Glutamate as a Neurotransmitter in the Brain: Review of Physiology and Pathology[1]

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ABSTRACT Glutamate is the principal excitatory neurotransmitter in brain. Our knowledge of the glutamatergic synapse has advanced enormously in the last 10 years, primarily through application of molecular biological techniques to the study of glutamate receptors and transporters. There are three families of *ionotropic* receptors with intrinsic cation permeable channels [*N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate]. There are three groups of *metabotropic*, G protein–coupled glutamate receptors (mGluR) that modify neuronal and glial excitability through G protein subunits acting on membrane ion channels and second messengers such as diacylglycerol and cAMP. There are also two glial glutamate transporters and three neuronal transporters in the brain. Glutamate is the most abundant amino acid in the diet. There is no evidence for brain damage in humans resulting from dietary glutamate. A kainate analog, domoate, is sometimes ingested accidentally in blue mussels; this potent toxin causes limbic seizures, which can lead to hippocampal and related pathology and amnesia. Endogenous glutamate, by activating NMDA, AMPA or mGluR1 receptors, may contribute to the brain damage occurring acutely after status epilepticus, cerebral ischemia or traumatic brain injury. It may also contribute to chronic neurodegeneration in such disorders as amyotrophic lateral sclerosis and Huntington's chorea. In animal models of cerebral ischemia and traumatic brain injury, NMDA and AMPA receptor antagonists protect against acute brain damage and delayed behavioral deficits. Such compounds are undergoing testing in humans, but therapeutic efficacy has yet to be established. Other clinical conditions that may respond to drugs acting on glutamatergic transmission include epilepsy, amnesia, anxiety, hyperalgesia and psychosis. J. Nutr. 130: 1007S–1015S, 2000.

KEY WORDS: • *glutamate* • *excitotoxicity* • *domoate* • *neuroprotection* • *cerebral ischemia*

The excitatory action of glutamate in the mammalian brain and spinal cord has been known since the 1950s (Curtis and Watkins 1960, Hayashi 1952). It was not until the late 1970s, however, that it became widely recognized that glutamate is the principal excitatory transmitter within the vertebrate nervous system. At the same time, it was proposed that glutamate acts postsynaptically on three families of ionotropic receptors, named after their preferred agonists, *N*-methyl-D-aspartate (NMDA),[2] α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate. These receptors all incorporate ion channels that are permeable to cations, although the relative permeability to Na^+ and Ca^{++} varies according to the family and the subunit composition of the receptor. Molecular biological studies subsequently confirmed that there are three types of receptor, which are multimeric, with subunits that show high sequence homology within the three types (Hollmann et al. 1989, Hollmann and Heinemann 1994, Keinanen et al. 1990; Laurie et al. 1997, Lomeli et al. 1994, Monyer et al. 1992, Van den Pol et al. 1994) (see **Fig. 1**). Interestingly, the glutamate recognition sites within the ionotropic receptors show sequence homology with bacterial periplasmic amino acid transporters and plant peptides that are glutamate sensitive and are involved in photic responses, indicating the long evolutionary history of glutamate receptors (Lam et al. 1998).

In the late 1980s, it was proposed that there are also glutamate metabotropic receptors that are G protein linked and operate either by releasing second messengers in the cytoplasm or by influencing ion channels through release of G

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[2] Abbreviations used: ALS, amyotrophic lateral sclerosis; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BMAA, β-methylamino-L-alanine; BOAA, β-*N*-oxalylamino-L-alanine; β-ODAP, β-*N*-oxalyl-α,β-diaminopropionic acid; CA_1, CA_3, cellular zones of hippocampus (*cornu ammonis*); GABA, γ-aminobutyric acid; EAAC1, neuronal glutamate and aspartate transporter in rat brain; EAAT1–5, excitatory amino acids transporters 1–5, clones from human brain; EC_{50}, 50% effective concentration; EM, electron microscopy; EPSP, excitatory postsynaptic potential; GLAST (also GLAST1), rat glial glutamate and aspartate transporter; GLT (also GLT1), rat glial glutamate transporter; GluR1–4, glutamate A-D peptide subunits of the AMPA receptor; mGluR, metabotropic glutamate receptors, mGluR1–8 (there are presently eight); MK-801, dizocilpine; MND, motor neuron disease; NAAG, *N*-acetyl-aspartylglutamate; 3-NPA, 3-nitropropionic acid; NR1, NR2A,B,C,D, peptide subunits of the NMDA receptor; NMDA, *N*-methyl-D-aspartate; TGF-β, transforming growth factorβ.

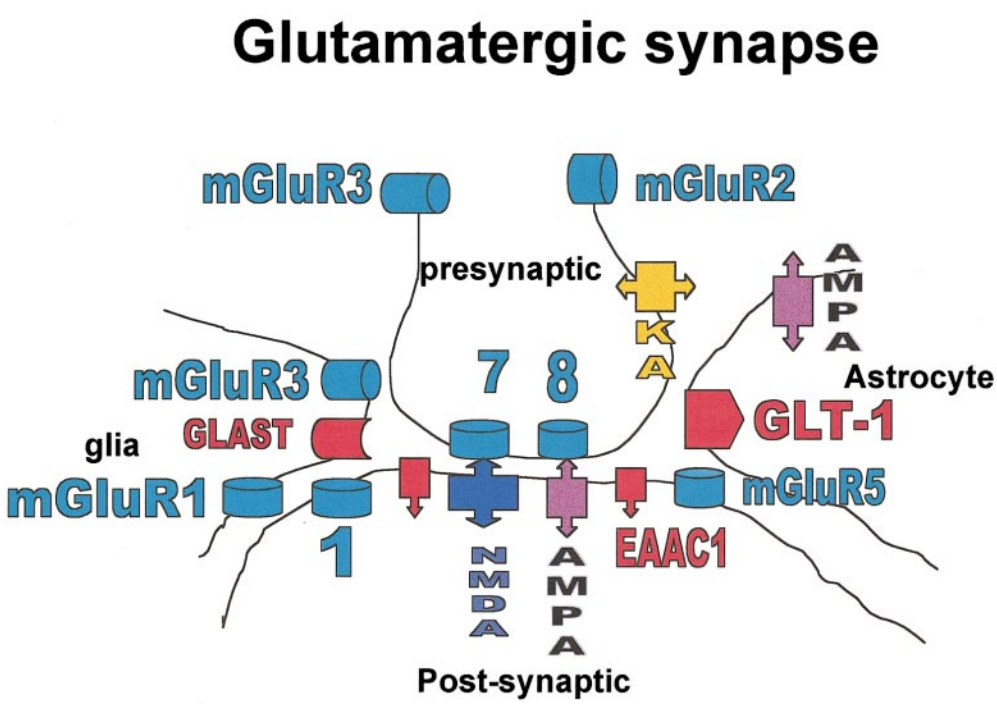

FIGURE 1 Diagram illustrating the plasma membrane location of glutamate receptors and transporters relative to the synaptic cleft. Note that metabotropic glutamate receptors (mGlu)R7 and mGluR8 are located within the presynaptic grid (Shigemoto et al. 1996), whereas mGluR3 and mGluR2 are located on the preterminal axon (Lujan et al. 1997). Group I mGluR (mGluR1, mGluR5) are predominantly perisynaptic on the postsynaptic membrane (Lujan et al.1996). The kainate ionotropic receptor acts presynaptically to decrease glutamatergic transmission in the hippocampus (Vignes et al. 1998) and is also functionally important presynaptically in γ-aminobutyric acid (GABA)ergic synapses (Rodriguez-Moreno and Lerma 1998). It also generates postsynaptic currents at specific sites in the hippocampus and amygdala (Li and Rogawski 1998). α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors and the neuronal glutamate and aspartate transporter in rat brain (EAAC)1 are all related to the postsynaptic density (Conti et al. 1998). The icon for EAAC1 is duplicated to emphasize that it is expressed at 15 times the density of AMPA receptors.

protein subunits within the membrane (membrane delimited effects) [see reviews by Conn and Pin (1997), Pin and Duvoisin (1995), Schoepp and Conn (1993)].

Other endogenous ligands for glutamate receptors

That L-aspartate has an excitatory role similar to that of glutamate has been known since the 1950s. Its possible neurotransmitter role remains controversial to this day. It has been claimed that, although it is taken up into neurons or into synaptosomes by the same carriers as glutamate, it is not transported into synaptic vesicles. Recent studies, however, strongly support the concept that aspartate is a neurotransmitter and is released from certain synapses (Gundersen et al. 1998). Sulfonic and sulfinic analogs of glutamate and aspartate (e.g., L-cysteine sulfinate, L-homocysteine sulfinate, L-homocysteate, L-cysteate) are also potential neurotransmitters (Thompson and Kilpatrick 1996) acting on "glutamate" receptors. Quinolinate is a compound synthesized by astrocytes that is a selective but relatively weak NMDA receptor agonist and produces a distinct pattern of neurodegeneration in the hippocampus and striatum after its focal injection (Beal et al. 1986, Schwarcz et al. 1983). When assayed on hippocampal neurons in the absence of Mg^{++}, glutamate is the most potent agonist [50% effective concentration (EC_{50}) 2.3 μmol/L] and quinolinate the least (EC_{50} 2.3 mmol/L) with the others intermediate (L-aspartate, EC_{50} 16.9 μmol/L; L-cysteate, EC_{50} 302 μmol/L) (Mayer et al. 1994). The endogenous dipeptide, *N*-acetyl-aspartyl-glutamate, (NAAG, spaglumic acid) is an agonist for NMDA receptors in the lateral geniculate nucleus (Harata et al. 1999).

NMDA receptors also have glycine or D-serine as coagonists (Johnson and Ascher 1992). The in vivo agonist may vary with the brain region, with D-serine playing a role in the forebrain but glycine being most significant in the cerebellum (Matsui et al. 1995).

For metabotropic receptors, NAAG is a selective agonist at metabotropic glutamate receptor (mGluR)3; L-serine-*O*-phosphate is a selective agonist for Group III receptors (mGluR4, mGluR6, mGluR7, mGluR8) (Thomsen and Suzdak 1993) and L-cysteine sulfinic acid is an agonist at the metabotropic receptor coupled to phospholipase D. (Boss et al. 1994).

Glutamate release

Glutamate is released from vesicles in presynaptic terminals by a Ca^{++}- dependent mechanism that involves N- and P/Q-type voltage-dependent Ca^{++} channels (Birnbaumer et al. 1994) that appear to be closely linked to vesicle docking sites. The glutamate concentration within the vesicle is thought to be ~100 mmol/L; release of a single vesicle produces an excitatory postsynaptic potential (EPSP) that is related primarily to AMPA receptor activation.

Glutamate may also be "released" by reverse operation of the glutamate transporters. This will occur when the Na^+ and K^+ gradient across the membrane is reduced during cerebral ischemia (Levy et al. 1998, Obrenovitch and Urenjak 1997).

The synaptic release of glutamate is controlled by a wide range of presynaptic receptors. These include not only the

Group II and Group III glutamate metabotropic receptors (see Fig. 1 and below) but also cholinergic (nicotinic and muscarinic) receptors, adenosine (A1), kappa opioid, γ-aminobutyric acid $(GABA)_B$, cholecystokinin and neuropeptide Y (Y2) receptors (see Meldrum 1998).

Glutamate ionotropic receptors

The three families of ionotropic receptors were first defined by their pharmacology and subsequently by their molecular biology. The receptors appear to be tetrameric (Laube et al. 1998) or pentameric and the subunits that comprise these are specific for each of the three families (Dingledine and Conn 2000). The subunit composition determines the biophysical properties of the receptor and to a variable extent its pharmacology. The most notable modification in AMPA receptor function is provided by the presence of a GluR2 (also known as GluRB) subunit, which prevents the open channel from showing a Ca^{++} conductance. Receptors expressing only GluR1 and GluR3 subunits show a significant Ca^{++} conductance. AMPA receptors have a lower glutamate affinity than NMDA receptors (see **Table 1**), but they have faster kinetics and are responsible for the fast initial component of the EPSP. The crystal structure of GluR2 bound to kainate was determined recently (Armstrong et al. 1998). Four α-helices form a bilobed extracellular structure with the agonist located in an interdomain crevice.

For NMDA receptors, the presence of NR1 (with several possible splice variants) appears invariant, whereas the selection of NR2 A, B, C or D subunits determines the time constants of opening of the channel and modifies the effect of various antagonists.

A distinctive feature of the NMDA receptor is its voltage-sensitive block by Mg^{++}. This is operative under normal circumstances but is overcome by partial depolarization of the resting membrane potential. A further specific feature is the need for glycine as a coagonist. Each receptor unit appears to have two glycine and two glutamate binding sites (Laube et al. 1998).

Ionotropic receptors have functional properties beyond that of opening ion channels. These are provided by the capacity of the intracellular carboxy terminal to interact with a variety of intracellular proteins. These include proteins involved in the spatial and functional organization of postsynaptic densities, but also proteins involved in signal transduction. For example, the AMPA receptor activates a protein tyrosine kinase, Lyn, that activates the mitogen-activated protein kinase pathway (Hayashi et al.1999).

TABLE 1

Glutamate: concentrations and affinities[1]

Approximate concentration in	
CSF	<1 μmol/L
Brain ECF	0.5–2 μmol/L
Plasma	30–100 μmol/L
Synaptic cleft	2–1,000 μmol/L
Brain (homogenate)	10 mmol/L
Synaptic vesicle	100 mmol/L
"Affinity" (ED_{50})	
GLT-1	1–20 μmol/L
NMDAR	2.5–3 μmol/L
mGluR2,3,4,8	5 μmol/L
mGluR1,5	10 μmol/L
AMPAR	200–500 μmol/L
mGluR7	1,000 μmol/L

[1] CSF, cerebrospinal fluid; ECF, extracellular fluid; ED_{50}, 50% effective dose; GLT, rat glial glutamate transporter; NMDAR, *N*-methy-D-aspartate receptor; mGluR, metabotropic glutamate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid receptor.

Glutamate metabotropic receptors (mGluR)

These receptors share a common molecular morphology with other G protein–linked metabotropic receptors, i.e., they are presumed to have seven *trans*-membrane domains with an extracellular N-terminal and intracellular COOH terminal. They have little sequence homology with other metabotropic receptors, except for a modest resemblance to $GABA_B$ receptors. Group I receptors activate phospholipase C, producing diacylglycerol and inositol triphosphate as second messengers. Groups II and III are negatively coupled to adenylyl cyclase. Studies using oocyte or human embryonic kidney cells expressing specific mGluR show marked variation in the sensitivity of the receptors to glutamate, with mGluR7 being remarkably insensitive (see Table 1). The sensitivity to glutamate has to be considered in relation to the location of the receptor on the cell membrane relative to the synaptic cleft. Immunochemistry at the electron microscopy (EM) level reveals a highly selective expression of mGluR (Lujan et al. 1996 and 1997, Shigemoto et al. 1996), with some occurring presynaptically in close relationship to the presynaptic density (mGluR7, mGluR8) and some occurring on the presynaptic axon, relatively distant from the synaptic cleft (mGluR2, mGluR3) (Fig. 1).

Glutamate transporters

Five glutamate transporters have been cloned from the mammalian central nervous system. Two are expressed predominantly in glia [glial glutamate and aspartate transporter (GLAST) and glial glutamate transporter (GLT)] and three in neurons [EAAC1, excitatory amino acid transporter (EAAT)4 and EAAT5] (in humans, these are referred to as EAAT1–5, respectively) (Seal and Amara 1999). They are all Na^+ dependent; in fact, the transmembrane gradients of Na^+ and K^+ provide the driving force for the transport. The suggested stoichiometry (for GLT) is one molecule of glutamate coupled to the cotransport of three Na^+ and one H^+ and the countertransport of one K^+ (Levy et al. 1998). Interestingly, the neuronal transporters seem to be linked to a Cl^- channel, which opens when glutamate binds, thereby tending to hyperpolarize the postsynaptic membrane and diminish synaptic activity. This phenomenon is thought to be functionally significant in Purkinje cells, which express EAAT4 prominently (Kataoka et al. 1997). The glial glutamate transporters have a marked differential regional distribution; GLT is predominant in the rat hippocampus, whereas GLAST is predominant in the cerebellum (Lehre and Danbolt 1998). There are also differences in the proximity of astrocytic processes to glutamatergic synapses, such that synaptic cross-talk may be possible at certain sites in the hippocampus. The rat neuronal transporter EAAC (equivalent to the human EAAT3 transporter) is highly expressed in the postsynaptic neuronal membrane (with up to 15 times the density of AMPA receptors); glutamate binding to this transporter contributes to termination of the excitatory postsynaptic current.

Plasma membrane glutamate transporters also transport D-aspartate and L-aspartate. The vesicular glutamate transporter has very different properties. It is driven by the proton gradient and appears to be selective for L-glutamate.

TABLE 2

Dietary "excitotoxins"[1]

Compound	Receptor action	Dose producing severe neurotoxicity: Rodent	Human	Brain pathology
Domoate	Kainate agonist	3–6 mg/kg ip	3 mg/kg orally	Limbic
Kainate	Kainate agonist	10 mg/kg ip	Unknown	Limbic (rodent)
Glutamate	Ionotropic agonist	0.5–4.0 g/kg ip (neonatal)	No known pathology	Hypothalamus (rodent)
3-Nitroproprionic acid	Mitochondrial poison	10–30 mg/kg sc (rat) 120 mg/kg ip (mouse)	No known pathology	Striatal (rodent)
BOAA, β-ODAP	AMPA agonist	No pathology in adult rodents	Unknown	Upper motorneurons in humans?

[1] ip, intraperitoneal; sc, subcutaneous; BOAA, β-*N*-oxalylamino-L-alanine; β-ODAP, β-*N*-oxalyl-α, β-diaminoproprionic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid.

Glutamate and neurodevelopment

Glutamate clearly plays an important role in neuronal differentiation, migration and survival in the developing brain. This is largely through facilitating the entry of Ca^{++} (Hack and Balázs1994, Yano et al. 1998).

Blockade of NMDA receptors during the prenatal period [as by dizocilpine (MK-801), phencyclidine or ethanol] can induce apoptosis in vulnerable neurons (the selectivity of the vulnerability depending on developmental stage) (Ikonomidou et al. 1999).

Glutamate and neurodegeneration

Glutamate is of particular interest to neurologists because of its possible involvement in acute or chronic neurodegenerative processes. It is useful to consider three distinct possible mechanisms. One is the possibility that exogenous glutamate, or related compounds acting on glutamate receptors, can be consumed in the diet and damage the brain. There is one well-documented example of such a phenomenon in humans.

Second, there is the possibility that endogenous glutamate released from neurons can contribute to acute neurodegeneration occurring in relation to cerebral ischemia or traumatic brain injury. Third, there is the possibility that activation of glutamate receptors contributes to the process of cell death in chronic neurodegenerative disorders, such as motor neuron disease (MND) or amyotrophic lateral sclerosis (ALS), Huntington's disease, Parkinson's disease and Alzheimer's disease.

Glutamate can be neurotoxic through an agonist effect on NMDA, AMPA, kainate or Group I metabotropic receptors. The relative contribution of these different classes of receptor varies according to the neurons involved and a variety of other circumstances. Selective neuronal death subsequent to status epilepticus appears to be highly dependent on NMDA receptor activation. Acute neuronal degeneration after transient global or focal cerebral ischemia seems to be dependent on both NMDA and AMPA receptors.

Susceptibility to excitotoxic cell death is under genetic control in a variety of ways. Single-gene defects may enhance vulnerability, as in the case of superoxide dismutase. Some induced gene defects in mice confer protection against excitotoxic damage (e.g., neuronal nitric oxide synthase-knockout mice show reduced sensitivity to focal ischemia). Genetic background can be protective. Thus C57BL/6 and BALB/c mice are relatively insensitive to the excitotoxic effect of kainic acid in the hippocampus (Schauwecker and Steward 1997).

Glutamate and related compounds as dietary toxins

This topic has been reviewed extensively (Meldrum 1993). Syndromes relating to acute toxicity are relatively easy to identify and validate by in vitro and in vivo animal experiments (**Table 2**). In cases in which chronic or delayed toxic effects are proposed, as with ALS of Guam, an excitotoxic mechanism is very difficult to validate.

Domoate poisoning. In humans, the only decisively documented example of a dietary toxin producing pathology through action on a glutamate receptor is that of domoic acid (Teitelbaum et al. 1990). Domoic acid is synthesized by marine diatoms (*Nitschia pungens*) and enters the food chain when it is concentrated by blue mussels (*Mytilus edulis*) feeding on the algae. In an outbreak of such poisoning in eastern Canada in 1987, affected individuals developed acute symptoms within 1–4 h of consuming 200–300 mg of domoate. An acute confusional state was the usual presenting feature, focal seizure signs were less commonly observed, but the picture was consistent with prolonged limbic seizure activity. A persistent anterograde amnesia was observed in some cases.

Neuropathologic studies in four elderly men who succumbed after days revealed extensive bilateral limbic system pathology with neuronal loss in cellular zones of hippocampus (CA_1, CA_3, dentate gyrus), amygdala, claustrum, septal area, thalamus and insular and subfrontal cortex. Similar patterns of damage can be induced by systemic injection of domoate or kainate in rodents, or by their focal injection into the hippocampus. The pathology is likely a consequence mainly of the limbic seizure activity rather than the effect of a direct excitotoxic action of domoate. This is shown by the observation that almost all of the pathology (commonly except for CA_3 cell loss and sometimes some amygdala damage) is prevented by the administration of an NMDA receptor antagonist (Jarrard and Meldrum 1993). It is likely that only the CA_3 neurons are dying as a direct result of the excitotoxic action of domoate. A similar protective effect against remote damage after kainate-induced limbic seizure activity can be obtained with diazepam (Ben-Ari et al. 1980).

Glutamate and hypothalamic lesions in neonatal rodents. In infant rats and mice (0–14 d old), the oral or intraperitoneal administration of high doses of glutamate or aspartate can be followed by acute neuronal degeneration in the retina (ganglion cells) and in various periventricular structures in the brain, including the arcuate nucleus of the hypothalamus (Olney 1971 and 1983, Olney et al. 1971). Whether this also occurs in primates is somewhat uncertain. Degeneration was reported by Olney et al. (1972) but not seen by several other authors (see Meldrum 1993). The effect, in infant rodents,

might possibly be related to the lesser capacity of their intestinal epithelium and liver to transaminate glutamate and aspartate, or to a lesser expression of the glial glutamate transporters GLT and GLAST in the hypothalamus at this developmental stage (Ullensvang et al. 1997). Developmental changes in the expression of ionotropic glutamate receptors are known to influence excitotoxic phenomena (Mitani et al. 1998) and may contribute to the pattern of vulnerability in the neonatal rodent.

BOAA and neurolathyrism. β-*N*-Oxalylamino-L-alanine (BOAA), also referred to as β-*N*-oxalyl-α,β-diaminopropionic acid (β-ODAP), is a toxin found in chick peas that is thought to be responsible for the syndrome of neurolathyrism, which is seen predominantly in malnourished young men and can have an acute or semiacute onset (Spencer et al. 1986). The observed motor disability arises predominantly from loss of upper motoneurons.

BOAA is a selective agonist for AMPA receptors and can cause excitotoxic cell death in neonatal rodents or in tissue culture (Willis et al. 1993). However, it does not produce the specific pathology of neurolathyrism in rodents or primates, although a transient neurological syndrome has been described in monkeys. It is possible that the human syndrome depends on some vitamin or other deficiency that impairs mitochondrial metabolism and renders neurons unusually vulnerable to an AMPA agonist.

BMAA and ALS of Guam. β-Methylamino-L-alanine (BMAA) is present in the fruit of the cycad that grows in Guam; it has been proposed that it could be the dietary toxin responsible for ALS of Guam (Spencer et al. 1987). BMAA is not directly excitotoxic; in cultures, it becomes toxic in the presence of bicarbonate (Weiss and Choi 1988). In rats, acute excitotoxicity is seen in the cerebellum after very high doses (1–4 g/kg). The low level of consumption and the very long latent period make it extremely unlikely that BMAA is acting as an excitotoxin to produce the ALS syndrome of Guam.

Mitochondrial toxins and excitotoxic lesions in the striatum. Another mechanism whereby activation of glutamate receptors leads to neurodegeneration involves mitochondrial toxins, such as malonate and 3-nitropropionic acid (3NPA), which inhibit succinate dehydrogenase and impair electron transport and ATP synthesis. A consequence of impairing the electron transport chain is that the neuron becomes vulnerable to excitotoxic and free radical damage. 3-Nitropropionic acid can be synthesized by fungi (*Arthrinium*) growing on sugar cane and thereby enter the food chain. It produces a pattern of selective damage in the striatum very similar to that seen in Huntington's disease, with preferential loss of GABAergic neurons (Alexi et al. 1998, Beal et al. 1993, Schulz et al. 1996). Activation of NMDA receptors clearly plays a part in this selective neuronal degeneration because NMDA receptor antagonists such as MK-801 can prevent the damage induced by systemically administered 3-NPA (Schulz et al. 1996). It is possible that reduction in resting membrane potential leads to reversal of the Mg^{++} block so that low concentrations of glutamate activate the NMDA receptor directly.

Endogenous glutamate and acute neurotoxicity

Glutamate acting on AMPA, NMDA and probably also mGluR1 receptors is thought to play an important role in cell death subsequent to status epilepticus, cerebral ischemia, perinatal asphyxia and traumatic brain injury. When the stress is severe, it leads to necrotic cell death; when it is less severe, apoptosis may be the consequence. The primary mechanism involved is ionic disequilibrium related to the excessive entry of Na^{+} and Ca^{++} through ligand-gated and voltage-sensitive channels. Raised intracellular [Ca^{++}] activates various enzymes (e.g., proteases, phospholipases, nitric oxide synthases or endonucleases) that contribute to cell death by various mechanisms (Meldrum and Garthwaite 1990). There is a complex interaction between the ionic changes, altered energy metabolism with poisoning of mitochondria and oxidative or free radical–mediated damage (Beal 1992). The role of the ligand-gated channels can be shown by using selective antagonists; thus NMDA receptor antagonists of all types (glutamate receptor competitive antagonists, glycine site competitive antagonists, open channel blockers and selective antagonists acting preferentially on a polyamine site or on the NR2B subunit of the NMDA receptor) protect against ischemic brain damage (Meldrum 1990). NMDA receptors have different subunit composition according to their site of expression. Receptors with NR2B subunits are expressed particularly on GABAergic interneurons, so that antagonists acting selectively on these NMDA receptors may have effects differing from those of antagonists acting on NMDAI/NR2A receptors.

Chronic neurodegeneration

It has been proposed that neurodegeneration in a variety of late onset neurological disorders is at least partially dependent on endogenous glutamate activating NMDA or AMPA receptors. These include motor neuron disease, Huntington's disease, Parkinson's disease and Alzheimer's disease.

The evidence that AMPA receptors on spinal motoneurons are involved in MND (ALS) is of several types (Leigh and Meldrum 1996, Ludolph et al. 1998). There appears to be a reduction in the expression of GLT-1, a glial glutamate transporter, in the spinal cord and brain regions showing loss of motoneurons (Rothstein et al. 1995). In organotypic cultures of spinal cord, glutamate transport inhibitors cause degeneration of motoneurons. This can be prevented by AMPA receptor antagonists such as GYKI 52466 (Hirata et al. 1997, Rothstein and Kuncl, 1995). AMPA receptor antagonists protect against the toxic effects of mutations in Cu/Zn superoxide dismutase in cultured mouse neurons (Roy et al. 1998).

Huntington's disease may involve a primary metabolic or mitochondrial defect that causes striatal neurons to become vulnerable to excitotoxic effects of NMDA receptor activation.

Glutamate metabotropic receptors and neurodegeneration

The predominant effect of Group I receptor activation is excitatory; agonists acting on mGluR1 or mGluR5 (such as 1*S*,3*R*-1-amino-1,3-cyclopentanedicarboxylate and 3,5-dihydroxyphenylglycine) when injected focally into the brain produce epileptic activity and focal neurodegeneration (Sacaan and Schoepp 1992).

This is probably related to reductions in several potassium conductances producing membrane depolarization. There is also potentiation of NMDA receptor–mediated conductance changes and excitotoxicity (McDonald and Schoepp 1992). There is, however, one Ca^{++} sensitive K^{+} channel that is opened by mGluR1 activation leading to hyperpolarization (Fiorillo and Williams 1998). That activation of group I mGluR contributes to cell death after cerebral ischemia and traumatic brain injury is suggested by reports that Group I receptor antagonists can be neuroprotective in model systems (Mukhin et al. 1996).

In cell cultures, a variety of effects have been described. In hippocampal cells expressing Group I mGluR but not iono-

tropic receptors, a protective effect of glutamate can be demonstrated against oxidative stress and against glucose deprivation (Sagara and Schubert 1998). Glutamate preexposure has the effect of up-regulating mGluR1 and mGluR3.

Nicoletti and his colleagues, in a remarkable series of studies employing cocultures of neurons and astrocytes, showed that activation of Group II receptors on astrocytes is neuroprotective via release of a neurotrophic factor, transforming growth factor β (TGF-β) (Bruno et al. 1997 and 1998a). NAAG, the endogenous mGluR3 agonist, is neuroprotective against striatal quinolinate lesions (Orlando et al. 1997) and against NMDA excitotoxicity in mixed cortical cultures (Bruno et al. 1998b). TGF-β and Group II mGlu agonists also protect against apoptosis induced by β-amyloid (Ren and Flanders 1996).

Glutamate synapses as therapeutic targets

On the basis of multiple animal models and limited human clinical data, it is clear that the glutamate synapse is a potential target for drug intervention in a very wide range of neurological and psychiatric disorders. These include epilepsy, amnesia, motor neuron disease, stroke, traumatic brain injury, pain, anxiety and psychosis. The relevant strategies are listed in **Table 3**. All of the approaches listed in the table have been shown to be effective in relevant animal models. Not all of the approaches have been tried in humans. An unsuccessful clinical trial is indicated as "−", but lack of initial success does not of course mean that the approach is not valid.

Epilepsy. Epilepsy appears to be an excellent target on the grounds that NMDA and AMPA receptor antagonists are powerful anticonvulsants in a wide range of animal models of epilepsy (see Meldrum and Chapman 1999). No pure NMDA or AMPA receptor antagonists have been introduced clinically, although several agents that show such properties mixed with other actions have been introduced recently (e.g., felbamate, NMDA antagonism; topiramate, AMPA antagonism) or are under trial (remacemide, NMDA antagonism). Antagonists at Group I and agonists at Group III metabotropic receptors also appear to be potential candidates for clinical trial in epilepsy (Chapman et al. 1999a and 1999b).

Amnesia. Amnesia is a particularly interesting target in that it has been proposed that compounds that potentiate glutamate's action at AMPA receptors, such as AMPAkines, may be useful. Memory enhancement has been demonstrated not only in animal models (Shors et al. 1995) but also in elderly humans (Lynch et al. 1997). D-Cycloserine, a partial agonist at the glycine site of the NMDA receptor, has been shown to enhance performance in various animal memory tasks and has been proposed as a therapy in Alzheimer's disease.

Motor neuron disease (amyotrophic lateral sclerosis). Motor neuron disease as explained above may involve defective glutamate transporters and enhanced AMPA receptor activation (Leigh and Meldrum 1996). Thus, antiglutamate strategies have been proposed and Riluzole has been shown to decrease mortality (Lacomblez et al. 1996).

Pain. Hyperalgesia clearly involves NMDA receptors in the spinal cord. Attempts at the clinical use of NMDA-antagonists have been limited by side effects, but it is possible that intrathecal administration may be a useful approach (Kristensen et al. 1992). Kainate receptors are expressed in C fibers, and GluR5-selective antagonists such as LY 294,486 appear to be analgesic (O'Neill et al. 1998).

Cerebral ischemia (stroke) and traumatic brain injury. NMDA and AMPA receptor antagonists have been shown to

TABLE 3

Therapeutic targets at the glutamate synapse

Disorder	Approach	Drug	Clinical Trial[1]	Reference
Epilepsy	NMDA antagonist	D-CPPene	−	Sveinbjornsdottir et al. (1993)
	AMPA antagonist	GYKI 52466		Chapman et al. (1991)
	mGluR1 antagonist	LY 367385		Chapman et al. (1999b)
MND (ALS)[2]	"glutamate antagonist" & Na^+ channel block	Riluzole	+	Lacomblez et al. (1996)
Amnesia	AMPA potentiation	CX 516	+	Lynch et al. (1997)
	NMDA potentiation	D-cycloserine		
Schizophrenia	NMDA potentiation	D-cycloserine		Goff and Wine (1997)
	NMDA gly antagonist	L-701,324		Bristow et al. (1996)
	Metabo. Gp II agonist	LY354740		Moghaddam and Adams (1998)
Stroke	NMDA antagonist	CGS 19755	−	Grotta (1995)
	AMPA antagonist	Eliprodil	−	Gotti et al. (1990)
		GV 150526		Bordi et al. (1997)
		NBQX		
Traumatic brain	NMDA antagonist	CGS 19755	−	Schmutz et al. (1997)
Injury	AMPA antagonist	Cerestat, CNS1102	−	Knapp et al. (1997)
	mGluR1 antagonists	Eliprodil		Toulmond et al. (1993)
	mGluR Gp III agonists	D-CPPene		Okiyama et al. (1997)
		CP 100581		Mukhin et al. (1996)
				Faden et al. (1997)
Hyperalgesia	NMDA antagonist	CPP		Kristensen (1997)
	KA GluR5 antagonist	LY294486		O'Neill et al. (1998)
Anxiety	NMDA antagonist	2-APH		Stephens et al. (1986)
	Metabo Group II agonist	LY 354740		Helton et al. (1998)

[1] Minus sign (−) indicates no clinical benefit; plus sign (+) indicates that a clinical benefit was seen, relative to placebo.
[2] MND, motor neuron disease; ALS, amyotrophic lateral sclerosis.

be powerfully neuroprotective in animal models of stroke (for reviews see Gill 1994, Meldrum 1990). In permanent or reversible occlusion of the middle cerebral arteries, these antagonists consistently reduce the volume of cortex that is infarcted 24 h or one or more weeks later. They do not protect the striatum. The protection is greatest if the antagonist is given close to the time of onset of the ischemia; efficacy is diminished with delay, and protection usually disappears with drug administration at 90–120 min post-arterial occlusion. These preclinical data, and similar data for rodent models of traumatic brain injury, have led to major clinical trials of NMDA receptor antagonists in stroke and in head injury. Some of these are listed in Table 3. To date, none of the trials has shown therapeutic benefit. Problems have concerned effects on cardiac rhythms and blood pressure (both hypotension and hypertension and cognitive side effects). A key problem is knowing the therapeutic time window in humans (many recent trials have assumed that it is 6 h). Some major trials are still in progress (e.g., the glycine site antagonist, GV 150526). The AMPA antagonists initially shown to be effective in the animal models have proved unsuitable for clinical trial, but several compounds under development are likely to go forward to clinical trial in the near future.

Psychosis. The similarity of the features of phencyclidine poisoning and acute schizophrenia has given rise to the hypothesis that impaired function or inactivation of some NMDA receptors may be a contributory factor in schizophrenia. This has also led to the suggestion that potentiation of NMDA receptor function may be a valid therapeutic approach and the clinical trial of glycine and D-cycloserine (Goff and Wine 1997). It is also suggested that standard antipsychotic drugs such as haloperidol and clozapine may be effective partially through NMDA receptor potentiation (Banerjee et al. 1995). A glycine site NMDA antagonist, L-701324, however, has a neuroleptic-like action in several animal models of psychosis (Bristow et al. 1996). It was reported very recently that the acute signs of phencyclidine intoxication in the rat can be reversed by the Group II metabotropic agonist LY 354740 (Moghaddam and Adams 1998).

Conclusion: future prospects

Glutamate ionotropic and metabotropic receptors can now be studied in expression systems in *Xenopus* oocytes or in mammalian cell lines. AMPA, NMDA and kainate receptors with specific subunit composition can be studied biophysically and used for screening novel drugs. In this way, it should be possible to identify powerful novel agents with highly selective actions in terms of function and the target brain region or cell type. Similarly, all of the cloned human metabotropic receptors can be used for screening novel compounds. The prospects for identifying novel therapeutic agents acting on glutamatergic transmission that are effective in the conditions described above are now exceptionally good.

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