

NEUROCHEMISTRY OF SCHIZOPHRENIA: GLUTAMATERGIC ABNORMALITIES

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Multiple neurotransmitters have been implicated in schizophrenia. Dopamine is the neurotransmitter most often hypothesized to be associated with the pathophysiology of schizophrenia for two reasons. First, dopaminergic agonists can cause or exacerbate psychotic symptoms. Second, the correlation between antipsychotic efficacy and D2 dopamine-receptor blockade is excellent. For these reasons, a number of postmortem studies have focused on the dopaminergic system in schizophrenic brain. Although the results of these studies have generally been negative, the few positive findings have rarely been replicated, with the notable exception of increased striatal D2-receptor expression, which may be secondary to prior neuroleptic treatment. These studies of dopaminergic abnormalities in postmortem brain in schizophrenia have been recently reviewed (1,2).

Given the lack of findings associated with the dopamine system in the brain in schizophrenia, the elucidation of other potential neurotransmitter substrates of this illness has been an area of recent investigation. Glutamatergic dysfunction has been hypothesized to occur in schizophrenia, and this has been one of the most active areas of neurotransmitter research in this illness during the past few years. In this chapter, the glutamate hypothesis of schizophrenia is reviewed, the complexity of the molecules associated with the glutamate synapse is outlined, and postmortem neurochemical data suggesting glutamatergic abnormalities in schizophrenia are presented.

GLUTAMATE AND SCHIZOPHRENIA

Several lines of evidence have implicated glutamatergic dysfunction in schizophrenia. Dissociative anesthetics, espe-

cially phencyclidine (PCP) and ketamine, can cause psychotic symptoms in normal humans (3,4), and worsen these symptoms in persons with schizophrenia (5–7). Unlike catecholamine agonists, PCP can produce both the positive and negative (deficit) symptoms associated with this illness. PCP and related compounds are uncompetitive inhibitors of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor. Hence, this pharmacologic literature has been interpreted as suggesting that schizophrenia may be associated with decreased NMDA-receptor activity (5,8).

Several other reasons make a glutamate-receptor hypothesis of schizophrenia attractive. Schizophrenia is believed to have a neurodevelopmental component, and the NMDA receptor is critical in guiding axons to their targets in development (9). Further, NMDA receptors may be important in processes that lead to synaptic pruning seen in adolescence, which has been hypothesized to be abnormal in schizophrenia (10). Cognitive functioning depends on the plasticity mediated in part by NMDA receptors, and schizophrenics often have cognitive deficits (11). Finally, the reduction of gray matter in several brain regions seen in schizophrenia has been suggested to be the result of neurotoxicity mediated by NMDA receptors (12). A constellation of symptoms, findings, and hypotheses of schizophrenia can be parsimoniously explained by NMDA-receptor dysfunction.

The NMDA receptor is one of multiple subtypes of the glutamate receptor, however, and all these subtypes have functional interrelationships. Thus, although NMDA-receptor abnormalities have been hypothesized in schizophrenia, apparent NMDA-receptor dysregulation could be associated with abnormalities of another receptor subtype that interacts with the NMDA receptor, which in turn results in a breakdown of normal glutamatergic transmission in schizophrenia.

GLUTAMATE-RECEPTOR SUBTYPES

The four classes of glutamate receptors are functionally and pharmacologically distinct (Figs. 52.1 and 52.2). The iono-

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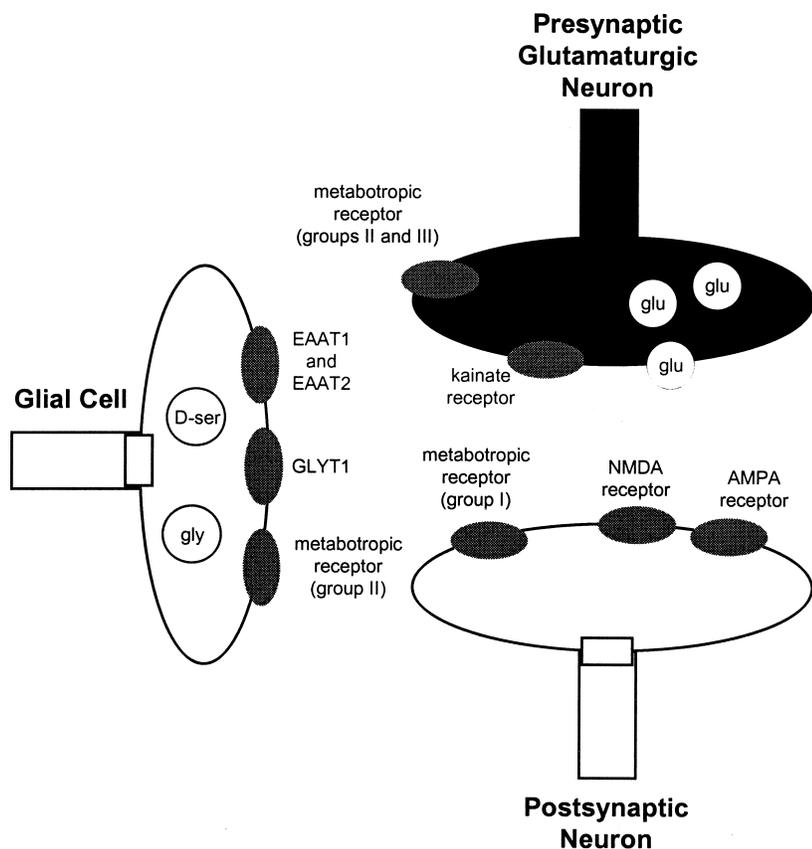


FIGURE 52.1. Diagram of a typical glutamatergic synapse. Recent data suggest that glutamatergic transmission requires three cells: a presynaptic glutamate-releasing cell, a presynaptic glial cell that releases the endogenous agonist for the glycine coagonist site (recently reported to be D-serine), and a postsynaptic neuron. The various glutamate receptors and transporters are differentially expressed by these three distinct cell populations. The glutamate uptake transporter EAAT₃ (excitatory amino acid transporter 3), which is not shown on this figure, appears to be expressed primarily on the cell body and dendrites.

Glutamate Receptors			
Ionotropic			Metabotropic
NMDA	AMPA	Kainate	
subunits	NR1 (& isoforms) NR2A NR2B NR2C NR2D NR3	GluR1 GluR2 GluR3 GluR4	GluR5 GluR6 GluR7 KA1 KA2
binding sites	glutamate glycine polyamines PCP/MK801	AMPA	Kainate
			receptors
			mGluR1 mGluR5
			group I
			mGluR2 mGluR3
			group II
			mGluR4 mGluR6 mGluR7 mGluR8
			group III

FIGURE 52.2. Subtypes of glutamate receptors. Three families of ionotropic glutamate receptors (N-methyl-D-aspartate, AMPA, and kainate) are known, each of which is composed of distinct subunits and has identifiable binding sites. The metabotropic receptors cluster into three groups, members of which share pharmacologic and structural features.

tropic glutamate receptors, AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), kainate, and NMDA are each composed of four or five subunits that form ligand-gated ion channels. The metabotropic glutamate receptors (mGluRs) are all seven transmembrane-domain, G protein-coupled receptors (13,14).

The AMPA-receptor subunits are derived from a family of four genes that have been named *GluR1* through *GluR4*. The transcripts from each of these genes are expressed in one of two isoforms, termed *flip* and *flop*, that result from alternative splicing. In addition, the final subunit protein of the AMPA receptor subunits has amino acids at specific locations in the ion channel that can vary according to RNA editing (13,14). Thus, a potential exists for considerable heterogeneity in the final assembled AMPA receptors, based on subunit composition and post-translational modification. The assembled AMPA receptors contain several binding sites: one for glutamate, another at which competitive antagonists such as CNQX (6-cyano-7-nitro-quinoxalindione) act, and yet another where desensitization modulators exert their influence. Subunit composition appears to confer unique pharmacologic properties to the final receptors (15–19). For example, decreased calcium influx in AMPA receptors that contain the GluR₂ subunit drastically diminishes the electrophysiologic activity of these receptors.

Kainate receptors are also ligand-gated ion channels composed of subunits derived from genes for the low-affinity GluR₅ through GluR₇ and high-affinity KA₁ through KA₂ subunits (13,14). The transcripts associated with these five subunits also undergo alternative splicing and editing. Final assembled kainate receptors may be composed of five identical subunits, or they may be heteromers composed of low- and high-affinity subunits, with pharmacologic properties that differ from those of low-affinity or high-affinity homomers.

The NMDA receptor subunits are encoded by five genes termed *NR1* and *NR2A* through *NR2D* (13,14). An *NR3* gene has also been identified, although this subunit appears to be expressed primarily during early development (20–22). NR₁ is expressed as one of eight isoforms because of alternative splicing of exons 5, 21, and 22 (13,14,23, 24). As in the case of the AMPA and kainate receptors, transcription of the NR₁ subunit presents an important level for the regulation of the expression of functional NMDA receptors. This regulation can influence certain properties of the final functional NMDA receptors, including the pharmacology of their binding sites.

The pharmacologic regulation of the NMDA receptor depends on the unique combination of binding sites (13, 14). A primary agonist site exists for the binding of glutamate. A separate glycine co-agonist site must also be occupied before glutamate can activate the ion channel; recent reports suggest that D-serine produced by astrocytes is the endogenous ligand for this site (25–28). Modulatory binding sites for polyamines, protons, neuropeptides including

dynorphin, and zinc have also been identified. Additionally, magnesium ions block the ion channel of the NMDA receptor complex at physiologic concentrations. This blockade is voltage-dependent; partial depolarization of the cell membrane extrudes the magnesium ion. Therefore, presynaptic glutamate release and postsynaptic pre-depolarization are both required for NMDA receptor activity. Finally, a site within the ion channel itself is associated with the binding of uncompetitive antagonists of the NMDA receptor, such as PCP, ketamine, and MK-801. These antagonists are use-dependent (i.e., the ion channel must be opened for these compounds to bind to the receptor), so cooperativity between multiple sites is necessary for occupancy by uncompetitive antagonists.

These binding sites are associated with different subunits, and their affinities can vary depending on subunit composition. NR₁ homomers have been shown to form glycine binding sites, but an NR₂ subunit appears to be required to form both glutamate and MK-801 binding sites (29–32). Further, receptors containing NR_{2A} subunits have a higher affinity for compounds that bind to the glutamate agonist site, whereas receptors with NR_{2A} or NR_{2B} subunits have higher affinities for MK-801 binding than do receptors with NR_{2C} or NR_{2D} subunits (31). In addition, NMDA receptors containing particular NR₁ splice variants have a higher affinity for MK-801 than do receptors with others, irrespective of NR₂ co-assembly (33). Receptors with NR_{2B} subunits are associated with a higher affinity for polyamine modulators (31,34). Therefore, differential subunit combinations confer unique binding properties to the NMDA receptors and probably are associated with subtle electrophysiologic differences within a population of NMDA receptors.

Eight mGluRs have been cloned and are grouped (group I, group II, and group III) based on pharmacology, sequence homology, and linkage to signal transduction pathways (35–40). These mGluRs belong to a unique subset of G protein-coupled receptors with seven transmembrane domains and large, extracellular amine termini. When expressed in heterologous systems, group I mGluRs have been shown to stimulate phospholipase C, phosphoinositide hydrolysis, and the formation of cyclic adenosine monophosphate (cAMP) (41–44). In heterologous systems, groups II and III mGluRs inhibit forskolin-stimulated cAMP formation and adenylyl cyclase, possibly via a G_i protein (39,40, 45,46). The metabotropic receptors have been the target of considerable recent interest because a functional relationship appears to exist between the group II metabotropic and NMDA receptors (47).

Each glutamate receptor subtype appears to have a unique role in glutamatergic neurotransmission. Glutamate receptors interact at multiple levels, as AMPA, kainate, and metabotropic receptors all affect NMDA-receptor activity. Accordingly, although the NMDA receptor is typically hypothesized to be dysregulated in schizophrenia, disturbances of any of the glutamate receptors could result in a condition

that produces the appearance of an abnormally functioning NMDA receptor.

ABNORMALITIES OF GLUTAMATE RECEPTORS IN SCHIZOPHRENIA

Given the possibility of glutamate-receptor dysfunction in schizophrenia, the expression of all four families of the glutamate receptor have been studied in schizophrenic brain. As would be expected, these investigations have primarily targeted limbic regions that have been implicated in schizophrenia, particularly limbic cortex, striatal areas, medial temporal lobe structures, and, more recently, the thalamus. These investigations have also targeted multiple levels of gene expression, including subunit messenger RNA (mRNA) and protein levels, and final binding sites have been studied. In the following sections, the studies that have been published for each receptor subtype in postmortem brain in schizophrenia are reviewed.

AMPA Receptors

Of all of the glutamate receptors in schizophrenia, the AMPA receptor has been studied the most, as summarized in Table 52.1. When the AMPA-associated subunits were first cloned, Harrison et al. (48) examined the expression of the mRNA encoding the GluR₁ subunit in medial temporal lobe structures in schizophrenia. A consistent decrease in the expression of this subunit transcript was found in hippocampal regions, an abnormality that was statistically significant only in the CA3 region. These investigators subsequently extended their finding and demonstrated that GluR₁-subunit mRNA is decreased in multiple hippocampal subfields (dentate gyrus, CA3, and CA4) and also in the subiculum (49). They also reported that GluR₂-subunit mRNA is decreased in the medial temporal lobe in schizophrenia, particularly in the parahippocampal gyrus (49), and continued their examination of AMPA-receptor expression in the medial temporal lobe by determining the patterns of expression of the flip and flop isoforms of the GluR₁ and GluR₂ subunits. Decreased expression of GluR₂-sub-

TABLE 52.1. AMPA RECEPTOR BINDING AND SUBUNIT EXPRESSION IN SCHIZOPHRENIA

	Ligand or Subunit	Findings	Brain Regions Studied	Reference
Receptor binding sites	[³ H]CNQX		caudate	57
	[³ H]CNQX	none	putamen, nucleus accumbens	57
	[³ H]AMPA	none	caudate, putamen, nucleus accumbens	55
	[³ H]CNQX		CA4, CA3	53
	[³ H]AMPA	none	frontal cortex, putamen, nucleus accumbens	58
	[³ H]AMPA	none	caudate, putamen, nucleus, accumbens	56
	[³ H]AMPA		CA2	54
	[³ H]AMPA	none	dentate gyrus, CA1, CA3, subiculum	54
	[³ H]AMPA	none	thalamus	61
	Subunit protein expression	GluR ₁		parahippocampal gyrus
		none	CA1, CA3, CA4, subiculum	50
GluR _{2/3}			CA4	50
		none	dentate gyrus, CA1, CA3, subiculum	50
			parahippocampal gyrus	
			hippocampus	52
Subunit mRNA expression	GluR ₂ , GluR ₃	none	cingulate cortex	52
	GluR ₁		dentate gyrus, CA3, CA4, subiculum	49
		none	CA1, parahippocampal gyrus	49
	GluR ₂		dentate gyrus, CA3, CA4, subiculum	49
		none	CA1	49
	GluR ₁ , GluR ₂ , GluR ₃ , GluR ₄	none	caudate, putamen, nucleus accumbens	55
	GluR ₁		CA3	48
		none	dentate gyrus, CA1, CA4, subiculum	48
	GluR ₁ , GluR ₂ , GluR ₃ , GluR ₄	none	caudate, putamen, nucleus accumbens	56
	GluR ₁ , GluR ₃		thalamus	61
	GluR ₂ , GluR ₄	none	thalamus	61
	GluR ₁		frontal cortex	59
	GluR ₁	none	frontal cortex	59
GluR ₂ flip	none	hippocampus	51	
GluR ₂ flop		hippocampus	51	
flip-flop ratio		hippocampus	51	

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; CNQZ, 6-cyano-7-nitro-quinolindione

unit mRNA was again found in hippocampal structures, and both the flip and flop variants were reduced, the flop to a greater extent (50).

Several studies have examined the expression of the AMPA-subunit proteins in the medial temporal lobe in schizophrenia. Using quantitative immunocytochemical analyses, Eastwood et al. (51) reported decreased expression of the AMPA subunits in medial temporal lobe structures. In particular, GluR₁ immunoreactivity was noted to be significantly reduced in the parahippocampal gyrus, and combined GluR_{2/3} immunoreactivity was decreased in the CA4 subfield of the hippocampus. On the other hand, Breese and co-workers (52) found no differences in GluR₁, GluR₂, or GluR₃ immunoreactivity in schizophrenia when they used Western analysis in hippocampal samples.

AMPA-receptor binding has also been studied in medial temporal lobe structures. Using [³H]CNQX to label the AMPA receptor, Kerwin et al. (53) noted decreased binding to the AMPA receptor in the schizophrenic hippocampus, particularly in the CA3 and CA4 subfields. More recently, Gao and colleagues (54) found decreased [³H]AMPA binding in CA2, but not in other hippocampal fields or associated structures. The convergence of these data is that AMPA-receptor expression is decreased in the medial temporal lobe in schizophrenia, a decrease that involves alterations of subunit gene expression in addition to the final binding site.

Although the medial temporal lobe data are the most robust, AMPA-receptor expression has also been examined in other brain regions in schizophrenia. In two studies, none of the AMPA-associated subunit transcripts were changed in striatal subregions (caudate, putamen, and nucleus accumbens) in schizophrenia (55,56). To date, subunit protein levels have not been reported in striatal regions. Binding to the AMPA receptor has been determined in striatal regions, but results have not been consistent. Although Noga and colleagues (57) reported an increase in AMPA binding, determined with [³H]CNQX, in caudate, putamen, and accumbens in schizophrenia, no differences in [³H]AMPA binding were found in striatal regions in schizophrenia in three other reports (55,58,56).

The cortex has also been studied for alterations of AMPA-receptor expression in schizophrenia. In one study, no differences in the expression of any of the AMPA-associated subunit mRNAs were found in prefrontal or occipital cortex in schizophrenia (55), although Sokolov (59), using reverse transcriptase polymerase chain reaction (RT-PCR), reported decreased GluR₁ mRNA in superior frontal gyrus. Breese et al. (52) found no differences in GluR₂ or GluR₃ protein in cingulate cortex as determined by Western analysis. Several groups have studied [³H]AMPA binding in cortical areas in schizophrenia (55,60), with generally negative results.

Recently, the neurochemical anatomy of the thalamus has become a subject of interest in schizophrenia research. The AMPA receptor is expressed in multiple nuclei of the

human thalamus. In a recent report (61), although [³H]AMPA binding was not different in limbic thalamic nuclei in schizophrenia, the transcripts encoding the GluR₁ and GluR₃ subunits were both found to be reduced in the face of normal levels of GluR₂ and GluR₄ mRNA. These results suggest that alterations in the stoichiometry of subunit composition may be associated with the AMPA receptor in the schizophrenic thalamus.

Kainate Receptors

The kainate receptor has been the subject of study in the brain in schizophrenia, as summarized in Table 52.2. Although the medial temporal lobe has been the best-studied region in the schizophrenic brain for AMPA-receptor expression, fewer studies have systematically focused on the kainate receptor in these structures. Porter and colleagues (62) found decreased expression of GluR₆ and KA₂ mRNA in several hippocampal regions, results paralleling similar data for the AMPA subunits in the medial temporal lobe. In this same study, GluR₆ mRNA was not found to be changed in the schizophrenic cerebellum. Only one study to date has examined any of the kainate subunit proteins; GluR₅ was studied by Western analysis and was not changed in schizophrenic hippocampus (52), although the antisera used in this study cross-reacts with GluR₆ and GluR₇.

Kainate-receptor expression has been examined in multiple cortical regions. Sokolov (59) has published data suggesting that GluR₇- and KA₁-subunit transcripts are decreased in the superior frontal gyrus in schizophrenia, similar to the decreases this investigator noted for some of the subunits associated with the AMPA and NMDA receptors. In a recent study examining transcripts of kainate-receptor subunits in the prefrontal cortex (63), a shift in subunit stoichiometry was found in multiple cytoarchitectural regions of the prefrontal cortex, with increased expression of GluR₇ mRNA and decreased expression of KA₂ mRNA in the face of normal expression of the other kainate subunits. In this same study, no changes in transcripts of kainate-receptor subunits were noted in Brodmann area 17.

Several studies have examined the expression of transcripts of the kainate-receptor subunit in subcortical structures. Two reports (56,63) noted no alterations of these subunits in multiple striatal regions in schizophrenia. On the other hand, a recent study noted decreased levels of KA₂ mRNA but normal levels of other transcripts of kainate-receptor subunits in limbic thalamic nuclei in schizophrenia.

Kainate-receptor binding has been studied in multiple brain regions in schizophrenia by several independent groups. All these studies have used [³H]kainate to label this receptor. In general, kainate-receptor binding has been reported to be altered in multiple cortical areas in schizophrenia (63–65). Data on the expression of kainate binding sites in medial temporal lobe structures are inconsistent;

TABLE 52.2. KAINATE RECEPTOR BINDING AND SUBUNIT EXPRESSION IN SCHIZOPHRENIA

	Ligand or Subunit	Findings	Brain Regions Studied	Reference	
Receptor binding sites	[³ H]kainate	none	caudate, putamen, nucleus accumbens	57	
	[³ H]kainate	none	thalamus	61	
	[³ H]kainate		CA4, CA3, CA2, CA1, dentate gyrus, parahippocampal gyrus	53	
	[³ H]kainate	none	CA3, CA2, CA1, dentate gyrus, subiculum	54	
	[³ H]kainate	none	prefrontal cortex	65	
	[³ H]kainate		frontal cortex	64	
	[³ H]kainate	none	temporal cortex	64	
	[³ H]kainate		prefrontal cortex	60	
	[³ H]kainate		prefrontal cortex	63	
	[³ H]kainate	none	striatum, occipital cortex	63	
	[³ H]kainate	none	caudate, putamen, nucleus accumbens	56	
	Subunit protein expression	GluR _{5,6,7}	none	hippocampus	52
	Subunit mRNA expression	GluR ₆		dentate gyrus, CA3	62
GluR ₆		none	cerebellum	62	
KA ₂			dentate gyrus, CA2, CA3	62	
KA ₂		none	CA4, CA1	62	
KA ₂			thalamus	61	
GluR _{5,6,7} , KA ₂		none	thalamus	61	
GluR ₇			prefrontal cortex	63	
KA ₂			prefrontal cortex	63	
GluR _{5,6} , KA ₁		none	prefrontal cortex	63	
GluR _{5,6,7} , KA ₁ , KA ₂		none	striatum	63	
GluR ₇			frontal cortex	59	
GluR ₇		none	frontal cortex	59	
KA ₁			frontal cortex	59	
KA ₁		none	frontal cortex	59	
GluR _{5,6,7} , KA ₁ , KA ₂		none	caudate, putamen, nucleus accumbens	56	

one study reported decreased [³H]kainate binding in the hippocampus and parahippocampal gyrus (53), but another found no differences in binding in medial temporal lobe structures (54). Although kainate-receptor binding has been reported to be abnormal in cortical structures, it has not been found to differ in subcortical regions in schizophrenia; [³H]kainate is unchanged in both striatal subregions (56, 57,63,65) and limbic thalamic nuclei (61) in this illness.

NMDA Receptors

Although most hypotheses of glutamatergic dysfunction in schizophrenia invoke the NMDA receptor, relatively few studies of this receptor subtype have been carried out to date (Table 52.3). Only several studies have been published that examine the expression of the NMDA subunits in schizophrenic brain, and all these have focused on mRNA levels. In a comprehensive examination of all the NMDA subunits in prefrontal cortex, Akbarian et al. (66) found no absolute differences between controls and schizophrenic patients for any of the NMDA subunits, but the contribution of NR_{2D} to the total pool of NR₂ transcripts was elevated in the schizophrenic patients. Recently, Gao et al. (54) found an altered stoichiometry of NMDA subunits

in hippocampus, with decreased NR₁ and increased NR_{2B} mRNA expression but normal NR_{2A} expression, in schizophrenia. Several other studies have been published in which only the NR₁ transcript was measured; in one study, this molecule was reported to be decreased in superior temporal cortex (67), and in another, it was decreased in superior frontal cortex (59).

Several recent studies have examined the expression of the NMDA receptor subunits in subcortical structures in schizophrenia. In one study (56), NR₁, NR_{2A}, NR_{2B}, NR_{2C}, and NR_{2D} mRNAs were measured in the caudate, putamen, and nucleus accumbens in schizophrenia; no significant differences were found in comparison with control striata. On the other hand, significant reductions of NR₁, NR_{2B}, and NR_{2C} transcripts (but not of NR_{2A} and NR_{2B} transcripts) were found in dorsomedial and anterior thalamic nuclei in this disorder (61).

Because of the myriad binding domains of the NMDA complex, studies of receptor binding are difficult to interpret and are subject to the selection of radioligand. Further, it has become apparent that certain subunit compositions are associated with specific binding sites, so it is possible that some but not all binding sites on the NMDA receptor are altered in schizophrenia. The best-studied of the

TABLE 52.3. NMDA RECEPTOR BINDING AND SUBUNIT EXPRESSION IN SCHIZOPHRENIA

	Ligand or Subunit	Findings	Brain Regions Studied	Reference
Receptor binding sites	[³ H]MK-801	none	caudate, putamen, nucleus accumbens	57
	[³ H]MK-801		putamen	68
	[³ H]MK-801	none	frontal cortex, entorhinal cortex, hippocampus, amygdala	68
	[³ H]L-689, 560		caudate, putamen,	72
	[³ H]L-689, 560	none	nucleus accumbens	
	[³ H]L-689, 560		temporal cortex	30
	[³ H]L-689, 560	none	motor cortex	30
	[³ H]CGP39653	none	temporal cortex, motor cortex	30
	[³ H]ifenprodil		temporal cortex	30
	[³ H]ifenprodil	none	motor cortex	30
	[³ H]ifenprodil		thalamus	61
	[³ H]MDL105,519		thalamus	61
	[³ H]MK-801	none	thalamus	61
	[³ H]CGP39653	none	thalamus	
	[³ H]ifenprodil	none	caudate, putamen, nucleus accumbens	56
	[³ H]MDL105,519			
	[³ H]MK-801			
	[³ H]CGP39653			
Subunit mRNA expression	NR _{2D}	none	prefrontal cortex	66
	NR _{1,2A,2B,2C}	none	prefrontal cortex	66
	NR _{1,2A,2B,2C,2D}	none	cerebellum, parietotemporal cortex	66
	NR _{1,2B,2C}		thalamus	61
	NR _{2A,2D}	none	thalamus	61
	NR ₁		temporal cortex	67
	NR ₁		frontal cortex	59
	NR ₁	none	frontal cortex	59
	NR ₁		dentate gyrus, CA3	54
	NR _{2A}	none	hippocampus, subiculum	54
	NR _{2B}		CA2, CA3	54
	NR _{1,2A,2B,2C,2D}	none	caudate, putamen, nucleus accumbens	56

NMDA-associated sites is the ion channel/PCP site. In general, studies in which [³H]MK-801 was used have been relatively unimpressive. In an early study (68), increased [³H]MK-801 binding was reported in the schizophrenic putamen, but no differences were noted in frontal cortex or multiple medial temporal lobe regions, including the hippocampus, amygdala, and entorhinal cortex. A more recent study (57) found no differences in caudate, putamen, or nucleus accumbens. The ion channel site has also been studied with the ligand [³H]TCP, and again minimal changes were noted. In one study (69), no changes were found in multiple cortical areas, putamen, or cerebellum. A subsequent report (70) observed no differences between controls and schizophrenic patients in hippocampus, amygdala, or polar frontal cortex (Brodmann area 10), but increased [³H]TCP binding was noted in orbitofrontal cortex (Brodmann area 11) in the schizophrenic patients.

The other NMDA-associated binding sites have been studied more recently. The primary agonist site for glutamate has been studied with [³H]glutamate in the hippocampus, and no differences have been found in schizophrenia (53,54). The glycine co-agonist site has also been studied.

Using [³H]glycine, Ishimaru and colleagues (71) reported increased binding in multiple cortical areas in schizophrenia. Recently, the glycine site was studied in striatum with [³H]L-689,560, and increased binding was noted in putamen, but not caudate or accumbens, in schizophrenia (72).

Several comprehensive studies examining multiple binding sites associated with the NMDA receptor complex in subcortical structures have recently been reported. In one of them (56), binding to the glutamate (measured with [³H]CGP39653) and glycine (measured with [³H]MDL105,519) agonist sites, the intrachannel/PCP site ([³H]MK-801), and the polyamine modulatory site ([³H]ifenprodil) were determined in caudate, putamen, and nucleus accumbens in schizophrenia. In this study, no differences were noted between controls and schizophrenic subjects. On the other hand, a study in thalamus from this same group (61), in which the same ligands were used to label the four sites, found decreased expression of binding associated with the glycine and polyamine sites, but not the intrachannel/PCP site or glutamate binding domain in limbic nuclei, in schizophrenia. These changes in some but not all binding sites in the thalamus were also associated

TABLE 52.4. METABOTROPIC GLUTAMATE RECEPTOR RNA EXPRESSION IN SCHIZOPHRENIA

Receptor	Findings	Brain Regions Studied	Reference
mGluR _{1,2,3,4,5,7,8}	none	thalamus	74
mGluR ₃	none	prefrontal cortex	73
mGluR ₅		prefrontal cortex	73

with changes in the stoichiometry of the various NMDA-associated subunit transcripts in these nuclei.

Metabotropic Receptors

Very little has been published about this family of receptors in schizophrenic brain (Table 52.4). In one study, the mRNAs encoding the metabotropic receptors mGluR₃ and mGluR₅ were measured in prefrontal cortex (73). Although mGluR₃ mRNA was not changed in schizophrenia in multiple areas of the prefrontal cortex, mGluR₅ was increased in the orbitofrontal cortex (Brodmann area 11), but not in Brodmann areas 9 or 10. Cell-level analysis revealed that this increase was secondary to increased expression of mGluR₅ mRNA in pyramidal cells in lamina III of this area of prefrontal cortex. More recently, the expression of the transcripts encoding seven of the eight cloned metabotropic receptors was reported in schizophrenic and control thalamus (74). No differences were found in the expression of the mGluRs in six different thalamic nuclei in schizophrenia in this study.

GLUTAMATE TRANSPORTERS

In addition to the glutamate receptors, other molecules at the glutamate synapse are critical for normal glutamatergic neurotransmission (Fig. 52.1). At least five glutamate up-

take transporters, excitatory amino transporter 1 (EAAT₁) through EAAT₅, are expressed in the glutamate synapse (75). EAAT₁ is predominantly expressed in astrocytes of the cerebellum, although expression is also significant in the forebrain. EAAT₂ is expressed in both astrocytes and neurons but has a more widespread distribution in the brain (76). Severe neuropathology and epilepsy develop in knockout mice for the *EAAT2* gene, which confirms its importance in normal glutamatergic function. EAAT₃ is a neuronal transporter expressed in multiple limbic regions. EAAT₄ expression is restricted to Purkinje cells of the cerebellum, and EAAT₅ is confined to the retina.

Although glutamate transporters affect the function of all four glutamate receptor subtypes, the glycine transporter family may specifically affect NMDA receptor-mediated activity. Glycine is an NMDA receptor co-agonist, and glycine transporter inhibitors affect normal NMDA-receptor function and reverse PCP-induced behaviors (77–81). The two families of glycine transporters are GLYT₁ and GLYT₂; three isoforms of GLYT₁ have overlapping expression in astrocytes throughout the human brain, whereas GLYT₂ is restricted to the hindbrain and spinal cord (82,83). By altering the availability of glutamate for its receptors, changes in the expression of the transporters may induce profound changes at the level of receptor function. Further, given that the NMDA receptor may depend on glycine as a co-agonist, abnormal synaptic levels of this amino acid may be associated with disturbed function of the NMDA receptor.

Initially, the quantification of glutamate uptake sites in schizophrenia preceded the identification of the EAAT subtypes, and conflicting data have been obtained in schizophrenic prefrontal cortex and basal ganglia with use of the nonselective transporter ligand [³H]D-aspartate (Table 52.5). Early studies found decreases in striatal uptake sites (84,85); however, later studies did not replicate these findings (57,86). Similarly, increases in frontal cortical uptake sites (64) were not confirmed in follow-up studies (84,87). The discrepancies in this literature may be in part a consequence of the nonselectivity of [³H]D-aspartate for the mul-

TABLE 52.5. EXCITATORY AMINO ACID BINDING IN SCHIZOPHRENIA

Ligand	Findings	Brain Regions Studied	Reference
[³ H]glutamate	none	caudate, putamen, nucleus accumbens	57
[³ H]aspartate		frontal cortex	64
[³ H]aspartate	none	temporal cortex	64
[³ H]aspartate		anterior cingulate gyrus	87
[³ H]aspartate	none	hippocampus, temporal cortex	87
[³ H]glutamate	none	CA4, CA3, CA2, CA1, dentate gyrus, parahippocampal gyrus	53
[³ H]glutamate	none	CA3, CA2, CA1, dentate gyrus, subiculum	54
[³ H]aspartate		putamen, globus pallidus caudate,	70
[³ H]aspartate	none	nucleus accumbens	70

multiple transporter subtypes; shifts in transporter subtype expression may occur in the absence of changes in total uptake sites. Consistent with this interpretation is the recent demonstration of decreased EAAT₂ mRNA levels in prefrontal cortex of schizophrenics (73). This change is in the opposite direction of that noted in previous studies examining radioligand binding to the transporters (64,84), which suggests that a shift from EAAT₂ to EAAT₁/EAAT₃ expression may occur in prefrontal cortex in schizophrenia.

OTHER NEUROMODULATORS AND MARKERS

An alternative mechanism for altering glutamate neurotransmission involves neuropeptide modulators of glutamate-mediated neurotransmission (88–91). For instance, cholecystokinin (CCK) augments glutamate-mediated neurotransmission (88,91). CCK is expressed in subgroups of γ -aminobutyric acid (GABA)- and glutamate-containing neurons in the entorhinal cortex (92–94). Several postmortem studies have found abnormalities in CCK, CCK receptors, and CCK mRNA expression in schizophrenia, both in the frontal and temporal lobes (95–98). A cell-based silver grain analysis confirmed the involvement of layer VI, finding a reduction in the level of CCK mRNA expression per pyramidal cell (99). This is further supported by other molecular studies involving the measurement of complexin I and complexin II mRNAs, which suggest preferential involvement of excitatory pyramidal neurons in the mesial temporal lobe in schizophrenia (100,101).

A second neuropeptide neuromodulator concentrated in glutamate neurons, *N*-acetylaspartylglutamate (NAAG), antagonizes the effects of glutamate at NMDA receptors (102). NAAG is cleaved by glutamate carboxypeptidase II (formerly referred to as *N*-acetyl- α -linked acidic dipeptidase), a membrane-spanning glial enzyme, to yield glutamate and *N*-acetylaspartate (NAA). One study of NAAG and glutamate carboxypeptidase II found decreased glutamate carboxypeptidase II activity in prefrontal cortex and hippocampus and increased NAAG levels in the prefrontal cortex of schizophrenic patients relative to normal controls (103). Moreover, *in vivo* magnetic resonance spectroscopic imaging has revealed selective reductions in NAA in the dorsolateral prefrontal cortex and hippocampal formation of schizophrenic subjects (104,105). This suggests that NAA, a marker of neuronal integrity, may be decreased specifically and regionally in schizophrenia secondary to decreases in glutamate carboxypeptidase II.

CONCLUSIONS

Converging evidence indicates that abnormalities of glutamatergic neurotransmission occur in specific brain regions

in schizophrenia. Although the hippocampus and associated structures have been the best studied, emerging data point to glutamatergic abnormalities in other areas of the brain that are likely to be associated with the pathophysiology of schizophrenia, including limbic cortex, striatal regions, and thalamus. Pharmacologic evidence suggests involvement of the NMDA receptor in schizophrenia, but other studies and theoretic considerations indicate that other molecules associated with glutamatergic transmission are also abnormal in this illness.

Studies in postmortem samples of the molecules associated with the glutamate synapse have not been conducted in a systematic and comprehensive fashion; however, several general principles are emerging from available data. First, although abnormalities of the glutamate synapse have been reported primarily in hippocampal regions, recent data suggest that thalamocortical circuits may also be abnormal. Interestingly, the striatal subregions appear to be less affected than medial temporal lobe and thalamocortical pathways. Second, all four families of glutamate receptors have been reported to be abnormal in brain in schizophrenia, although in region- and circuit-specific patterns. Third, changes are apparent at both transcriptional and translational levels of gene expression. Fourth, the ionotropic glutamate receptors have been studied most, and results thus far reveal changes in ionotropic receptor binding sites in addition to subunit changes suggestive of altered stoichiometry of subunit composition. The metabotropic receptors are just beginning to be studied, but the few available reports do suggest abnormalities of these receptors.

The literature on postmortem neurochemical studies of glutamatergic molecules in schizophrenia supports the hypothesis of abnormal glutamatergic neurotransmission in this illness that particularly involves the ionotropic receptors. These data suggest that novel strategies that permit the modulation of these receptors may prove to be of therapeutic utility in this illness, and may also provide clues about the pathophysiologic substrate of schizophrenia.

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