

THE DIVERSE ROLES OF L-GLUTAMIC ACID IN BRAIN SIGNAL TRANSDUCTION

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L-Glutamic acid (Glu) is accepted as the major excitatory neurotransmitter in the nervous system, although other acidic amino acids such as L-aspartic acid and L-homocysteic acid may also participate (1). Nevertheless, ongoing research reveals that the functions of Glu are much more diverse and complex than simply generating excitatory postsynaptic currents (EPSCs). It plays a major role in brain development, affecting neuronal migration, neuronal differentiation, axon genesis, and neuronal survival (2–4). In the mature nervous system, Glu is central to neuroplasticity, in which there are use-dependent alterations in synaptic efficacy as well as changes in synaptic structure. These latter actions are intimately implicated in memory and related cognitive functions. Finally, persistent or overwhelming activation of glutamate-gated ion channels can cause neuronal degeneration (5) depending on the circumstances, this occurs by means of necrosis or apoptosis (6). Known as “excitotoxicity,” this phenomenon has been linked to the final common pathway of neuronal death in a range of disorders including Huntington’s disease, Alzheimer’s disease, amyotrophic lateral sclerosis (ALS), and stroke (7,8).

This chapter provides an overview of the physiology and pharmacology of brain glutamatergic systems. There is a special emphasis on glutamate receptors because their rich diversity confers physiologic and pharmacologic specificity for this single neurotransmitter, which is used by up to 40% of all brain synapses. Finally, the potential role of glutamatergic system dysfunction in the pathophysiology of neuropsychiatric disorders is addressed.

AMPA-KAINATE RECEPTORS

Glutamate receptors mediating fast EPSCs have been distinguished from the voltage-dependent NMDA receptors through the effects of conformationally restricted agonists. The former glutamate-gated ion channels (iGluRs) have been segregated into two types: the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and KA receptors; however, cloning of the genes that encode the proteins comprising these iGluRs and the analysis of their pharmacology and biophysics in various expression systems indicate that each family of receptors represents complex heteromeric proteins consisting of multiple subunits with differential representation resulting in diverse functional attributes (9).

The AMPA receptor family consists of four genes that encode proteins of approximately 900 amino acids with 70% homology among themselves (GluR1–4) (1); however, cell-specific exon splice variants and posttranscriptional editing result in a complex range of physiologic responses. Two exon splice variants of GluR1–4 known as “flip” and “flop” affect desensitization, with the flip form associated with larger, more sustained currents (10). Splice variants truncated at the carboxy terminus have been described for GluR2 and GluR4 as well as the kainate subunits, GluR5–7 (9). Furthermore, nuclear editing of the mRNA encoding GluR2 transforms this receptor channel from one permeable to Ca^{2+} to one impermeable to the cation. Double-stranded RNA adenosine deaminase converts the adenosine in a CAG codon for glutamine (Q) to an inosine, thereby creating a CIG codon for arginine (R) (11,12). The regulatory site for the editing process is located downstream in an intron that aligns with the exon to form the secondary structure recognized by the enzyme. Although the other AMPA receptor subunits are generally Ca^{2+} permeable, the presence of edited GluR2 dominates a heteromeric receptor

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complex in that the multisubunit AMPA receptor behaves like GluR2 (i.e., has low calcium permeability). In the mature brain, the vast majority of GluR2 is edited and the majority of AMPA receptors have low calcium permeability, suggesting that GluR2 is reasonably ubiquitous (see the following). Mice in which the GluR2 editing process has been inactivated by a null mutation exhibit increased Ca^{2+} permeability with AMPA receptor activation, epilepsy, and early death (13). Stroke is associated with the suppression of GluR2 expression in the penumbra, resulting in increased Ca^{2+} permeability through the remaining AMPA receptors, thereby causing delayed neuronal degeneration (14).

Five separate genes encode the components of the kainate receptor: GluR5–GluR7 and KA1–KA2 (1). Homomeric complexes of GluR5, GluR6, and GluR7 form ion channels in the *Xenopus* expression system that are activated by KA but not by AMPA. However, homomeric complexes of KA1 or KA2 do not generate functional ion channels, although they exhibit high-affinity binding for kainic acid. It appears that KA1 or KA2 in conjunction with GluR5, GluR6, or GluR7 form functional KA receptors. GluR5 and GluR6 possess the Q/R editing site in their second transmembrane (M2) domain, which modulates Ca^{2+} permeability as for GluR2. Additional editing sites on GluR5–7 have complex effects on the receptor-channel function (15,16). GluR5–7 are also widely represented in the brain, particularly in neocortex and hippocampus.

A number of modulators of AMPA/KA receptors have been identified that act by attenuating their rapid and profound desensitization. Cyclothiazide selectively inhibits desensitization of AMPA receptors, whereas the lectin, concanavalin A, blocks desensitization at KA receptors (17). “Ampakines” also enhance AMPA receptor activity by attenuating desensitization and have been shown to facilitate glutamatergic neurotransmission *in vivo*, thereby improving performance in several cognitive tasks (18). Polyamines, first noted for their effects on NMDA receptors, mediate rectification of Ca^{2+} -permeable AMPA and KA receptors by inducing a voltage-dependent channel blockade (19).

A number of conformationally restricted analogues have assisted in characterizing the activity of subsets of AMPA/KA receptors. Prior to development of AMPA, quisqualic acid was used as a non-NMDA receptor agonist; however, its specificity for AMPA receptors is poor as it also activates mGluR 1 and 5 metabotropic receptors and inhibits glutamate carboxypeptidase II (GCP II) (20,21). The conformational rigidity of AMPA provides good specificity for AMPA receptors, whereas the more flexible kainic acid interacts with KA receptors as well as other types of iGluRs. The orientation, length, and saturation of the side chain of kainic acid and its analogues play a critical role in their binding to the receptor site with domoic acid exhibiting higher affinity and dihydrokainic acid having much lower affinity than kainate (22). The first potent selective antagonists at AMPA/KA receptors with negligible effects at NMDA re-

ceptors to be developed were the dihydroxyquinoxalines: CNQX, DNQX, and NBQX. With the exception of NBQX, which has a somewhat higher selectivity for AMPA receptors, they do not distinguish between AMPA and KA receptors. More recently 2,3-benzodiazepines have been demonstrated to act as selective noncompetitive AMPA receptor antagonists. The most potent in this family, GYKI53655, blocks AMPA receptors with an IC_{50} of 1 μM and has a 200-fold lower affinity for KA receptors (23). The recently developed LY294486 has tenfold specificity for inhibiting GluR5, indicating that iGluR subtype specific ligands can be developed.

Most of our initial knowledge concerning the regional brain expression of KA/AMPA receptors was based on autoradiographic studies. Specific binding of [^3H]-KA is relatively enriched in the hippocampal CA 3, striatum, deep layers of the neocortex, the reticular nucleus of the thalamus, and the cerebellar granular cell layer. When considered in aggregate, immunohistochemical staining for the five KA receptor subunits shares this ligand binding distribution (26).

Audioradiographic studies of the distribution of [^3H]-AMPA binding show high density in the CA 1 stratum radiatum, the dentate gyrus, superficial layers of the neocortex, and the molecular layer of the cerebellum (25). Consistent with its role in mediating EPSCs, moderate levels of binding are observed throughout the rest of the central nervous system (CNS). The distribution of [^3H]-AMPA binding corresponds with the regional expression of GluR1 and GluR2, because levels of GluR3 and GluR4 are much lower in the adult rat brain and have a more restricted distribution (27).

Although the autoradiographic approaches have been very informative with respect to general distribution of the KA/AMPA receptors, they have been less informative with respect to cellular and synaptic distribution and differential subunit distribution. It has recently become possible to overcome these limitations on both spatial and biochemical resolution, and begin the arduous process of delineating the GluR subunit profile of identified neurons and circuits in specific brain structures such as the neocortex and hippocampus. This capacity is a direct result of molecular neurobiological analyses that have fostered a detailed understanding of the subunit protein constituents of the three classes of inotropic GluRs, and thus allowed for *in situ* hybridization to be used to localize specific mRNAs (28) and with the use of class and subunit-specific antibodies for immunocytochemistry, it is now feasible to analyze GluR distribution at the highest levels of cellular and synaptic resolution (29,30).

AMPA receptor subunit distribution in the hippocampus and neocortex offer an instructive example of such an approach. Although early studies demonstrated a wide distribution of AMPA subunits GluRs 2/3 in the brain and spinal cord (31–34), it became clear early on that the relationship

between GluRs and specific circuits needed to be analyzed at a high level of resolution; colocalization studies directed at subsets of neurons (29,35–38) and ultrastructural dissection of the synapse (39–44). A key theme that emerges from these studies is that regional distribution and cellular colocalization patterns should not be extended to a synaptic interpretation: Such interpretations must be founded on ultrastructural data as seen in the following examples.

The cellular distribution of GluR2 has been linked to heterogeneity in calcium permeability of AMPA receptors (45,46). For example, electrophysiological analyses have demonstrated that pyramidal cells have AMPA receptors with low calcium permeability, and interneurons have AMPA receptors with relatively high calcium influx (47,48), and these properties are linked to the relative abundance of GluR1 versus GluR2 mRNAs (45,46). In addition, early reports using polyclonal antisera that did not differentiate among GluR2, 3, and 4c, obtained results implying that GABAergic interneurons might not contain GluR2, 3, and 4c (49–51).

Definitive conclusions regarding selective distribution of GluR2-specific protein were not possible until 1996, when an exclusively GluR2-specific monoclonal antibody (52), followed by a rabbit polyclonal (53) were developed. The GluR2 antibodies showed that virtually all pyramidal cells and the majority of GABAergic interneurons in the neocortex (e.g., S1) contain GluR2. A similar pattern was found in hippocampus, suggesting that the majority of the GABAergic interneurons in hippocampus are GluR2-positive, although a subset of GABAergic neurons lacks any detectable GluR2 (52,53), as in neocortex. These results are in excellent accord with the GluR2 mRNA results obtained by single cell RT-PCR studies (45,46), and suggest that a minority of the GABAergic interneurons lack GluR2 mRNA/protein. Thus, the differences in calcium permeability between GABAergic interneurons and pyramidal cells could not be the result of a widespread lack of GluR2 in GABAergic interneurons.

A double label GABA/GluR2 analysis that was extended to the ultrastructural level further clarified the issue of GluR2 representation in GABAergic interneurons (54). It was hypothesized that if the difference in calcium permeability between pyramidal and GABAergic neurons was related to differences in GluR2 expression, then it would likely be more apparent on the synaptic than the cellular level (54). Ultrastructural analysis revealed that there is a consistently lower number of immunogold particles at the labeled asymmetric synapses on GABAergic dendrites than those on pyramidal cell dendrites or spines, suggesting that a cell class-specific difference in synaptic abundance of GluR2 is the substrate for the observed differences in calcium permeability across these two cell classes revealed electrophysiologically (45,46).

As demonstrated in the GluR2 studies discussed in the preceding, cellular colocalization may not adequately reflect

the localization patterns at the synaptic level. This was reinforced in studies of GluR2/NR1 colocalization in hippocampus and neocortex, designed to delineate the degree of synaptic colocalization of NMDA and AMPA receptors in asymmetrical synapses (39–43). Although NR1 and GluR2 are broadly colocalized on a cellular level, extensive synaptic heterogeneity exists in their representation. NR1 and GluR2 are often colocalized at the same synapse; however, there are also a large group of NR1-containing synapses that lack GluR2 labeling (33% in [39]), many of which were on spines. These may be candidates for the “silent synapses” that have been described electrophysiologically (55,56) that might be activated by insertion of AMPA subunits (40,42, 55–57).

It has been generally recognized that AMPA receptors play a dominant role in mediating EPSCs. A physiologic role for KA receptors has been elucidated only recently with the development of more selective agonists and antagonists. In the hippocampal slice in which the AMPA, NMDA, and GABA receptors have been blocked pharmacologically, stimulation of the mossy fibers generates a slow excitatory synaptic current system with the biophysical properties of the KA receptor (58). This current is absent in mice homozygous for null mutation of the GluR6 subunit and less vulnerable to the epileptogenic effects of systemic KA (59). The presynaptic inhibitory effect on GABA release in the CA 1 region of the hippocampus is mediated by the GluR5 subunit (60).

Although KA subunits have not been localized as extensively at the ultrastructural level as have AMPA or NMDA receptors, immunocytochemical studies have demonstrated their broad distribution in the hippocampus and neocortex and broad colocalization with AMPA and NMDA receptor subunits (38,61) in both pyramidal and GABAergic interneurons (35,36).

NMDA RECEPTORS

The NMDA receptor, as its name indicates, was identified by the selective excitatory effects of the synthetic analogue of glutamate, N-methyl-D-aspartic acid (1). A number of properties distinguishes the NMDA receptor from the non-NMDA iGluRs. First, its activity is voltage dependent. At resting membrane potential, the channel is blocked by Mg^{2+} , which is relieved by membrane depolarization. Second, the receptor requires occupancy of another ligand binding site, the so-called glycine modulatory site, in order for glutamate to gate channel opening. Recent evidence indicates that not only glycine but also D-serine, which is synthesized in astrocytes by serine racemase, is a potent endogenous agonist at the glycine site (62). Third, the NMDA receptor possesses a number of modulatory sites of physiologic significance. Zn^{2+} is a potent inhibitor of NMDA receptor conductance, especially those containing the NR2A subunit (24). Zn^{2+} is concentrated in some gluta-

matergic terminals (e.g., the mossy fibers) and released with glutamate (63). A binding site for polyamines, when occupied, enhances conductance in part through increasing the affinity of the glycine modulatory site on the NMDA receptor (64). Receptor function is also modulated by redox status (65). Within the channel, there is a binding site for the dissociative anesthetics such as phencyclidine (PCP), MK-801, and ketamine, which serve as noncompetitive inhibitors (66). The effects of the dissociative anesthetics occur only with open channels, thereby causing a use-dependent inhibition. Finally, the NMDA channel provides ready passage of Ca^{2+} , a cation involved in a number of intracellular signaling processes.

Molecular cloning has disclosed at least six genes that comprise a family of polypeptides that form the various subtypes of the NMDA receptor (1). NR1 was the first component cloned and, when expressed in *Xenopus* oocytes, was shown to possess the primary electrophysiologic and pharmacologic features of the NMDA receptor-channel complex. Seven splice variants of NR1 have been described, which reflect the exclusion or inclusion of three exons, two in the C terminal and one in the N terminal portion (1). These splice variants significantly impact the biophysical characteristics of the receptor. The NR2 subunits, NR2A-D and the recently identified NMDARL or NR3A, do not form channels (67); however, when coexpressed with NR1, the heteromeric channels exhibit a markedly increased current as compared to the homomeric NR1 channels. Each of the NR2 subunits, when complexed with the NR1 subunit, exhibits different biophysical and pharmacologic properties. The NR2A and NR2B subunits, more highly expressed in adult cortex in contrast to NR2C/D receptors, appear to be less sensitive to NMDA receptor antagonists, not as vulnerable to Mg^{2+} blockade, and have lower Ca^{2+} conductance (1).

NR1 is expressed in most neurons, whereas the NR2 mRNAs exhibit different regional and developmental patterns of expression (68,69). The NR2A subunit is highly expressed in the neocortex, hippocampus, cerebellum, and several thalamic nuclei. The NR2B subunit is found in the neocortex, hippocampus, striatum, septum, and thalamic nuclei of the adult rat brain. The expression of the NR2C subunit is much more restricted in the adult brain, being enriched in the olfactory bulb, thalamic nuclei, and cerebellum. Finally, the NR2D subunit is enriched in brainstem nuclei, midline thalamic nuclei, and bipolar cells of the retina. The NR2B and NR2D subunits appear early in brain development, followed by a decline in NR2D expression in the third week after birth of the rat, whereas the acquisition of NR2A and NR2C subunits appears primarily postnatally in the rat (1).

With respect to NMDA receptor localization, as in the case of AMPA/kainate receptors, the early *in situ* hybridization studies discussed in the preceding offered important information as to the regional distribution of NMDA recep-

tors in the brain. These studies have been followed with very extensive immunocytochemical analyses, particularly of the obligatory subunit NR1. With respect to hippocampus and neocortex, NR1 is very broadly distributed and present in virtually all pyramidal neurons and nonpyramidal GABAergic interneurons (29,38), and in fact, appears to be present in over 90% of asymmetric synapses (30,41). NR1 distribution has also been shown to be modifiable on both the cellular and synaptic level with respect to plasticity. For example, deafferentation causes rearrangements of NR1 distribution at the cellular and synaptic level in a matter of days (70,71). In addition, although NMDA receptors have a very broad distribution on a cellular level, they can display a high degree of specificity on a synaptic basis. The most dramatic example of this is in CA3 of the hippocampus, where NMDA receptors are present postsynaptically in the distal dendrites (i.e., stratum moleculare), yet are absent in the stratum lucidum terminal zone, which is noted for LPT being NMDA receptor-independent (37,56,72). This suggests that there are intracellular trafficking mechanisms or local synthesis that can position NMDA receptors in a subset of synapses receiving a particular input to a given cell while not mediating other inputs.

The data on the distribution of other subunits are less well developed, and this is partly owing to the fact that it has been very difficult to develop antibodies that differentiate NR2A from NR2B, the two dominant subunits in the NR2 group in the hippocampus and neocortex. In general, it appears that NR2A and NR2B overlap in their distribution with NR1 to a large degree (73–75); however, there are regions such as CA3 where they differ in their distribution with NR1 (75,76), but this has yet to be worked out at the synaptic level. The detailed delineation of the synaptic distribution of NR2A and NR2B is an important task for the future given that the presence of these subunits confer different functional attributes on the receptor, and the analysis of genetically manipulated mice have suggested that up- or down-regulation of one of these subunits can profoundly impact their function. For example, NR2B overexpression in mouse enhances learning and memory (77). Thus, as is the case for AMPA and kainate receptors, delineating the subunit representation and potential stoichiometry at specific circuits and synapses for the NMDA receptor is of paramount importance if this receptor is to be definitively linked to circuits that mediate specific behaviors and suffer under certain pathologic conditions.

Several endogenous amino acids, aside from glutamate, are selective agonists at the NMDA receptor, including L-homocysteic acid, L-aspartic acid, L-cysteine sulfate, L-serine-O-sulfate, L-cysteic acid, and quinolinic acid in order of decreasing potency (78). Given the multiple modulatory sites and agonist binding sites for the NMDA receptor, it is not surprising that the antagonist pharmacology for this receptor is complex. Several phosphonate analogues of glutamate, including D-aminophosphonovaleric acid (APV),

D-aminophosphonoheptanoic (APH) acid, D-aminoadipic acid, and the cyclic analogue of AHP, (2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP), are competitive inhibitors to glutamate. As described, the NMDA receptor channel has a binding site for dissociative anesthetics, which shares pharmacologic features with the σ receptor. The glycine modulatory site, which must be occupied for glutamate gating of the ion channel, is subject to inhibition by the endogenous metabolite of tryptophan, kynurenic acid as well as synthetic analogues such as 7-chlorokynurenate (79). For greater selectivity, attention is now directed at developing subtype specific antagonists such as ifenprodil, which inhibits NMDA receptors bearing the NR2B subunit (80).

METABOTROPIC GLUTAMATE RECEPTORS

It was generally believed that the neurophysiologic effects of glutamate were mediated exclusively by iGluR, until Sladeczek and colleagues (81) reported that glutamate catalyzed phosphoinositide hydrolysis through a receptor coupled to a G-protein. Since then, research has disclosed the existence of a family of glutamate receptors whose effects are largely mediated by G-proteins, the so-called metabotropic glutamate receptors (mGluRs). The mGluRs appear to play an important role modulating both presynaptically and postsynaptically the effects of glutamate at glutamatergic synapses where iGluRs are also engaged (82).

Cloning of mGluR 1 indicated that the predicted amino acid sequence shared negligible homology with any of the other G-protein coupled receptor (GPCR) families except the parathyroid Ca^{2+} -sensing receptor (83) and the GABA-B receptor (84). On the basis of pharmacology, physiologic effects, and sequence homology, the eight mGluRs have been subdivided into three groups (85). Group I includes mGluR 1 and mGluR 5, which act via phospholipase C; group II includes mGluR 2 and mGluR 3, which are negatively coupled to adenylyl cyclase; and group III, which includes mGluR 4, 6, 7, and 8, and are also negatively coupled to adenylyl cyclase. Adding to this complexity, mGluR 1 has four splice variants, whereas mGluR 4 and 5 each have two. The mGluRs within a group exhibit greater than 70% sequence homology, whereas the homology falls to approximately 45% between groups (82).

Like other GPCRs, the mGluRs have seven putative transmembrane domains separated by short intracellular and extracellular loops and an unusually large extracellular domain that contains nearly a score of cysteine residues (86). In further contradistinction to other GPCRs, the agonist binding sites for the mGluRs are located in the extracellular domain. As a family, mGluRs are broadly expressed in the nervous system, with group I having primarily a postsynaptic localization, whereas groups II and III primarily have a presynaptic localization where they serve as autoreceptors

and heteroreceptors that negatively regulate neurotransmission (82). mGluR 1 is prominently expressed in the hippocampal granule and pyramidal cells, the Purkinje cells of the cerebellum, the thalamus, and the lateral septum (87). mGluR 5 exhibits somewhat of a complementary distribution with high levels expressed in the neocortex, the pyramidal cells in CA1 sector of the hippocampus, lateral septum, striatum, and nucleus accumbens (88). Although studies *in vivo* suggest a low level of expression of mGluR 5 in glia, cultured astrocytes exhibit high expression (89). The expression of group II receptors is more restricted than group I, with mGluR 2 found in the cerebellum, pyramidal cells in the entorhinal cortex and the dentate gyrus, and presynaptically on corticostriatal afferents (90). The majority of the cerebellar Golgi cells have mGluR 2 but a minor subset express mGluR 5 in a complementary fashion (91). mGluR 3 is found in astrocytes throughout the brain and in neurons in the neocortex, caudate putamen, thalamic reticular nucleus, and granule cells of the dentate gyrus (92). With regard to group III, mGluR 4 is found in the thalamus, lateral septum, dentate gyrus, and cerebellar granule cells (92). mGluR 7 has a widespread distribution with prominent representation in sensory afferent systems including the dorsal root ganglia and the trigeminal nucleus in addition to the cerebral cortex, hippocampus, striatum, thalamus, and Purkinje cells (93). mGluR 6 is restricted to on-bipolar cells in the inner nuclear layer of the retina (94), with a highly specific synaptic distribution (95). Finally, mGluR8 is found in the mitral cells of the olfactory bulb, piriform cortex, and lateral thalamic reticular nucleus (96).

Ultrastructural studies have demonstrated that mGluRs differ in their predominant synaptic distribution from AMPA and NMDA receptors. Although AMPA and NMDA receptors are localized predominantly in the postsynaptic density, mGluRs are principally localized presynaptically and perisynaptically (97–99). In addition, the mGluRs exhibit a high degree of specificity in this regard with mGluR7 (100,101) and mGluR4 (102) (which are primarily present presynaptically) and mGluRs 1 and 5 (which are primarily present perisynaptically) (103–105). These patterns suggest that synaptic localization is partially segregated by mGluR group (99), with group I primarily perisynaptic, surrounding the postsynaptic density, group II primarily presynaptic or extrasynaptic, and group III optimally situated as an autoreceptor on the presynaptic terminal. These synaptic distribution patterns position mGluRs to play a critical role in modulating excitatory neurotransmission.

Group I mGluRs stimulate phospholipase C and the hydrolysis of phosphoinositide. These two receptors have been reported to stimulate cAMP formation in different model systems (106). They also increase the excitability of neurons by reducing the K^+ currents, through a mechanism that appears to be independent of G-protein action (107). Although the AMPA receptor agonist, quisqualic acid, is the

most potent agonist at the group I mGluRs, 3,5-dihydroxyphenylglycine (3,5-DHPG) is the most specific agonist. Group II mGluRs inhibit adenylyl cyclase by coupling with the G_{T} -protein. (See ref. 43 for a detailed review of mGluR pharmacology.) Group II receptors also inhibit the N-type Ca^{2+} channels through $G\text{I}$ -protein coupling. The group III mGluRs inhibit adenylyl cyclase via $G\text{I}$ -protein, although they may utilize other transduction mechanisms.

The most specific agonist at group I mGluRs appears to be 2R, 4R-4-aminio pyrrolidine-2, 4 dicarboxylate (APDC). LY354740 is an exceptionally potent and selective agonist at group II mGluRs and is effective with systemic administration (108). Notably, N-acetylaspartyl glutamate (NAAG), an endogenous neuropeptide, is a relatively potent agonist at mGluR 3, although it also serves as an antagonist at the NMDA receptor (109). L-aminophosphonobutyric acid (L-AP4) is the most potent and selective agonist at the group III mGluRs with the exception of mGluR 7, where L-serine-O-phosphate has greater potency. Presently, 2-carboxy-3-phenylcyclopropyl glycine (CPCG) is the most potent antagonist against the group II mGluRs, and α -methyl-4-phosphonophenyl-glycine appears to be an effective antagonist against the group III mGluRs, although the pharmacology of these receptors remains less well developed than the group I mGluRs.

The heterogeneity of the mGluRs and their role in modulating glutamatergic neurotransmission make them attractive potential therapeutic targets for drug development. Thus, activation of group II and group III mGluRs has been associated with protection against excitotoxicity, whereas activation of group I mGluRs may actually enhance NMDA receptor-mediated neuronal degeneration (110,111). Recently it has been shown that inhibition of GCP II, the enzyme that degrades the selective mGluR 3 agonist NAAG, provides potent protection against neuronal degeneration caused by transient occlusion of the middle cerebral artery (112). A similar reciprocal relationship has been observed with regard to the effects on epilepsy with group I agonists exacerbating and group II and III agonists attenuating seizures (113). Finally, metabotropic receptors, both in the dorsal root and thalamus, have been implicated in modulating neuropathic pain (114).

GLUTAMATE TRANSPORTERS

The demonstration of sodium-dependent high-affinity transport of glutamate in synaptosomal preparations was the first evidence supporting the hypothesis that glutamate serves as a neurotransmitter (115). The presence of these transporters on excitatory nerve terminals was exploited to label putative glutamatergic pathways such as the climbing fibers of the cerebellum through the autoradiographic visualization of retrogradely transported radiolabel in axons and cell bodies. The transporters are capable of maintaining ex-

traordinary gradients with the extracellular concentration of glutamate in brain low μM (i.e., below the threshold for iGluRs) in the face of a tissue concentration in the mM range (116). Energy deprivation not only collapses the sodium gradient across the membrane that drives the transporter, thereby inhibiting glutamate uptake, but also results in reverse transport with massive efflux of glutamate stores (117). Pharmacologic inhibition of glutamate transport in tissue culture models has been shown to promote excitotoxic neurodegeneration (118).

Early pharmacologic studies pointed to the existence of subtypes of sodium-dependent glutamate transporters in brain with cerebellum exhibiting a form that is much more sensitive to inhibition by L- α -amino adipate and forebrain sensitive to dihydrokainate (119). Both the pharmacologic heterogeneity as well as the diverse cellular distribution of the sodium-dependent glutamate transporters have been illuminated recently by their cloning and molecular characterization. (See refs. 120 and 121 for review.) Although acronyms have varied with the sequence of discovery, five excitatory acidic amino acid transporters (EAAT) that are sodium dependent and chloride independent have been cloned. EAAT 1 or GLAST has its highest expression in brain but is also found in peripheral tissue and placenta. The cerebellum appears to have the highest level within brain, depending on the species. EAAT 2 (GLT 1) is primarily expressed in brain, although low levels have been reported in pancreas and placenta; the highest expression occurs in the forebrain, the lowest in the cerebellum. Its expression is predominantly if not exclusively astroglial in localization. The predominant neuronal transporter is EAAT 3, which is also expressed in kidney and to a lesser extent in other peripheral tissues. Consistent with the broad distribution of glutamatergic neuronal systems in brain, the levels of EAAT 3 are fairly uniform. EAAT 3 is not consistently expressed in all glutamatergic systems, and some non-glutamatergic systems express it (122). Thus, EAAT 3 does not appear to be a specific marker for glutamatergic neurons. Two additional minor forms have been identified: EAAT 4, which is expressed in cerebellar Purkinje cells; and EAAT 5, which is limited to the retina.

Like the mGluRs, the neuronal transporter EAAT3 (i.e., EAAC1) has been shown to have a synaptic distribution different from the AMPA or NMDA receptors in that it is primarily perisynaptic and presynaptic (44,123). A double-label postembedding immunogold study demonstrated the value of such ultrastructural data for revealing the importance of differential distribution of these proteins with respect to the synapse (44). The double-label analysis targeted the AMPA subunit GluR2 and the glutamate transporter, EAAT3 (i.e., EAAC1), the neuron-specific glutamate transporter. This study revealed differential spatial distribution of these two proteins very clearly. At the light microscopic level, as expected, GluR2 was broadly colocalized with EAAC1 in hippocampal projection neurons, and both pro-

teins had substantial cytoplasmic pools. However, the post-embedding immunogold localization offered additional insights into the spatial relationships between EAAT3 and GluR2 localization in and near the synapse, revealing morphologic and molecular constraints on excitatory synaptic transmission. Specifically, synaptic GluR2 was present primarily in the postsynaptic specialization, appropriately positioned to mediate the synaptic effects of glutamate. EAAT3 was not intermingled with GluR2 postsynaptically, but was generally present perisynaptically, often immediately outside the synaptic specialization, with a small but significant presynaptic pool as well (44). This arrangement positions EAAT3 to both confine glutamate to the synaptic site that contains the ionotropic receptor molecules, as well as to regulate its levels in immediately adjacent presynaptic and postsynaptic domains. This distribution is also interesting with respect to the distribution of mGluRs, which are located perisynaptically and extrasynaptically (see the preceding). This suggests that EAAT3 is also optimally positioned to regulate the exposure of perisynaptic and presynaptic mGluRs to glutamate (44).

The EAATs exhibit affinities for glutamate in the low μM range, two orders of magnitude more avid than the vesicular transporter for glutamate, which is not sodium dependent. A generally accepted model for transport involves the binding of three Na^+ , one H^+ , and glutamic acid, which is linked to the counter transport of one K^+ (124). In addition, there is increasing evidence that individual EAAT subtypes may also subserve signal transduction activity; thus, EAATs have been implicated in inhibition of adenylyl cyclase, altering Ca^{2+} levels and Cl^- flux by mechanisms independent of glutamate transport (121).

Pharmacologic inhibition of EAAT activity potentiates excitotoxic effects in tissue culture (118). Mice homozygous for the nul mutation of EAAT 2 develop fatal epilepsy and exhibit increased vulnerability to excitotoxic insults (125), and mice homozygous for the nul mutation for EAAT 1 exhibit cerebellar dysfunction (126). Thus, in contrast to the original hypothesis of the essential role of neuronal glutamate transport in terminating excitatory neurotransmission, it is now apparent that the glial transporters play the dominant role in neuroprotection from glutamate. In this regard, astroglial processes are tightly interdigitated with glutamatergic and GABAergic synapses, where the transporters are expressed in high density, which accounts for the previous identification of the "synaptosomal" localization of glutamate transport (127).

The activity of the glutamate transporters is regulated by transcriptional as well as posttranslational mechanisms. (See ref. 128 for review.) GLT-1 expression, which is normally low in primary astrocyte cultures, is significantly increased in astrocytes by coculture with neurons (129). Furthermore, subsequent destruction of the neurons results in down-regulation of GLT-1 but an up-regulation of GLAST protein (130). Fornix transection results in down-regulation of both

GLT-1 and GLAST in the innervation field (131). The neuronal signal mediating this interaction has proved to be somewhat elusive but may in fact be glutamate itself acting at AMPA/KA receptors on astrocytes. PKC has also been implicated in the regulation of glutamate transporter activity both directly and by altering trafficking (136).

GLUTAMATE AND GLIA

The neuroprotective action of glutamate transporters expressed by astroglia represents only one facet of the critical role astroglia play in modulating glutamatergic neurotransmission. Astrocytes express a high-affinity Na^+ -dependent transporter for glycine, GlyT-1, which maintains concentrations of glycine that are subsaturating for its modulatory site ($K_d = 20 \text{ nM}$) on the NMDA receptor in spite of μM levels in cerebrospinal fluid (CSF) (133). In addition, forebrain astroglia express serine racemase, which generates D-serine, a potent agonist at the glycine modulatory site (31). The metabolism of tryptophan by a series of enzymes expressed in glia generates both positive and negative modulators of the NMDA receptor (134). Of these, quinolinic acid is an agonist at NMDA receptors. Although its affinity for the receptor is relatively low, lack of efficient clearance mechanisms renders it a pathophysiologically significant NMDA receptor agonist. For example, the levels can achieve toxic concentrations in HIV encephalopathy with the activation of microglia and infiltration of macrophages highly expressing quinoline (135). Kynurenic acid, another metabolite of tryptophan, is a noncompetitive antagonist at the NMDA receptor acting at the glycine modulatory site. The synthesis of kynurenic acid in brain takes place almost exclusively in astrocytes and is regulated by cellular energy status, ionic environment, local 2-oxo acid concentration, and dopaminergic neurotransmission (136). Altered levels of kynurenic acid in disease states such as Huntington's disease (HD) and schizophrenia appear to correlate with NMDA receptor dysregulation (137).

N-acetylaspartyl glutamate is an abundant neuropeptide found in many but not all glutamatergic systems and several well-characterized nonglutamatergic systems such as the locus ceruleus and motor neurons. Located in storage vesicles and subject to evoke release by a Ca^{2+} -dependent process, NAAG is a noncompetitive antagonist at NMDA receptors (138) and a selective agonist at the mGluR 3 (60). It is metabolized to N-acetylaspartate and glutamate by GCP II, which is selectively expressed by astrocytes (139). Postmortem studies indicate reduced activity of GCP II in hippocampus, temporal cortex, and frontal cortex in schizophrenia, a disorder potentially involving hypofunction of NMDA receptors (140), and increased activity in motor cortex and dorsal horn, which would increase extracellular glutamate generated from NAAG in the neurodegenerative disorder ALS (141).

GLUTAMATE AND NEURODEGENERATION

The term “excitotoxicity” was coined by Olney 30 years ago to designate a selective form of neurodegeneration caused by systemic treatment of newborn rodents with monosodium glutamate (5). Degeneration of neurons with their perikarya in the arcuate nucleus of the hypothalamus was apparent 90 minutes after injection; notably, axons passing through the lesion and glia were spared. As the neurotoxic effects of glutamate analogues correlated with their excitatory potency, Olney hypothesized that the neuronal degeneration resulted from excessive activation of glutamate receptors expressed on neurons. Through the use of potent excitatory analogues of glutamate, the relevance of this phenomenon to neurodegenerative diseases became apparent. Thus, Coyle and Schwarcz reported that intrastriatal injection of kainic acid in the rat replicated the neuropathologic and synaptic neurochemical alterations that occur in HD (142). Systemic treatment with kainic acid produced a behavioral syndrome and histopathology associated with temporal lobe epilepsy. GluR-mediated neurodegeneration now has been implicated in a broad range of neurodegenerative conditions including stroke, ALS, cerebellar degeneration, and trauma (5,143,144).

Our knowledge of the role of iGluRs in neurodegenerative diseases has greatly increased since the realization that excitotoxicity is linked to programmed cell death (PCD) in many neurodegenerative disorders. A seminal paper by Kerr and colleagues (145) illustrated the morphological differences between apoptosis and necrosis. Subsequently, several genes that regulate cell death in *C. elegans* were identified, leading to the discovery of the caspases and Bcl-2 and homologues (146). As a consequence, the idea that cells could control their own death through the synthesis of new proteins was formulated. Subsequently, inhibitors of macromolecular synthesis were proved to prevent the naturally occurring cell death in both sensory and motor neurons. Recently, numerous molecular pathways and their components that activate or prevent neuronal cell death in response to iGluR activation have been identified.

A major effort has been undertaken to identify the specific iGluRs and the downstream events following receptor stimulation that mediate the death processes. Nevertheless, effective therapies to prevent or limit neuronal damage in neurodegenerative diseases remain elusive, reflecting an incomplete understanding of the mechanisms of neuronal death *in vivo*. It has become apparent that the boundary between apoptosis and necrosis is not well defined, leading to the realization that there exists a gradual shift from an apoptotic to a necrotic cell death in many cases, referred to as the “apoptotic-necrotic” continuum (6). Weaker insults typically promote apoptosis, whereas stronger ones favor necrosis. In other cases the apoptotic mechanism is activated along with the necrotic one, hampering attempts to distinguish the two (147).

Apoptotic cell death is typically associated with caspase activation, chromatin condensation, DNA laddering, and cell membrane blebbing that lead to cell shrinkage (6). Necrosis, on the other hand, is usually associated with the failure of ion pumps that causes cells to swell and burst, and is identified in tissues by the presence of invading macrophages and disruption of plasma membrane integrity, whereas in cell culture the absence of apoptotic markers and the rapid time course of death are the best indicators. Proteolysis by calpain and the caspases is often an early event following iGluR activation (148). Calcium overload mediated by iGluRs has a significant role in neurodegeneration (149). In addition, reactive oxygen species (ROS) play an important role in neuronal death mediated by iGluRs (150).

Mitochondria are almost invariably involved in the pathways triggered by iGluR activation. There are several general mechanisms at play, including release of proteins that activate caspases, disruption of electron transport, and alteration of cellular redox potential. The discovery that Bcl-2 is localized to the mitochondria directed attention toward the role of this relationship in cell death (151). Subsequently, it was found that Bcl-2 could prevent cytochrome c release, an inducer of apoptosis, from mitochondria. Bcl-2 also blocks the onset of the mitochondrial permeability transition (MPT) (152). The MPT represents an increase in permeability of the mitochondrial inner membrane to solutes of 1,500 daltons or less that results in membrane depolarization, uncoupling of oxidative phosphorylation, ion release, and mitochondrial swelling (153). Many of the insults that lead to the opening of the MPT function in a positive feedback manner (154). Glutamate can induce either early necrosis or delayed apoptosis in cultures of cerebellar granule cells, with mitochondrial function a critical factor that determines the mode of neuronal death (6).

Excitotoxicity is more commonly associated with necrosis because activation of iGluRs results in cation flux into the cell; however, apoptosis can follow stimulation of both AMPA/KA and NMDA receptors. The iGluRs may activate apoptosis using existing cellular components; for example, Simonian and colleagues demonstrated that kainate neurotoxicity in cerebellar granule cells was apoptotic but independent of protein synthesis (155). Alternatively, cells may require a prior insult or the addition or withdrawal of a trophic factor to become sensitive to iGluR activation that ordinarily would not be toxic. Cultured cerebellar granule cells remain resistant to AMPA-receptor-mediated toxicity when maintained in medium containing serum or insulin-like growth factor I (IGF-I), but become sensitive 4 to 5 days following the removal of trophic factors (156). In other cases, iGluR activation triggers PCD that utilizes a signaling pathway. Jiang and associates reported that NMDA receptor-mediated influx of extracellular Ca^{2+} rapidly and transiently activated ERK1/2, leading to apoptosis in cultured rat cortical neurons (157). Activation of the NMDA recep-

tor up-regulated p53 expression in cultured cerebellar granule cells, whereas blockade of p53 induction by an antisense oligonucleotide resulted in a complete inhibition of apoptosis (158). Similarly, systemic administration of kainate increased p53 mRNA levels in neurons exhibiting morphological features of damage within kainate-vulnerable brain regions (159).

Apoptosis may be a favored route in PCD partly because the reactive microglia that usually accompany necrosis often stimulate secondary cell death. Caspase-mediated degradation of AMPA receptor subunits occurs early during periods of cell stress in cultured rat hippocampal neurons (160). This may favor apoptosis, because levels of the AMPA receptor subunits GluR1 and GluR4 are rapidly decreased in neurons undergoing apoptosis in response to withdrawal of trophic support, whereas levels of NMDA receptor subunits NR1, NR2A, and NR2B are unchanged. Activation of calpain I by NMDA in cultured hippocampal neurons prevented the entry of cells into a caspase-dependent cell death program after the mitochondrial release of cytochrome c, possibly by inhibiting the processing of procaspase-3 and -9 into their active subunits (161). Thus, moderate NMDA receptor activation can prevent apoptosis without stimulating caspase-independent cell death, whereas a more severe stimulus favors apoptosis.

Necrotic cell death following iGluR activation is often attributed to alterations in receptor desensitization, subunit expression or other regulatory mechanisms. Human NT2-N neurons, which express calcium-permeable AMPA receptors, become vulnerable to excitotoxicity when desensitization is blocked with cyclothiazide (162). Necrosis is induced by insulin treatment within 48 hours in cultured mouse cortical neurons (163). Insulin exposure increased the level of the NR2A subunit of the NMDA receptor without altering NR1 or NR2B levels. Macromolecular synthesis inhibitors and NMDA antagonists blocked cell death, suggesting that an activity-dependent emergence of excitotoxicity contributed to insulin neurotoxicity. Cultured rat hippocampal neurons pretreated with BDNF exhibited increased levels of NR1 and NR2A, greater calcium responses to NMDA, and enhanced vulnerability to excitotoxic necrosis and reduced vulnerability to apoptosis (164). Cultured cerebellar granule cells, which show primarily an apoptotic death following KA treatment, undergo necrosis when L-type voltage-dependent calcium channels are blocked (147).

GLUTAMATE AND BRAIN DISORDERS

Neurodegenerative Diseases

HD is an autosomal dominant, progressive neurodegenerative disease that typically has its symptomatic onset in mid-life. Its manifestations include chorea, dementia, and death 15 to 20 years after onset. Afflicted individuals have an expanded CAG repeat in the gene encoding huntingtin on

chromosome 4, resulting in an elongated series of glutamines. The number of CAG repeats is 10 to 34 in normal individuals and 37 to 100 in HD patients (165). The identification of the HD gene has enabled the production of mouse models transgenic for huntingtin such as line R2/6, which has exon 1 with 92 repeats as well as transgenic cell lines that reiterate cellular characteristics of the disease (see the following).

HD was the first neurodegenerative disease for which iGluR-mediated neurodegeneration was implicated. Intrastriatal injection of kainate in the rat caused a striatal neuronal degeneration resembling HD (142); however, subsequent studies revealed that NMDA receptor agonists replicated the selective neuronal vulnerability in HD much more faithfully (166). Chronic treatment of rats with the mitochondrial toxin 3-nitropropionic acid elevated striatal lactate and selective striatal neuronal degeneration mediated by NMDA receptors (167). In this regard, lactate is elevated in the cerebral cortex and basal ganglia of HD patients. There is also reduced phosphocreatine/inorganic phosphate in resting muscle of HD patients, and mitochondrial electron transport enzymes are reduced in HD postmortem tissue. (See ref. 168 for review.) Consistent with these observations, mitochondria from HD lymphoblasts and fibroblasts display an increased tendency to depolarize (169). It may be that as a consequence of lowered energy levels, striatal neurons in HD can not maintain the resting membrane potential (thereby relieving the Mg^{2+} block), leading to increased $[Ca^{2+}]_i$ via NMDA receptors and ultimately cell death. Noteworthy is a report by Ferrante and associates that dietary creatine supplementation improved survival, slowed the development of striatal atrophy, and delayed the formation of huntingtin-positive aggregates in mice transgenic for huntingtin exon 1 with the expanded CAG repeat (170). Creatine may exert neuroprotective effects by increasing phosphocreatine levels or stabilizing the MPT, either of which could mitigate excitotoxicity mediated by GluRs.

Altered expression or composition of iGluR subunits may also contribute to neuronal death in HD. The editing of GluR2 mRNA is compromised in a region-specific manner in HD as well as in schizophrenia and AD, although there is still a large excess of edited GluR2 in each of these disorders (171). Chen and co-workers found that coexpression of huntingtin containing 138 repeats with NMDA receptors resulted in an increased number of functional NR1/NR2B-type receptors at the cell surface as compared to cells with normal huntingtin (172). Striatal spiny neurons are selectively vulnerable in HD and ischemia, whereas large aspiny (LA) cholinergic interneurons of the striatum are spared in these pathologic conditions. Because NR1/NR2B is the predominant NMDA receptor expressed in medium spiny neostriatal neurons, this may contribute to the selective vulnerability of these neurons in HD (172). Calabresi and associates found that membrane depolarization and inward currents produced by AMPA, KA, and NMDA were

much larger in spiny neurons than LA interneurons (173); moreover, concentrations of agonists producing reversible membrane changes in LA interneurons caused irreversible depolarization in spiny cells. The striatal and cortical neurons of R6/2 mice and mice with 94 CAG repeats displayed more rapid and increased swelling following NMDA treatment than controls, whereas AMPA and KA treatments had no differential effects. These findings suggest that NMDA antagonists or compounds that alter sensitivity of NMDA receptors may be useful in the treatment of HD (174).

The mGluRs may adversely affect iGluR function within the striatum in HD. The selective group I mGluR agonist 3,5-DHPG strongly enhanced membrane depolarization and intracellular calcium accumulation induced by NMDA application in striatal spiny neurons but not in LA interneurons, indicating a positive interaction between NMDA receptors and group I mGluRs, which are differently expressed between these two neuronal subtypes (175). Cha and colleagues found that 12-week-old R6/2 mice displayed decreased expression of AMPA- and KA- but not NMDA-type iGluR receptors compared to age-matched littermate controls. These mice also had decreased expression of mGluR1-3 that preceded the appearance of motor symptoms; therefore, altered mGluR function may contribute to subsequent pathology (176).

Approximately half of the variation in onset age for HD can be explained by the size of the repeat expansion. MacDonald and associates examined a TAA repeat polymorphism, which is closely linked to the GluR6 gene, in 258 unrelated HD-affected persons and found that younger onset age of HD was associated with linkage disequilibrium for this polymorphism (177). Rubinsztein and co-workers found that 13% of the variance in the age of onset of HD that was not accounted for by the CAG repeat size could be attributed to GluR6 genotype variation (178). These data implicate GluR6-mediated excitotoxicity in the pathogenesis of HD in addition to NMDAR-mediated neurodegeneration.

Alzheimer's disease (AD) is a progressive dementia characterized by a cortical neurodegeneration, particularly in the entorhinal cortex, hippocampal CA1 region, and subiculum. The etiology of AD is complex, with age, trauma, health, and environmental and genetic factors all playing a role (179). iGluR-mediated excitotoxicity is postulated to play a role in the neurodegeneration of AD (5). Mutations in the presenilin-1 (PS1) gene are causally linked to many cases of early-onset inherited autosomal dominant AD. Mice transgenic for the PS1M146V gene are hypersensitive to seizure-induced synaptic degeneration and necrotic neuronal death in the hippocampus (180). Cultured hippocampal neurons from PS1M146V knock in mice display increased vulnerability to glutamate, which is correlated with perturbed calcium homeostasis, increased oxidative stress, and mitochondrial dysfunction. Glutamate toxicity is potentiated by ROS mediated inhibition of EAATs; two stud-

ies have shown that ROS generated by A β peptide inhibits astrocyte glutamate uptake (181,182).

Immunocytochemical studies indicate that virtually all projection neurons in the hippocampus express iGluR subunits from each receptor class; however, regional differences in immunoreactivity were apparent in AD versus normal brain. In the vulnerable regions (i.e., CA1), GluR1, GluR2(4), GluR5/6/7, and NR1 were reduced, presumably owing to cell loss (183). In contrast, GluR2(4) immunolabeling appeared to be increased in the inner portion of the molecular layer of the dentate gyrus. Quantitative receptor autoradiography was also used to measure the laminar distribution of NMDA and AMPA receptors in three areas of visual cortex in control and AD postmortem human brains. The hierarchical pattern of the laminar loss of NMDA receptor binding correlated with the increasing complexity of associational visual cortices and increasing numbers of neurofibrillary tangles; however, AMPA receptor losses did not directly correlate with the pathology (184). Hyman and colleagues found no difference for the pattern of immunostaining between control and AD in either hippocampi or adjacent temporal cortices for GluR1, GluR2/3, and GluR4 (185); however, age-related loss of GluR2/3 immunoreactivity prior to degeneration has been reported in nucleus basalis of Meynert (186) and entorhinal cortex (187), suggesting that an increase in calcium permeability of AMPA receptors may leave these neurons vulnerable to degeneration in AD. Western blot analysis revealed average reductions of approximately 40% for GluR1 and GluR2/3 in the entorhinal cortex of patients with AD pathology versus age-matched controls, but neither GluR1 nor GluR2/3 protein concentration correlated significantly with tangle density (188). Thus, the relationship between excitotoxicity and neuronal loss in AD is complex and requires additional investigation.

Amyotrophic lateral sclerosis is a disorder characterized by a selective and progressive degeneration of motor neurons in the spinal cord and pyramidal neurons in the motor cortex, with onset in midlife (189). Death results from complications of the progressive paralysis. The two forms of ALS, sporadic and familial (FALS), have similar clinical symptoms and neuropathology, although the latter only accounts for 10% of the cases. Rosen and associates first reported a tight genetic linkage between FALS and the gene encoding Cu/Zn superoxide dismutase (SOD1), and identified 11 different SOD1 missense mutations in 13 different FALS families (190). Expression of high levels of a mutant form of human SOD1 for which the glycine at position 93 was replaced with an alanine (G93ASOD1; a change that has little effect on enzyme activity) caused a progressive motor neuron disease resulting in death by 6 months in transgenic mice (191). Because the mouse gene for SOD1 is unaffected in the transgenic mice, the results indicate that these mutations in SOD1 cause a gain-of-function that results in motor neuron death.

The reason for the selective vulnerability of motor neurons in ALS is unknown. Various molecular and neurochemical features of human motor neurons may render this cell group differentially vulnerable to such insults. Motor neurons are large cells with long axonal processes that require a high level of mitochondrial activity and have greater neurofilament content than other neuronal groups. Motor neurons have a very high expression of the cytosolic free radical scavenging enzyme Cu/ZnSOD1, which may render this cell group more vulnerable to genetic or posttranslational alterations interfering with the function of this protein. The low expression of calcium binding proteins and GluR2 AMPA receptor subunit by vulnerable motor neuron groups may render them unduly susceptible to calcium-mediated toxic events following GluR activation (192).

High levels of mRNA for GluR1, GluR3, and GluR4 are expressed in normal human spinal motor neurons; however, GluR2 subunit mRNA was not detectable in this cell group, predicting that normal human spinal motor neurons express calcium-permeable AMPA receptors unlike most neuronal groups in the human CNS (193); however, this has not been borne out in studies of mouse spinal cord in the context of the mouse models of ALS, where GluR2 is well represented in spinal cord motor neurons (194). AMPA or kainate exposure triggers substantial mitochondrial calcium loading in motor neurons, but causes little mitochondrial accumulation in forebrain GABAergic interneurons, neurons that express large numbers of calcium-permeable AMPA/kainate channels but do not degenerate in ALS. Brief exposure to either AMPA or kainate caused mitochondrial depolarization in motor neurons, whereas these effects were only observed in the GABAergic neurons after exposure to the non-desensitizing AMPA receptor agonist kainate. Finally, blocking mitochondrial calcium uptake attenuated AMPA/kainate receptor-mediated motor neuron injury. Thus, mitochondrial calcium uptake and consequent ROS generation may be central to the injury process (195). Quantification of mRNA expression in spinal cord showed a significant widespread loss of NR2A from both dorsal and ventral horns with losses of 55% and 78%, respectively, in ALS as compared to control. These results were substantiated by analysis of spinal cord homogenates, which showed a significant total decrease of 50% in NR2A message for ALS as compared to control (196).

Riluzole, which attenuates the glutamate neurotransmitter system, has been shown to prolong survival in patients with ALS (197). Riluzole affects neurons using three mechanisms: by inhibiting excitatory amino acid release, inhibiting events following stimulation of iGluRs, and stabilizing the inactivated state of voltage-dependent sodium channels. Mennini and associates studied inotropic glutamate receptor subtypes and the effect of chronic treatment with NBQX in the spinal cord of motor neuron disease (mnd) mice. NBQX significantly improved the behavioral scores in mnd mice. These findings suggest that selective antagonism of

inotropic non-NMDA receptors may be of value in the treatment of motor neuron disease (198). Further research may allow the development of therapies that target specific glutamate receptor subunits and modulate “downstream” events within motor neurons, aimed at protecting vulnerable molecular targets in specific populations of ALS patients.

SCHIZOPHRENIA

Kim and associates reported diminished concentrations of glutamate in CSF of patients with schizophrenia and first proposed that hypofunction of glutamatergic systems might cause the disorder (199). This finding has been replicated by some studies but not by several others (200–203). In a postmortem study, Tsai and associates (140) studied eight brain regions and found decreased concentrations of glutamate and aspartate in the frontal cortex and decreased concentration of glutamate in the hippocampus of patients with schizophrenia as compared to controls. Furthermore, the concentration of NAAG was increased in the hippocampus and the activity of GCPII was selectively reduced in the frontal cortex, temporal cortex, and hippocampus of people with schizophrenia. Subsequent studies with magnetic resonance spectroscopy have revealed significant reductions in the level of N-acetylaspartate (NAA), the product of NAAG by GCPII, in the very same regions—frontal cortex, temporal cortex, and hippocampus (204).

Initial ligand binding studies in postmortem schizophrenic brain have revealed increases in the non-NMDA iGluRs in the prefrontal cortex (205,206) and decreases in the hippocampus (207,208). Strychnine-insensitive binding, which labels the glycine modulatory site on the NMDA receptor, is increased throughout the primary sensory cortex and related associational fields in schizophrenia (209). Molecular approaches have shown a reduction in mRNA encoding GluR2 in the hippocampus and parahippocampus of people with schizophrenia, and reduced editing of GluR2 in the prefrontal cortex (210,211). Although the density of NMDA receptors in the prefrontal cortex of people with schizophrenia was normal, the relative subunit composition differed significantly from controls with a large increase observed for NR2D (212).

A convincing link between glutamatergic dysfunction and schizophrenia came from anecdotal and subsequent controlled studies of the neuropsychologic effects of dissociative anesthetics, which are noncompetitive antagonists of the NMDA receptor (213). When chronically abused, PCP produces a syndrome in normal individuals that closely resembles schizophrenia and exacerbates symptoms in patients with chronic schizophrenia. Subanesthetic doses of ketamine administered to normal subjects produces positive symptoms, including delusions and thought disorder, negative symptoms, and frontal lobe cognitive impairments characteristic of schizophrenia (214). When administered

to schizophrenic subjects, subanesthetic doses of ketamine exacerbate delusion, hallucinations, and thought disorders that are consistent with the patient's typical pattern of psychotic relapse (215,216). These effects are attenuated by the atypical antipsychotic clozapine but not haloperidol.

Although acute administration of ketamine to normal subjects causes increased (217) prefrontal cortical perfusion, chronic exposure to PCP is associated with the classical "hypofrontality" of schizophrenia (218,219). Chronic PCP treatment produced more perseveration and fewer nonspecific cognitive deficits in monkeys that persisted after discontinuation. Notably, these memory deficits were prevented by clozapine treatment (220).

Administration of NMDA receptor antagonists markedly increased the release of dopamine and glutamate in prefrontal cortex and subcortical structures in rats (221, 222), which was associated with impaired performance on a memory task sensitive to prefrontal cortical function (217); these alterations could be ameliorated by treatment with an AMPA/KA receptor antagonist. Furthermore, administration of a group I mGluR agonist blocked PCP-induced glutamate release without affecting dopamine release (223). These effects of NMDA receptor antagonism observed in the rodent have been shown to be comparable to humans in a positron emission tomographic study in which [¹¹C]-raclopride binding in striatum was used to measure dopamine release; subanesthetic doses of ketamine cause increased dopamine release in human subjects (224).

If the symptoms of schizophrenia result from hypofunction of NMDA receptors, then agents that enhance NMDA receptor function would be predicted to reduce symptoms. Because full agonists could be excitotoxic, studies have focused primarily on agents that act via the glycine modulatory site (225). Electrophysiologic studies in the hippocampal slice indicate that the glycine modulatory site is not fully occupied because of efficient transport of glycine by the GLYT-1 transporter on astroglia so that the modulatory site is subject to pharmacologic manipulation (133). In most of the studies, the drugs were added to typical antipsychotics in stable patients with prominent negative symptoms. Javitt and colleagues have performed a series of placebo-controlled crossover trials in which high doses of glycine (30 to 60 g per day) were added to antipsychotic drugs. They demonstrated improvement in negative symptoms and cognitive function without effects on psychotic symptoms or extrapyramidal side effects (226–228). Tsai and associates added D-serine at a dose of 30 mg per kg to typical antipsychotic drugs for 8 weeks and found significant improvements in negative symptoms, cognitive function (as measured by the Wisconsin Card Sorting test), and psychosis (229). The more robust effect of D-serine may reflect the fact that it has better penetrance of the blood–brain barrier, is a full agonist and has a higher affinity than glycine.

Another drug that has been extensively studied is the antitubercular agent, D-cycloserine, which is a partial ago-

nist at the glycine modulatory site with 60% efficacy and readily crosses the blood–brain barrier (230). A blinded dose finding study in patients receiving typical antipsychotics and exhibiting prominent negative symptoms revealed a U-shaped dose–response curve with significant reductions in negative symptoms and improvement in cognitive function at 50 mg per day (231). van Berckel and associates observed improvement in negative symptoms at a D-cycloserine dose of 100 mg per day in a small open trial with medication-free schizophrenics (232). D-cycloserine at 50 mg per day significantly improved negative symptoms when added to conventional antipsychotics in an 8-week fixed dose placebo-controlled parallel group trial with patients meeting criteria for deficit syndrome of schizophrenia (233); however, performance on a cognitive battery did not change. Notably, full response for negative symptoms was not achieved until after 4 to 6 weeks of treatment.

It was of interest to determine whether the addition of D-cycloserine would have further ameliorative effects in clozapine responders because clozapine has substantial effects on negative symptoms in many patients who respond poorly to typical antipsychotics. To the contrary, two separate trials of D-cycloserine at 50 mg per day added to clozapine resulted in worsening of negative symptoms (234,235). In contrast, trials in which the full agonists, glycine or D-serine, were added to clozapine yielded no additional change in negative symptoms or cognitive function (236,237). A plausible explanation for these findings is that clozapine may exert its effects on negative symptoms and cognitive functions in part by increasing occupancy of the glycine modulatory site on the NMDA receptor, thereby transforming the partial agonist D-cycloserine into an antagonist. Support for this inference comes from electrophysiologic studies in the hippocampal slice where clozapine enhances NMDA receptor currents (238).

As hippocampal interneurons appear more sensitive to NMDA receptor antagonists owing to the presence of NR2C (239), hypofunction of these NMDA receptors because of an excess of endogenous antagonists such as NAAG or kynurenic acid could account for many features of schizophrenia. The disinhibition of cortico-hippocampal efferents appears to increase subcortical dopamine release associated with positive symptoms (240). This would also interfere with the precision of cortical/hippocampal activations consistent with schizophrenic subjects' inability to increase hippocampal neuronal activity in a memory task because of a ceiling effect (241). The effects of glycine modulatory site activation, particularly on negative symptoms and cognitive impairment, are consistent with this model. Finally, the fact that ketamine reproduces the eye tracking impairments found in schizophrenics and some of their first-degree relatives suggest that NMDA receptor hypofunction could be part of the endophenotype (242).

Age-Associated Memory Impairment

Glutamate receptors have also been implicated in the functional decline seen in normal aging in the absence of neurodegeneration. Spatial memory is particularly vulnerable to aging (243), and is also disrupted by pharmacologic blockade of NMDA receptor function (244) or hippocampal knockout of NR1 (245). Electrophysiologic investigations of aging in rat hippocampus have revealed that certain aspects of excitatory synaptic transmission are unaffected or even compensatory, whereas others are compromised (246). One component of synaptic transmission that is compromised is maintenance and induction of long-term potentiation (LTP) that could be related to impaired NMDA receptor-mediated processes and the decreased stability of spatial information coding by “place cell” in aged rats (247). At the regional level, receptor binding studies have reported decreases in NMDA binding in hippocampus (248) with confirmatory declines in mRNA expression (249); however, other studies suggested that there is no change in NMDA receptors with aging (250).

In studying age-related changes in receptors, it is particularly important to be able to take the analysis from the regional level to that of cell classes, circuits, individual neuronal compartments, and synapses, because the changes are very likely to be cell-, circuit-, and synapse-specific and therefore difficult to resolve at the regional level; for example, age-related shifts in NR1, have been reported in the molecular layer of the dentate gyrus (251). The projection from the entorhinal cortex (ERC) to the DG is strictly confined to the outer molecular layer (OML), that is, the distal dendrites of granule cells; whereas other excitatory inputs terminate in a nonoverlapping fashion in the inner molecular layer (IML), the proximal dendrites. Aged monkeys, compared to young adult monkeys, exhibit a decrease in the fluorescence intensity for NR1 in the OML of the DG as compared to the IML. Given the tight laminar organization of these circuits, this pattern means that decreased NR1 levels primarily affect the input from the ERC, pointing to the ERC input to the hippocampus as a key element in age-related changes, and suggests that the intradendritic distribution of a neurotransmitter receptor is modified in an age-related and circuit-specific manner (251).

Although these results suggest that age-related circuit-specific shifts in NMDA receptors might underlie memory defects, they were not done in behaviorally characterized animals, and need to be followed up in the context of behavior. In addition, the data on NR1 changes were limited to the dendrite and did not directly address the GluRs at the synapse. Thus, these data need to be extended, particularly in the nonhuman primate model. Species issues may be particularly relevant because in rat hippocampus decreases in presynaptic markers such as synaptophysin correlate with age-associated memory impairment more directly than do any age-related shifts in GluRs (252,253).

CONCLUSION

In closing, glutamate sits at the epicenter of signal transduction in brain, not only mediating excitatory neurotransmission, but also modulating neuroplasticity at the genetic, synaptic, and structural levels. Furthermore, insufficient glutamatergic signaling causes the degeneration of immature neurons through apoptosis (254), whereas excessive activation of iGluRs kills neurons through necrosis and/or apoptosis. Dysregulation of glutamatergic neurotransmission has been implicated in an expanding number of neuropsychiatric disorders. Although the clinical pharmacology of glutamate is currently embryonic, the remarkable advances in the molecular characterization of this system hold promise for the development of a rich array of specific drugs in the future.

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