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GLUTAMATERGIC MECHANISMS IN SCHIZOPHRENIA

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■ **Abstract** Schizophrenia is a chronic, severely disabling brain disorder with symptomatic onset in early adulthood. Typical antipsychotic medications that block dopamine D2 receptors are most effective in treating the psychosis but have limited effects on the negative symptoms and cognitive impairments. Considerable research has demonstrated that noncompetitive NMDA receptor antagonists, the dissociative anaesthetic like phencyclidine and ketamine, reproduce the cardinal symptomatic features of schizophrenia. Postmortem studies reveal variable alterations in glutamate receptors and their modulators in schizophrenia. Several clinical trials indicate agents that enhance NMDA receptor function via the glycine modulatory site reduce negative and variably improve cognitive function in schizophrenics receiving typical antipsychotics. Thus, hypofunction of a subpopulation of cortico-limbic NMDA receptors may participate in the pathophysiology of schizophrenia.

INTRODUCTION

Schizophrenia is a chronic, severe, disabling brain disorder that typically has its symptomatic onset in early adulthood and persists, in most cases, throughout life (1). Schizophrenia affects multiple cognitive-behavioral domains comprising the *positive symptoms* including delusions, hallucinations, and thought disorder; *negative symptoms* such as apathy and social incompetence; and *cognitive symptoms* with impairments of attention, memory, and executive functions. Schizophrenia results from a complex interplay of heritable risk factors and the environment as indicated by the concordance rate of approximately 50% in monozygotic twins but 12% to 17% for dyzogotic twins and first degree relatives (2). Ongoing linkage studies have revealed several sites in the human genome that are associated with increased heritable risk for schizophrenia (3).

Structural brain imaging has consistently demonstrated reduced cortical, hippocampal, and thalamic volume, and ventricular enlargement (4–7). Prospective

imaging studies suggest that these changes may be progressive, at least in the most impaired patients (8). Functional imaging studies also reveal the inability of schizophrenics to activate frontal cortex and hippocampus in cognitive tasks that require their engagement for optimal performance (9). Finally, nuclear magnetic resonance spectroscopic studies have found small but significant reductions in the levels of N-acetyl aspartate (NAA), a marker for neuronal integrity (10), in the prefrontal cortex, hippocampus, and temporal lobe (11, 12). Thus, schizophrenia is a disorder with structural and functional abnormalities distributed in the thalamo-cortico-limbic regions of the brain.

DOPAMINERGIC HYPOTHESIS

For nearly three decades, the dominant hypothesis about the pathophysiology of schizophrenia posited a dysregulation of dopaminergic neurotransmission (13). The hypothesis was based upon two fundamental observations. First, abuse of stimulants like amphetamines and cocaine, which enhance central dopaminergic neurotransmission, can cause a psychosis that resembles the positive symptoms of schizophrenia. Secondly, the antipsychotic medications, such as haloperidol and chlorpromazine, which have been the main stay for treatment for nearly 50 years, have in common their ability to block dopamine D2 receptors, and their affinity for these receptors correlates highly significantly with their clinical potency in ameliorating psychosis. However, this correlation has become weaker as a result of recently developed atypical antipsychotic medications that also show substantial affinity for 5HT 2 receptors.

Attempts in postmortem studies, as well as positron emission tomographic (PET) studies to demonstrate upregulation or excessive activation of D2 receptors, have not resulted in consistent findings (14). Thus, the most parsimonious interpretation of these findings is that excessive activation of the D2 receptor is most probably associated with positive symptoms and not negative symptoms or cognitive impairment (15). Furthermore, the nearly unique effects of clozapine, a weak D2 receptor antagonist that reduces negative symptoms and enhances cognitive function, supports the notion that additional neurotransmitter systems contribute to the psychopathology of schizophrenia (16). Indeed, the D2 antagonists are not specific for schizophrenia and are effective in reducing psychosis in bipolar disorder and in psychotic depression.

GLUTAMATERGIC SYSTEMS

Glutamate is the major excitatory neurotransmitter in the mammalian brain and is utilized by 40 percent of all synapses. Glutamate serves as the neurotransmitter of the pyramidal cells, which are the sources of efferent and interconnecting pathways of the cerebral cortex and limbic system—brain regions implicated in the

pathophysiology of schizophrenia (17). The effects of glutamate are mediated by two major classes of receptors: glutamate-gated ion channels and metabotrophic glutamate receptors, which are G protein–coupled receptors (for review, see 18). The metabotrophic glutamate receptors, eight of which have been cloned, act via phospholipase C (mGlu R1 and 5) or by inhibiting adenyl cyclase, thereby modulating glutamatergic neurotransmission in complex ways. The ionotrophic glutamate receptors can be further subdivided into two families: the AMPA/kainate receptors, which play a primary role in generation of excitatory postsynaptic currents, and the NMDA receptors. Cloning studies have revealed at least 15 different subunits, which have multiple splice variants and edited sites, that comprise the glutamate-gated cation channels (19).

The NMDA receptors have some important features that distinguish them from other glutamate ionotrophic receptors. First, they are voltage-dependent so that magnesium noncompetitively blocks the channel under normal resting membrane potential, a blockade that is relieved by depolarization. Second, the NMDA receptor possesses a binding site for glycine and another endogenous ligand D-serine, which must be occupied in order for glutamate to open an ion channel (20, 21). The intrasynaptic concentration of glycine in forebrain is regulated by sodium-dependent glycine transporter expressed on astrocytes (21). And, D-serine is synthesized by serine racemase, which is also expressed in astrocytes (22). The NMDA receptor and channel is encoded by NR-1, which forms a heteromeric receptor channel complex composed of one of four additional polypeptides (NR-2 A–D). The NR-2 subunits determine the pharmacologic and biophysical characteristics of the NMDA receptor (23). Recent studies are revealing an expanding number of modulators that affect both AMPA/kainate and NMDA receptor function (24).

THE PCP LINK

Phencyclidine (PCP), ketamine, and MK 801 are dissociative anaesthetics that, at pharmacologically relevant concentrations, act as noncompetitive antagonists of the NMDA receptor by binding to a site in the channel. Since PCP was first introduced, its psychotomimetic effects and their resemblance to schizophrenia were recognized (25). However, with the emergence of a more refined understanding of the multiple domains affected by schizophrenia, the similarities have become even more compelling. As was reviewed by Javitt & Zukin, stimulant abuse is associated with primarily positive symptoms of schizophrenia, whereas PCP abuse results in positive symptoms, negative symptoms, and cognitive impairments (26). Nevertheless, those conclusions must be considered cautiously as PCP-abusers likely use other substances, such as marijuana and may, in fact, have a latent risk for schizophrenia. To preclude this reservation, Krystal et al. carried out well-controlled studies in the laboratory setting on the effects of ketamine in normal human volunteers (27–29). With the exception of hallucinations, ketamine caused positive symptoms in the form of delusions and thought disorder, negative

symptoms characterized by withdrawal, blunted affect, and psychomotor retardation, and cognitive impairments. These cognitive deficits are subtle and characteristic of schizophrenia, including impaired performance on the Wisconsin Card Sorting Test, on the Verbal Declarative Memory Test, and on the Delayed Word Recall and Verbal Fluency without global cognitive impairment as assessed by the Mini Mental State Exam. Studies have also been carried out on remitted schizophrenics for whom the administration of ketamine produces delusions, hallucinations, and thought disorder resembling the pattern of psychotic relapse typical for that patient (30). Notably, treatment with clozapine but not with the typical antipsychotic haloperidol attenuated the exacerbation of clinical symptoms produced by ketamine (31).

Repeated administration of disassociative anaesthetics may provide a more valid model of schizophrenia than acute administration. Thus, the psychomimetic effects of single dose infusion of ketamine in normal subjects tends to be mild and somewhat variable; in contrast, chronic abuse of PCP is associated with severe and persistent psychotic symptoms more typical of schizophrenia. This distinction is reinforced by findings from functional brain imaging studies. Schizophrenics exhibit impairments in the ability to perform tasks that involve the frontal lobes, which corresponds to their inability to activate the frontal lobe while carrying out such tasks. The acute administration of ketamine to normal volunteers increases blood flow in the prefrontal cortex and anterior cingulate and decreases hippocampal perfusion (32, 33). In contrast, chronic PCP-abusers exhibit hypofrontality characteristic of schizophrenics (34).

An important question is which subpopulation of NMDA receptors are selectively affected by the subanaesthetic doses of the dissociative anaesthetics since near complete inhibition results in anaesthesia. In the acute hippocampal slice preparation, Grunze et al. found that GABAergic interneurons were tenfold more sensitive to NMDA receptor inhibitors than were the pyramidal neurons (35). This would result in a subtle loss of GABAergic inhibition that could interfere with localized processing, which would result in cognitive impairment. Consistent with this hypothesis, Heckers et al. (36) have used functional brain imaging on schizophrenics performing a memory task that requires activation of the hippocampus. The patients performed poorly and were unable to activate the hippocampus because its basal activity was elevated at rest to the level achieved by controls during the performance of the task (ceiling effect).

HUMAN FINDINGS

Among the first to propose a defect in glutamatergic neurotransmission in schizophrenia, Kim et al. in 1980 reported reduced concentrations of glutamic acid in the cerebral spinal fluid of patients with schizophrenia (37). This finding was replicated by some investigators but not by others (38). More recently, Tsai et al. (39) did not find a difference from controls but observed an inverse correlation between

cerebrospinal fluid glutamate levels and cortical atrophy in schizophrenia. In a postmortem study, Tsai et al. found decreased concentrations of glutamate and aspartate in the prefrontal cortex and a decreased concentration of glutamate in hippocampus of patients with schizophrenia as compared to controls (40). Furthermore, they found that the concentration of N-acetyl aspartyl glutamate (NAAG), an acidic dipeptide which acts as an antagonist at NMDA receptors and an agonist at mGluR 3 receptors (41), was increased in the hippocampus and that the activity of glutamate carboxypeptidase II (GCP II), the enzyme that catabolizes NAAG to glutamate, and NAA were selectively reduced in the frontal cortex, temporal cortex, and the hippocampus in schizophrenics. Consistent with this finding, magnetic resonance spectroscopic studies in schizophrenia have demonstrated small but highly significant reductions in the levels of NAA, the metabolite of GCP II, in the frontal cortex, temporal cortex, and hippocampus in schizophrenics (12). Kynurenic acid, another endogenous antagonist at NMDA receptors, has also been reported to be increased in the hippocampus in postmortem studies from schizophrenics (42).

Postmortem studies of glutamate receptor binding have yielded tantalizing results. Ligand-binding techniques have shown consistent increases in kainate receptors in the prefrontal cortex and decreased AMPA and kainate receptor-binding in the hippocampus, but they show no alterations in NMDA receptor density (43–47). Immunocytochemical analyses have replicated the decreased AMPA receptors in medial temporal lobe, although reductions in the hippocampus were not confirmed (48). Ligand-binding studies can also label the NMDA receptor at its glycine modulatory site or its channel at the PCP site. The latter has not been found to be altered in schizophrenia, but the former has been reported to be elevated throughout the primary sensory cortex (49, 50).

Studies of receptor expression generally support the findings from the ligand-binding studies. Reductions in the mRNA-encoding AMPA receptor subunits have been found in the hippocampus and in the parahippocampus of schizophrenics (51). Furthermore, Akbarian et al. (52) found a tenfold increase in the unedited form of GluR 2 in the prefrontal cortex of patients with schizophrenia (0.1% to 1.0%), which would increase AMPA receptor permeability to calcium and thereby potentiate neurotoxicity. The mRNAs-encoding kainate receptor subunits have also been found to be reduced in the hippocampus and parahippocampus (53, 54). While the ligand-binding studies have not revealed remarkable alterations in NMDA receptors, the expression of the NMDA NR-2D subunit was found to be increased in the prefrontal cortex of schizophrenics, whereas the NMDA NR-1 subunit was found to be decreased in the hippocampus of schizophrenic patients (55, 56). These alterations did not appear to be the result of neuroleptic exposure. Finally, Ibrahim et al. (57) reported decreased levels of mRNA encoding subunits of the NMDA, AMPA, and kainate receptors in the thalamus of schizophrenic patients and decreased ligand binding to the polyamine and glycine modulatory sites of thalamic NMDA receptors. Notably, these alterations were most evident in nuclei with reciprocal projections to the limbic regions of the brain.

ANIMAL STUDIES

Acute administration of NMDA receptor antagonists to rodents markedly increases the release of dopamine and glutamate in the prefrontal cortex and in subcortical regions. Moghaddam et al. (58) showed that ketamine-induced augmentation of dopamine release in the prefrontal cortex resulted in impaired performance on memory tasks sensitive to prefrontal cortical function. The cognitive and behavioral effects of ketamine could be attenuated by treatment with an AMPA/kainate receptor antagonist and with a group II mGluR agonist, LY 354740 (59). The PCP augmentation of subcortical dopamine release was also inhibited by glycine and the associated behaviors by an inhibitor of the glycine transporter (60).

NMDA antagonists increase the rate but decrease the variability of dopaminergic neuronal activity in the ventral tegmental area (VTA) in a projection-specific fashion; thus, burst firing increased in dopamine neurons projecting to the limbic regions but decreased in dopamine neurons projecting to the prefrontal cortex (61, 62). These findings of increased dopamine release with treatment with dissociative anaesthetics have been extended to human subjects by monitoring the striatal binding of [^{11}C]raclopride with positron emission tomography (63). Thus, acute administration of ketamine in normal volunteers resulted in [^{11}C]raclopride displacement comparable to that seen with administration of the dopamine-releasing amphetamine and the degree of displacement correlated with psychotic symptoms (64, 65).

Whereas the acute administration of dissociative anaesthetics increases dopamine turnover in the prefrontal cortex, chronic treatment results in decreased dopamine turnover. Treatment of rats for seven days with PCP resulted in a 75% reduction in the turnover of dopamine and a 40% reduction in extracellular dopamine measured by in vivo dialysis in the prefrontal cortex (58). Reinforcing this hypodopaminergic state, treatment with dissociative anaesthetics also causes decreased expression of the dopamine D1 receptor in prefrontal cortex of rats and monkeys (34, 66).

A more nuanced formulation of the dopamine hypothesis argues for diminished dopaminergic neurotransmission in the prefrontal cortex, which may contribute to cognitive impairment and negative symptoms and to a corresponding increase in dopaminergic neurotransmission in the mesolimbic pathways, which causes positive symptoms (67). In contrast to the decrease of frontal cortical turnover of dopamine, chronic administration of dissociative anaesthetics increases subcortical dopamine release, particularly in the nucleus accumbens, and produces sensitization to the behavioral effects of NMDA receptor antagonists, dopamine agonists, and stress (68–70). Thus, these findings of a prefrontal hypodopaminergic state and subcortical excessive dopamine release caused by dissociative anaesthetics indicate that the hypothesized hypofunction of NMDA receptors in schizophrenia is not inconsistent with current evidence of dopaminergic dysregulation in schizophrenia.

Mohn and her colleagues (71) created a mouse mutant by recombinant methods in which the expression of the NR-1 subunit was reduced by 95% (complete

knockouts of NR-1 do not survive). These mice were hyperactive and exhibited stereotypies, behavioral markers of dopaminergic overactivity, as well as profound impairments of social behaviors and sexual activity. Notably, the typical antipsychotic, haloperidol, a selective dopamine D2 receptor antagonist, reduced the hyperactivity and stereotypies but not the social impairments. In contrast, clozapine, an atypical antipsychotic that reduces the negative symptoms of schizophrenia, normalized the aberrant social behaviors.

CLINICAL INTERVENTIONS

The preclinical and clinical evidence supporting the role of NMDA receptor hypofunction in schizophrenia has prompted clinical trials of agents that may enhance an NMDA receptor function. Direct agonists have not been studied because of the concern that excessive activation of NMDA receptors may cause excitotoxic damage to neurons (72). Rather, most studies to date have focused on the glycine modulatory site on the NMDA receptor. Three agents have been examined in clinical trials: glycine, D-serine, and D-cycloserine (DCS). Most trials have examined the effects of adding these agents to ongoing neuroleptic therapy in poorly responsive patients as a preliminary step towards characterizing efficacy and as an ethical way to avoid depriving patients of treatment with known therapeutic benefit.

Early trials with glycine used relatively low doses of 5 g to 15 g per day, which resulted in variable therapeutic effects, probably because glycine crosses the blood brain barrier poorly. More recently, Javitt and his colleagues have carried out double-blind placebo controlled crossover trials using high doses of glycine ranging from 30 g to 60 g per day added to antipsychotic treatments. Their studies demonstrated selective improvement in negative symptoms and, in an extended trial, significant improvement in cognitive function (73–76).

Utilizing another full agonist at the glycine modulatory site, Tsai et al. (77) studied the addition of D-serine (30 mg/kg/day) to ongoing antipsychotic medication in a double-blind placebo controlled trial and reported a significant reduction in negative symptoms and in positive symptoms and improvement in cognitive function as assessed by the Wisconsin Card Sorting Test. D-serine has greater penetrance of the blood brain barrier and higher affinity for the glycine modulatory site, which may account for the greater efficacy in this trial (78).

D-cycloserine has attracted interest because it is an antitubercular drug that has been in clinical use for several decades and has been shown to be a relatively selective partial agonist at the glycine modulatory site over a narrow range of concentrations (79). D-cycloserine has 60% of the efficacy of glycine so that at low concentrations of glycine it behaves as an agonist, but at saturating concentrations of glycine it behaves as an antagonist (80). An initial dose-finding study revealed significant improvement in the performance of a cognitive task and in negative symptoms at a dose of 50 mg/day with loss of effect at 250 mg/day (81). Consistent with these results, another study demonstrated efficacy at 100 mg/day (82). In studies utilizing 15, 30, and 250 mg/day, other investigators found no

effect of D-cycloserine, consistent with the narrow U-shaped dose response curve (83–86). A large double-blind placebo controlled study of D-cycloserine added onto typical antipsychotics again revealed significant reductions in negative symptoms, although no significant differences were observed with regard to cognitive symptoms (87). Thus, the most consistent findings with the glycine modulatory site agonists are a reduction in negative symptoms of schizophrenia and variable improvements in cognitive function; only the most potent full agonist D-serine affected positive symptoms (77).

Clozapine is a novel antipsychotic medication noted for its efficacy in a substantial percentage of schizophrenics with prominent negative symptoms who do not respond to typical antipsychotics. Thus, it was of interest to determine whether glycine modulatory site agonists might further augment its effects. To the contrary, two trials with glycine and one with D-serine added to clozapine yielded no additional benefit in contrast to the response of patients receiving typical antipsychotics (88). Furthermore, two trials with the partial agonist D-cycloserine added onto clozapine revealed actual deterioration of negative symptoms so that their negative symptom scores appeared to be virtually identical to the scores of patients receiving typical antipsychotics alone (87, 89). The best explanation of these findings is that clozapine may exert its effects on negative symptoms and cognitive impairment by augmenting glycine modulatory site occupancy so that agonists have no additional effects, whereas the partial agonist, D-cycloserine, behaves like an antagonist.

As NMDA receptor recruitment involves membrane depolarization via AMPA receptors, agents that positively modulate AMPA receptor function might have therapeutic effects in the situation of NMDA receptor hypofunction (90). Ampakines are a family of drugs that act as positive modulators of the AMPA receptor complex, increasing the peak and the duration of glutamate-induced AMPA receptor-gated inward currents. They enhance long-term potentiation in the hippocampal slice, as well as learning and memory in several behavioral tasks (91). Preliminary results from placebo controlled trials in a small number of patients receiving clozapine revealed a consistent pattern of improved performance in tests of attention, memory, and distractibility (92).

CORTICAL ATROPHY

As noted above, schizophrenia is associated with significant atrophy of the cerebral cortex. Recent studies indicate that this atrophy may be progressive, at least in a subpopulation of patients with a severe form of the disorder (8). Olney and colleagues have carried out a series of studies demonstrating that dissociative anaesthetics cause neuronal damage as evidenced by the expression of heat shock proteins and even neuronal death in cingulate and retrosplenial cortex in rats, which appears to be due to disinhibition of glutamatergic neurons leading to

excessive activation of AMPA/kainate receptors (93–95). Notably, this vulnerability is age dependent, becoming apparent at puberty and maximal in early adulthood, the period of vulnerability for the symptomatic onset of schizophrenia. Furthermore, the psychotomimetic effects of ketamine in humans exhibits a similar developmental time course with minimal effects prior to puberty (96). The atypical antipsychotics, olanzapine and clozapine, prevent the neurotoxicity, whereas typical antipsychotics are less effective (97, 98). If these findings can be extended to schizophrenia, they suggest that appropriate and early pharmacologic intervention might prevent deterioration, an inference that is supported by clinical evidence (99).

CONCLUSION

Preclinical as well as clinical studies provide circumstantial but convincing evidence of hypofunction of NMDA receptors as a primary, or at least, a contributory process in the pathophysiology of schizophrenia. Given that the dissociative anaesthetics, at concentrations that cause subtle cognitive impairments, have schizophrenogenic-like effects, it is likely that only a subpopulation of NMDA receptors, presumably those on cortico-limbic GABAergic interneurons, are affected. This interpretation is consistent with neurophysiologic studies, as well as with the evidence of modest cortical disinhibition upon administration of ketamine, which mimics the functional abnormalities seen in brain imaging studies in schizophrenia. Several clinical trials with agents that act at the glycine modulatory site on the NMDA receptor have revealed consistent reductions in negative symptoms and variable effects of cognitive and positive symptoms. These studies also provide evidence that suggests the effects of clozapine on negative symptoms and cognition may be through activation of the glycine modulatory site on the NMDA receptor.

The NMDA receptor is part of an elaborate complex in the postsynaptic density with several modulatory sites so that the opportunities for subtle impairments of NMDA receptor function are multiple (100, 101). The pharmacologic targets to enhance NMDA receptor function are numerous and include the glycine modulatory site, the glycine transporter, serine racemase, the polyamine site, and the zinc binding site, and they are differentially sensitive depending upon the NR-2 subtype. Thus, allosteric positive modulators of the NMDA receptor with receptor subtype selective effects may become important treatment strategies for schizophrenia and other disorders of impaired cognitive function.

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