung and Blood Institute, National Institutes of Health, personal communication.

3 R. Schnaar, unpublished data.

Fig. 1. N18-RE-105 neuroblastoma × retina hybrid cells in culture. N18-RE-105 cells were grown under conditions described under "Methods" and the photomicrograph was prepared using an Olympus IMT phase microscope fitted with an Olympus OM-2 camera body at a magnification of × 33 (to the film). Bar = 100 μm.

Table I

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**FIG. 2.** Association isotherm for the binding of [3H]glutamate to N18-RE-105 neuroblastoma membranes. Washed membranes, prepared from confluent cell cultures grown in 75-cm² tissue culture flasks, were incubated with varying concentrations of [3H]glutamate at 37 °C and bound radiolabel was measured using the centrifugation assay described under "Methods." Non-specific binding was measured in the presence of 100 μM unlabeled glutamate. Each point represents the average of six determinations. The inset presents a Scatchard plot of the data from the saturation isotherms. The $K_D$ is expressed as a nanomolar concentration and the $B_{max}$ as picomoles/mg of protein.

**FIG. 3.** Competition curves for the displacement of [3H]glutamate by excitatory amino acids. Washed membranes prepared from confluent cell cultures grown in 75-cm² tissue culture flasks were incubated with 100 nM [3H]glutamate in the presence of various concentrations of L-glutamate (O), quisqualate ( ), N-methyl-DL-aspartate ( ), and kainate ( ). Specific glutamate binding is determined as described under "Methods." Each data point is the mean of triplicate determinations from four separate experiments.

Using hippocampal membranes (22), NaCl was about a 10-fold more potent inhibitor than other monovalent cations, producing a 50% decrease in binding when added at a concentration of 10 mM (Fig. 5).

Scatchard analysis of the [3H]glutamate binding data collected using N18-RE-105 membranes preincubated with 10 mM CaCl₂ or 10 mM NaCl for 10 min at 37 °C revealed that

**TABLE II**
Affinities of amino acid agonists and antagonists for the [3H]glutamic acid binding site on isolated N18-RE-105 and rat brain membranes

Washed membranes were prepared from rat cerebellum (7) or N18-RE-105 cells and incubated with 100 nM [3H]glutamate in the presence of 10 nM to 1 mM concentrations of the substances listed. ICₕ values were extrapolated from the logit-log plots of the resulting data and inhibition constants were calculated using the following formula: $K_I = IC_50/(1 + f/K_D)$; where $f$ is the free ligand concentration (100 nM) and $K_D = 654$ nM (see Fig. 2).
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**FIG. 4.** Effect of calcium chloride and lanthanum chloride preincubation on \[^{3}H\]glutamate binding to isolated N18-RE-105 membranes. Washed membranes from N18-RE-105 cells were resuspended in Tris-HCl buffer containing the indicated concentrations of calcium chloride (○) or lanthanum chloride (□) and preincubated for 10 min at 37 °C before being assayed for \[^{3}H\]glutamate binding as described in the text. The data are expressed as the amount of glutamate binding compared to that in Tris-HCl buffer alone. Each data point is the mean of triplicate determinations from each of four to eight separate experiments.

**FIG. 5.** Effects of monovalent cations and magnesium on \[^{3}H\]glutamate binding to isolated N18-RE-105 membranes. Washed membranes from N18-RE-105 cells were resuspended in Tris-HCl buffer containing the indicated concentrations of sodium chloride (O), potassium chloride (▲), lithium chloride (■), or magnesium sulfate (□) and preincubated for 10 min at 37 °C before assaying for \[^{3}H\]glutamate binding. The data are expressed as the amount of glutamate binding compared to that in Tris-HCl buffer alone. Each data point is the mean of triplicate determinations from each of four to eight separate experiments.

the respective increase and decrease in \[^{3}H\]glutamate binding was the result of a change in receptor number relative to control values (Fig. 6). The \(B_{\text{max}}\) values for control, 10 mM CaCl\(_2\), and 10 mM NaCl-treated membranes were 15.5 pmol/mg of protein, 46.9 pmol/mg of protein, and 6.97 pmol/mg of protein, respectively, while \(K_D\) values were 654 nM, 523 nM and 435 nM, respectively. These data reveal a remarkable range of regulation of the glutamate receptor concentration by mono- and divalent cations, perhaps indicative of a link between binding sites and ion channels (22).

One possible explanation for the rapid calcium-induced increase in binding observed in N18-RE-105 and hippocampal membranes is that the glutamate receptor can be activated by coupling with calmodulin in response to increasing intracellular calcium concentrations. Trifluoperazine, a phenothiazine widely used as a specific inhibitor of calmodulin-mediated events (23), was therefore added to membrane homogenates along with CaCl\(_2\) during the preincubation period. Concentrations of 0.01–10 μM trifluoperazine (which did not affect binding when added alone) were ineffective at blocking CaCl\(_2\) stimulation of binding when the two compounds were added together (data not shown). Since the half-maximal dose for trifluoperazine inhibition of calmodulin's action is usually around 1 μM, it appears unlikely that calmodulin mediates the CaCl\(_2\) effect on glutamate binding.

**Regulation of Glutamate Binding by Anions—**All of the binding experiments described above were performed in 50 mM Tris-HCl buffer. In order to assess possible binding regulation by anions, the membranes were washed and resuspended in either Tris-HCl (as above), 50 mM Tris acetate buffer, pH 7.1 (Tris-Ac), or in 50 mM Tris citrate, pH 7.1 (Tris-Cit). In each of the three buffer systems, glutamate binding was performed in the appropriate buffer alone (control) or after a 10-min preincubation at 37 °C in the same buffer supplemented with either 1 mM Ca(Ac)\(_2\), 1 mM KCl, or 1 mM CaCl\(_2\). Control binding in Tris-Ac or Tris-Cit buffers was only about one-tenth of that measured using Tris-HCl buffer (Fig. 7). The addition of low concentrations of potassium chloride caused a marked increase in binding in the chloride-free buffer background. In Tris-Ac buffer, addition of calcium acetate also caused a sharp increase in glutamate binding, somewhat less than that caused by the addition of KCl. The addition of both calcium and chloride ions (CaCl\(_2\)) resulted in a synergistic effect leading to a 25-fold overall increase in glutamate binding. These data demonstrate that both calcium and chloride ions act to increase glutamate binding. In Tris-Cit buffer, calcium acetate did not increase glutamate binding, probably because of citrate's ability to chelate calcium. In this buffer, only the chloride effect was seen when CaCl\(_2\) was added. One mM CaCl\(_2\) (2 mM Cl\(^-\)) was no more effective at increasing glutamate binding than 1 mM KCl (1 mM Cl\(^-\)), suggesting that the chloride effect is saturated at 1 mM concentration. The data provide further evidence that chloride ions can act in addition to calcium ions to up-regulate the glutamate receptor.

**DISCUSSION**

Among several homogeneous neuronal cell lines tested, the neuroblastoma x rat embryonic retina cell line, N18-RE-105,
was shown to possess the highest concentration of specific membrane glutamate binding sites. Saturation isotherms reveal a single class of saturable sites having a $K_D$ of 650 nM and a $B_{max}$ of 16 pmol/mg of protein (Fig. 2), in excellent agreement with analysis of glutamate binding to rat cerebellar membranes. The pharmacology of these glutamate binding sites is also remarkably similar to that in rat brain (Table II). Most notably, quisqualate is the most potent inhibitor of $[^3H]$glutamate binding to N18-RE-105 membranes closely followed by unlabeled glutamate itself (Fig. 3). N-Methyl-D-aspartate and kainate showed negligible ability to inhibit binding of labeled glutamate to these neuroblastoma membranes (Fig. 3), supporting the hypothesis that they bind to sites which are pharmacologically distinct from the quisqualate/glutamate site defined here. In addition, the diaminodicarboxylic acid analogs such as D-α-aminoadipate are potent inhibitors of glutamate binding to both brain and N18-RE-105 membranes, as are the phosphono acid derivatives, all of which show a rank order of potency which mirrors that reported in electrophysiologic studies in brain. These data demonstrate a remarkable kinetic and pharmacologic similarity between the glutamate binding sites on N18-RE-105 cell membranes and those on rat brain, constituting the first report of such excitatory amino acid receptors on clonal neuronal cells in culture.

Glutamate receptors on N18-RE-105 cell membranes are subject to cation regulation similar to that reported for receptors on hippocampal membranes (14) and on forebrain synaptosomes (24). Membrane suspensions which are preincubated with calcium for 10 min at 37°C prior to being added to the glutamate binding assay mixture show sharply increased ligand binding. This effect is maximized using 10 mM Ca$^{2+}$ and declines at higher concentrations. Lanthanum ions, which are known to bind to calcium channels, produce a similar type of binding stimulation which is maximal at 1 mM La$^{3+}$. As with hippocampal and forebrain membranes, the calcium-induced increase in binding was shown, by Scatchard analysis, to be due to an increase in the number of receptors available to interact with free ligand. Although hippocampal membrane up-regulation is maximal at somewhat lower calcium concentrations (approximately 1 mM), the effect appears to be very similar to that on N18-RE-105 membranes.

When chloride ions were eliminated from the basic binding assay buffer, a stimulation of glutamate binding by added chloride ions was also revealed (Fig. 7). The stimulatory effect was even greater than that produced by calcium. As with brain membranes (13, 24), calcium and chloride produce a synergistic increase in glutamate binding on neuroblastoma membranes. However, while calcium appears to require the presence of chloride ions to produce an effect on hippocampal and forebrain membranes, it is capable of stimulating glutamate binding to neuroblastoma membranes in the absence of chloride ions. Monovalent cations and magnesium inhibited $[^3H]$glutamate binding on neuroblastoma membranes apparently by decreasing the $B_{max}$. As reported for hippocampal membranes (13), NaCl is approximately 10-fold more potent than LiCl, KCl, or MgSO4, at reducing glutamate binding. This finding also supports the pharmacologic data demonstrating that $[^3H]$glutamate is binding to a neurotransmitter receptor and not an uptake site, since sodium has been shown to stimulate binding to uptake sites while it inhibits binding in our system. Furthermore, amino acid transport studies in the N18-RE-105 clone did not reveal the avid uptake of L-$[^3H]$glutamate observed in brain synaptosomal preparations. Thus, cation regulation of neuroblastoma glutamate receptors is remarkably similar to the regulation of these receptors on hippocampal membranes.

Baudry and Lynch (13) have speculated that the inhibition of glutamate binding by monovalent cations may reflect an association between the receptor and sodium channels. Electrophysiological studies have in fact shown that glutamate produces its neuroexcitatory action by opening sodium channels and it is entirely reasonable that some form of autoregulation exists to limit damage which might result from overstimulation. Since glutamate also stimulates the conductance of calcium ions, an increased intracellular calcium concentration may trigger the up-regulation of the glutamate receptor which, in turn, is responsible for producing LTP in hippocampal neurons. As discussed in the introduction, there is a strong correlation between the formation of LTP and the calcium regulation of this receptor.

We have begun to probe the possible biochemical mechanisms underlying the described glutamate regulation. A calcium binding protein, calmodulin, regulates many calcium-mediated cellular events, and it seemed a likely candidate for involvement in the glutamate receptor regulatory mechanism. The use of trifluoperazine, a phenothiazine tranquilizer which has been shown to specifically inhibit the interaction of
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REFERENCES


