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, especially 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-
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 co-agonist 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.

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 bind-
ing sites: one for glutamate, another at which competitive
agonists 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 dimin-
ishes the electrophysiologic activity of these receptors.

Kainate receptors are also ligand-gated ion channels com-
posed 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 identi-
cal 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 homo-
mers.

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, transcrip-
tion 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 occu-
pied before glutamate can activate the ion channel; recent
reports suggest that t-serine produced by astrocytes is the
endogenous ligand for this site (25–28). Modulatory bind-
ing sites for polyamines, protons, neuropeptides including
dynorphin, and zinc have also been identified. Additionally,
magnesium ions block the ion channel of the NMDA recep-
tor complex at physiologic concentrations. This blockade
is voltage-dependent; partial depolarization of the cell mem-
brane 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 be-
tween multiple sites is necessary for occupancy by uncom-
petitive antagonists.

These binding sites are associated with different subunits,
and their affinities can vary depending on subunit composi-
tion. NR1 homomers have been shown to form glycine
binding sites, but an NR3 subunit appears to be required
to form both glutamate and MK-801 binding sites (29–32).
Further, receptors containing NR2A subunits have a higher
affinity for compounds that bind to the glutamate agonist
site, whereas receptors with NR2A or NR2B subunits have
higher affinities for MK-801 binding than do receptors with
NR2C or NR2D subunits (31). In addition, NMDA recep-
tors containing particular NR1 splice variants have a higher
affinity for MK-801 than do receptors with others, irre-
pective of NR2 co-assembly (33). Receptors with NR2B
subunits are associated with a higher affinity for polyamine
modulators (31,34). Therefore, differential subunit combin-
ations confer unique binding properties to the NMDA
receptors and probably are associated with subtle electro-
physiologic differences within a population of NMDA re-
ceptors.

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 do-
 mains and large, extracellular amine termini. When ex-
pressed in heterologous systems, group I mGluRs have been
shown to stimulate phospholipase C, phosphoinositide hy-
drolysis, and the formation of cyclic adenosine monophos-
phate (cAMP) (41–44). In heterologous systems, groups II
and III mGluRs inhibit forskolin-stimulated cAMP forma-
tion and adenylyl cyclase, possibly via a G, protein (39,40,
45,46). The metabotropic receptors have been the target of
considerable recent interest because a functional relation-
ship 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 hy-
pothesized 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.

**TABLE 52.1. AMPA RECEPTOR BINDING AND SUBUNIT expression IN SCHIZOPHRENIA**

<table>
<thead>
<tr>
<th>Ligand or Subunit</th>
<th>Findings</th>
<th>Brain Regions Studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor binding sites</td>
<td>[1H]CNQX</td>
<td>caudate</td>
<td>57</td>
</tr>
<tr>
<td>[1H]CNQX</td>
<td>none</td>
<td>putamen, nucleus accumbens</td>
<td>57</td>
</tr>
<tr>
<td>[1H]AMPA</td>
<td>none</td>
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<td>55</td>
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<tr>
<td>[1H]CNQX</td>
<td>none</td>
<td>CA4, CA3</td>
<td>53</td>
</tr>
<tr>
<td>[1H]AMPA</td>
<td>none</td>
<td>frontal cortex, putamen, nucleus accumbens</td>
<td>58</td>
</tr>
<tr>
<td>[1H]AMPA</td>
<td>none</td>
<td>caudate, putamen, nucleus, accumbens</td>
<td>56</td>
</tr>
<tr>
<td>[1H]AMPA</td>
<td>none</td>
<td>CA2</td>
<td>54</td>
</tr>
<tr>
<td>[1H]AMPA</td>
<td>none</td>
<td>dentate gyrus, CA1, CA3, subiculum</td>
<td>54</td>
</tr>
<tr>
<td>[1H]AMPA</td>
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<td>thalamus</td>
<td>61</td>
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</tr>
<tr>
<td>GluR1</td>
<td>none</td>
<td>CA1, CA3, CA4, subiculum</td>
<td>50</td>
</tr>
<tr>
<td>GluR1</td>
<td>none</td>
<td>CA4</td>
<td>50</td>
</tr>
<tr>
<td>GluR1</td>
<td>none</td>
<td>dentate gyrus, CA1, CA3, subiculum parahippocampal gyrus</td>
<td>50</td>
</tr>
<tr>
<td>GluR1, GluR3</td>
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<td>hippocampus</td>
<td>52</td>
</tr>
<tr>
<td>GluR2, GluR3</td>
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<td>cingulate cortex</td>
<td>52</td>
</tr>
<tr>
<td>Subunit mRNA expression</td>
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</tr>
<tr>
<td>GluR2</td>
<td>none</td>
<td>CA1, parahippocampal gyrus</td>
<td>49</td>
</tr>
<tr>
<td>GluR2</td>
<td>none</td>
<td>dentate gyrus, CA3, CA4, subiculum</td>
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<tr>
<td>GluR1</td>
<td>none</td>
<td>CA1</td>
<td>49</td>
</tr>
<tr>
<td>GluR1, GluR2, GluR3, GluR4</td>
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<td>caudate, putamen, nucleus accumbens</td>
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<tr>
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<td>CA3</td>
<td>48</td>
</tr>
<tr>
<td>GluR1, GluR2, GluR3, GluR4</td>
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<td>dentate gyrus, CA1, CA4, subiculum</td>
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<tr>
<td>GluR1</td>
<td>none</td>
<td>caudate, putamen, nucleus accumbens</td>
<td>56</td>
</tr>
<tr>
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<td>thalamus</td>
<td>61</td>
</tr>
<tr>
<td>GluR2, GluR3</td>
<td>none</td>
<td>thalamus</td>
<td>61</td>
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<td>none</td>
<td>frontal cortex</td>
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<td>hippocampus</td>
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<td>51</td>
</tr>
<tr>
<td>flip-flop ratio</td>
<td>none</td>
<td>hippocampus</td>
<td>51</td>
</tr>
</tbody>
</table>

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; CNQZ, 6-cyano-7-nitro-quinoxalindione
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 GluR2/3 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 \[^{3}H\]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 \[^{3}H\]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 \[^{3}H\]CNQX, in caudate, putamen, and accumbens in schizophrenia, no differences in \[^{3}H\]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 \[^{3}H\]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 \[^{3}H\]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, GluR7 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 \[^{3}H\]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;
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 NR\(_2\) transcripts was elevated in the schizophrenic patients. Recently, Gao et al. (54) found an altered stoichiometry of NMDA subunits in hippocampus, with decreased NR\(_1\) and increased NR\(_{2B}\) mRNA expression but normal NR\(_{2A}\) expression, in schizophrenia. Several other studies have been published in which only the NR\(_1\) 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), NR\(_1\), 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 NR\(_1\), 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

<table>
<thead>
<tr>
<th>Ligand or Subunit</th>
<th>Findings</th>
<th>Brain Regions Studied</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Receptor binding sites</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$[^{3}H]$MK-801</td>
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<td>57</td>
</tr>
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<td>$[^{3}H]$MK-801</td>
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<td>68</td>
<td></td>
</tr>
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<td>none</td>
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<tr>
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<td>72</td>
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<td>$[^{3}H]$L-689, 560</td>
<td>temporal cortex</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>$[^{3}H]$L-689, 560</td>
<td>motor cortex</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>$[^{3}H]$CGP39653</td>
<td>none</td>
<td>temporal cortex, motor cortex</td>
<td>30</td>
</tr>
<tr>
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<td>temporal cortex</td>
<td>30</td>
<td></td>
</tr>
<tr>
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<td>none</td>
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<tr>
<th>Subunit mRNA expression</th>
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<td>NR2D</td>
<td>none</td>
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<td>66</td>
</tr>
<tr>
<td>NR1,2,2A,2B,2C</td>
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<td>prefrontal cortex</td>
<td>66</td>
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<tr>
<td>NR1,2,2A,2B,2C,2D</td>
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<td>cerebellum, parietotemporal cortex</td>
<td>66</td>
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<td>thalamus</td>
<td>61</td>
</tr>
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<td>none</td>
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<td>61</td>
</tr>
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</tr>
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</tbody>
</table>

NMDA-associated sites is the ion channel/PCP site. In general, studies in which $[^{3}H]$MK-801 was used have been relatively unimpressive. In an early study (68), increased $[^{3}H]$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 $[^{3}H]$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 $[^{3}H]$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 $[^{3}H]$glutamate in the hippocampus, and no differences have been found in schizophrenia (53,54). The glycine co-agonist site has also been studied. Using $[^{3}H]$glycine, Ishimaru and colleagues (71) reported increased binding in multiple cortical areas in schizophrenia. Recently, the glycine site was studied in striatum with $[^{3}H]$L-689,560, and increased binding was noted in putamen, but not caudate or accumens, 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 $[^{3}H]$CGP39653) and glycine (measured with $[^{3}H]$MDL105,519) agonist sites, the intrachannel/PCP site ($[^{3}H]$MK-801), and the polyamine modulatory site ($[^{3}H]$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

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Findings</th>
<th>Brain Regions Studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>mGluR1,2,3,4,5,7,8</td>
<td>none</td>
<td>thalamus</td>
<td>74</td>
</tr>
<tr>
<td>mGluR3</td>
<td>none</td>
<td>prefrontal cortex</td>
<td>73</td>
</tr>
<tr>
<td>mGluR5</td>
<td>prefrontal cortex</td>
<td>73</td>
<td></td>
</tr>
</tbody>
</table>

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 uptake 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

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Findings</th>
<th>Brain Regions Studied</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]glutamate</td>
<td>none</td>
<td>caudate, putamen, nucleus accumbens</td>
<td>57</td>
</tr>
<tr>
<td>[3H]aspartate</td>
<td>frontal cortex</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>[3H]aspartate</td>
<td>temporal cortex</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>[3H]aspartate</td>
<td>anterior cingulate gyrus</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>[3H]aspartate</td>
<td>hippocampus, temporal cortex</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>[3H]glutamate</td>
<td>CA4, CA3, CA2, CA1, dentate gyrus, parahippocampal gyrus</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>[3H]glutamate</td>
<td>CA3, CA2, CA1, dentate gyrus, subiculum</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>[3H]aspartate</td>
<td>putamen, globus pallidus caudate,</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>[3H]aspartate</td>
<td>nucleus accumbens</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>
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/EAAAT₃ 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 medial 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.

REFERENCES


16. Nakanishi S. Molecular diversity of glutamate receptors and spine density in mice lacking the NMDA receptor subunit NR3A. Nature 1998;393:377–381.


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50. Eastwood SL, Burnet PWJ, Harrison PJ, GluR2 glutamate receptor subunit flip and flop isoforms are decreased in the hippocampal formation in schizophrenia: a reverse transcrptase-polymerase chain reaction (RT-PCR) study. *Mol Brain Res* 1997;44:92–98.


