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Novel Targets for Development of Drugs for Treating Schizophrenia: Focus on Glycine, D-Serine and Nitric Oxide

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ABSTRACT

For many years the focus of neurochemical and pharmacological studies on schizophrenia was on dopamine, and that work yielded a great deal of information about the possible etiology of this debilitating disorder and contributed significantly to the development of antipsychotic drugs. However, it has become increasingly apparent that other neurotransmitters and neuromodulators must also play an important role, and in recent years there has been considerable interest in these other compounds and the systems related to them in the search for developing drugs that are more effective and have fewer side effects than the antipsychotics currently available. This review deals with three of those neurochemicals, namely glycine, D-serine and nitric oxide, and shows how an increased knowledge of them may be important in the future diagnosis and/or pharmacotherapy of schizophrenia.

Key words: *D serine, dopamine, glutamate, glycine reuptake, nitric oxide, schizophrenia, sodium nitroprusside*

Schizophrenia is a devastating illness that affects approximately 1% of the population worldwide and there is an urgent need to learn more about its causes and to develop more effective drugs for its treatment. Although the emphasis for many years in schizophrenia research has been on the biogenic amine dopamine (1-6), it has become obvious that other neurotransmitters/neuromodulators must be involved. Thus in recent years there has been exciting research on the involvement of neurochemicals such as 5-hydroxytryptamine (5-HT, serotonin) (7), gamma-aminobutyric acid (GABA) (8), acetylcholine (9), neurosteroids (10, 11), glutamate (12-15) and nitric oxide (NO) (16). The present paper focuses on the research that has been done related to glutamate and NO and the consequences it may have for more effective pharmacotherapy of schizophrenia in the future.

Dysfunction of glutamate transmission in schizophrenia

Glutamate is the major excitatory neurotransmitter in the brain and a ligand of both ionotropic and metabotropic receptors. Increasing evidence has supported the importance of the glutamate system as a target for novel antipsychotics. This evidence includes studies demonstrating that the antagonism of ionotropic glutamate N-methyl-D-aspartate (NMDA) receptors with phencyclidine (PCP), dizocilpine (MK-801), or ketamine produces cognitive and sensory-motor deficits in rodents and induces in healthy human subjects positive, negative, and cognitive symptoms resembling those seen in schizophrenia (17-19). The NMDA receptor is important in mediating the activity of neurons by gating the influx of calcium, and prolonged stimulation of this receptor with glutamate or agonists of the glutamate-binding site can result in excess calcium entering neurons initiating a cascade of events potentially leading to excitotoxicity (20). In view of this toxicity, the focus has now shifted to the development of drugs that modulate the NMDA receptor distant from the glutamate-binding site, including agonists of the glycine B (GlyB) binding site located on the NR1 subunit that must be occupied simultaneously by glycine (or D-serine) in order for glutamate to activate the NMDA receptor. Add-on treatments, with glycine, D-serine, or D-alanine agonists at the GlyB site, show efficacy in alleviating the positive, negative, or cognitive symptoms of schizophrenic patients (21,22). While high doses of glycine may be effective against some symptoms of schizophrenia

(23), subjects may be more sensitive to NMDA receptor internalization (24) and/or activation of inhibitory glycine receptors (25,26) triggered by high glycine levels. Therefore, a better approach is required to stimulate synaptic NMDA receptors to provide beneficial effects on the negative and cognitive deficits in subjects with schizophrenia.

Glycine transporter inhibitors in schizophrenia

A promising avenue in drug development for schizophrenia is the inhibition of the glycine transporter-1 (GlyT1), a transporter responsible for the removal of glycine from the synapse (27). Inhibition of the GlyT1 transporter increases the levels of glycine within the synapse and augments the activity of the NMDA receptor. In brain, GlyT1 transporters are highly co-localized with NMDA receptors, suggesting that these transporters are intimately involved in mediating the function of NMDA receptors (28). Glycyldodecylamide, a glycine derivative, was shown several years ago to reverse PCP-induced schizophrenic-like symptoms in rodents by inhibiting glycine transport at the synapses of the cortex (29,30). This finding led to the development of the high affinity sarcosine-based GlyT1 inhibitor, N[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)-propyl]sarcosine (NFPS). Sarcosine is an endogenous metabolic intermediate of glycine metabolism and inhibits GlyT1 transporters (31). NFPS has been shown to potentiate the activity of NMDA receptors (32), alleviate withdrawal and cognitive symptoms in rodent models of schizophrenia (33,34) and reverse PCP-induced changes in dopamine release in the striatum both in vivo and in vitro (35,36). A problem with NFPS is that it produces undesired side effects in rodents due to its irreversible binding kinetics and non-specificity for different GlyT1 isoforms in the brainstem, spinal cord, and cerebellum. The resulting increased glycine in these regions acts on glycine receptors to induce respiratory inhibition, ataxia, and motor deficits such as meaningless or compulsive movements (37). In order to minimize these side effects, nonsarcosine-based inhibitors with reversible binding kinetics for the GlyT1 transporter have been developed; however, high doses of these inhibitors still produce some undesired side effects (38) but are better tolerated when compared to sarcosine-based compounds such as NFPS. One of these inhibitors, SSR504734, reverses the persistent latent inhibition exhibited in models of schizophrenia in rodents following the antagonism of the NMDA receptor (39) and increases extracellular levels of dopamine in the prefrontal cortex (40). Consistent with the involvement of dopamine in cognitive processes governed by the prefrontal cortex, SSR504734's effect of enhancing frontal dopaminergic neurotransmission may facilitate the attenuation in the cognitive deficits of schizophrenia. Experimental animal studies with another non-sarcosine based compound, RG-1678, have also shown promising results. An oral dose of 10 mg/kg RG-1678 in rats produces a robust and sustained increase in brain glycine levels (2.3 fold over baseline levels) and shows potent GlyT1 transporter inhibition (41). On this basis, RG-1678 has advanced into clinical trials, and researchers from Hoffmann-La Roche have recently reported positive phase II results for RG-1678 in the treatment of negative symptoms in schizophrenia (41).

Another non-sarcosine based compound, ASP2535 (4-[3-isopropyl-5-(6-phenyl-3-pyridyl)-4H-1,2,4-triazol-4-yl]-2,1,3-benzoxadiazole), exhibits high brain permeability and potency for GlyT1 inhibition (IC_{50}= 92 nM) in the rat (42). Harada and colleagues (42) evaluated the effect of ASP2535 on rodent models of cognitive impairment and found that the working memory and visual learning deficits in MK-801- and PCP-treated mice, respectively, were attenuated by ASP2535; in addition ASP2535 attenuated the PCP-induced deficit in prepulse inhibition in rats. PF-3463275, also a non-sarcosine based GlyT1 inhibitor, alleviates the deficits in the spatial working memory of rhesus monkeys after antagonism of the NMDA receptor with ketamine, but fails to reverse the ketamine-induced hallucinatory-like behaviors in these primates (43).

Co-administration of GlyT1 inhibitors and antipsychotics

Add-on treatments to antipsychotics aimed at increasing glutamatergic transmission have shown mixed results. Combined treatment with risperidone and Org-24461, a GlyT1 inhibitor, results in a decrease in extracellular brain dopamine concentrations accompanied by sustained increases in extracellular glycine and glutamate levels in the rat, whereas risperidone alone increases dopamine levels and has no effect on glycine levels (44). This suggests that co-administration of this GlyT1 inhibitor with an antipsychotic may help counteract NMDA receptor dysfunction and result in a reduction in the side effects normally induced by elevations in dopamine. In human subjects, sarcosine-based GlyT1 inhibitors improve positive and negative symptoms when administered in combination with risperidone, but not with clozapine (45). GlyT1 inhibitors have been reported to have varied effects on antipsychotic actions and effects on prefrontal glutamatergic transmission in subjects with schizophrenia depending on whether they are administered with risperidone or olanzapine (46). A number of factors can account for these varied results including the severity of symptoms, stage of the illness, emergence and presence of negative symptoms, and possible differing effects of the antipsychotic involved on glutamate transmission in preclinical and clinical studies. For instance, animal studies have shown effects on extracellular glutamate levels and NMDA receptor activity following risperidone treatment (47,48), whereas human subjects show no such effects (44). Clearly, more preclinical experiments and clinical trials are required to elucidate the best possible treatments for each stage of schizophrenia.

D-Serine in schizophrenia

There is accumulating evidence that suggests abnormalities in D-serine-mediated neurotransmission in schizophrenia. D-serine is a co-agonist of the NMDA receptor with a three-fold greater affinity than glycine at the same binding site (49). Abnormalities in the synthesis, degradation, or uptake of D-serine can serve as possible factors resulting in NMDA receptor dysfunction and the emergence of schizophrenia. Animal models show that D-serine administration reverses the behavioral and cognitive deficits induced by the NMDA antagonists PCP or MK-801 (50,51). An agonist at the glycine/D-serine binding site, 7-chlorokyneurenic acid, results in the attenuation of deficits in sensorimotor gating when injected into the nucleus accumbens (52).

D-serine is derived from the isomerization of L-serine catalyzed by the enzyme serine racemase (SR). Genetically altered SR-null mutant mice express less that 10% of normal brain D-serine levels and exhibit impaired performance on a trace-conditioning memory task which is rescued by D-serine treatment (53). Other animal studies have corroborated the behavioral and cognitive deficits in SR-mutant mice (54,55) associated with reductions in long-term potentiation, numbers of dendritic spines in the prefrontal cortex (56) and cortical or hippocampal volume (57), resembling characteristics observed in the brains of patients with schizophrenia (58).

The interest in novel drugs that target enzymes involved in D-serine metabolism is increasing. The catabolic enzyme D-amino acid oxidase (DAAO), present in both neurons and astrocytes, metabolizes D-serine into an imino acid, releasing ammonia and hydrogen peroxide as by-products (56). Human DAAO activity and expression have been reported to be increased in schizophrenic patients, while D-serine concentrations in serum and cerebrospinal fluid are decreased (59). Infusion of the DAAO inhibitor, AS057278, elevates the levels of D-serine in the rat cortex, and this is accompanied by a reversal of PCP-induced behavior (60).

Clinical findings lend inconsistent support for D-serine as an add-on to antipsychotic drugs for the treatment of schizophrenia. Schizophrenic patients enrolled in a 6-week double blind, placebo-controlled trial showed significant improvements in their positive, negative, and cognitive symptoms when D-serine was added (30 mg/kg) to their stable antipsychotic regimens such as risperidone and

olanzapine, but not clozapine (61). Another study found that add-on D-serine treatment did not significantly affect the symptoms in schizophrenic patients in a similar 6-week controlled trial, whereas GlyT1 inhibitors did show beneficial outcomes (62). However, higher doses of D-serine (60 and 120 mg/kg) showed significant improvement in symptoms and neuropsychological measures in antipsychotic-stabilized schizophrenic patients with minimal safety issues resulting from the increased doses (63). These findings suggest that more research is required in order to establish optimal doses of D-serine.

Nitric oxide and schizophrenia

Nitric oxide (NO)-based therapies may also be promising for antipsychotic drug development. In the central nervous system (CNS), NO acts as a second messenger of glutamate signaling, through the NMDA glutamate receptor complex. It is produced by substrate binding of the semi-essential amino acid L-arginine to the neuronal NO synthase (nNOS) enzyme, a NO-producing enzyme found mainly in neurons (64,65). NO is a lipophilic soluble gas that is able to diffuse across cell membranes and increase the production of the second messenger cyclic guanosine monophosphate (cGMP) in neighboring neurons, inducing widespread signaling cascades. Downstream signaling mechanisms that are regulated through a fully functional NMDA receptor, including the production of NO, may be disrupted in schizophrenia (66).

Several neurotransmitters and ion channels that have been implicated in schizophrenia are modulated by NO via cGMP and protein kinase activation. Studies on NO donors in animal models suggest that the release and the reuptake of several neurotransmitters, including catecholamines, acetylcholine, and excitatory and inhibitory amino acids, may be regulated by NO (67-70).

Preclinical studies have been helpful in understanding the possible role of NO in pharmacological models of schizophrenia. The use of NO donors and nNOS inhibitors has provided some insight regarding the contribution of NO to PCP-induced psychosis. The primary pharmacological action of PCP involves binding to an inhibitory site within the ion channel pore of the NMDA receptor, acting as a non-competitive antagonist (71). The NMDA receptor blockade is consistent with the dysregulated glutamate mechanisms that have been proposed to contribute to the pathophysiology of schizophrenia. NO donors such as sodium nitroprusside (SNP) and molsidomine have been shown to reduce PCP-induced locomotor effects and improve cognitive deficits in animal models (72-76). Several studies have also shown that a decrease in NO production (by blocking nNOS activity with NOS inhibitors) can enhance locomotor effects of PCP (73,76,77). However, an intact nNOS enzyme is required for PCP to induce hyperlocomotor activity. In knock-out mice, it has been demonstrated that reduced expression of nNOS reduces effects of PCP (78). Taken together, these studies suggest that NO is directly implicated in the mechanism of action of PCP, and that the modulation of the glutamate-NO-cGMP pathway may have therapeutic benefit in correcting the clinical manifestations of NMDA receptor pathophysiology in schizophrenia.

There have also been various clinical studies suggesting that signaling within the glutamate-NO-cGMP pathway may be disrupted in schizophrenia. Although NO is difficult to measure directly, the amount of the NO metabolites nitrite and nitrate in both plasma and cerebrospinal fluid (CSF) has been considered as an indirect index of in vivo NO production. Reduced levels of NO metabolites in plasma of drug-free patients with schizophrenia as compared to normal controls have been reported (79). Nitric oxide metabolite levels measured both pre- and post-treatment with risperidone have also been reported to be lower in patient groups (80), and treatment with risperidone can significantly increase NO metabolite plasma levels after 6-8 weeks of therapy (79,80), suggesting that an increase in NO production may contribute to the therapeutic effects of antipsychotic medications. Plasma NO metabolite levels have also been reported to be significantly lower in schizophrenic patients with prominent negative symptoms when compared to schizophrenic patients without such symptoms (81), and positively

correlated with the Positive and Negative Syndrome Scale (PANSS) negative symptom scores prior to antipsychotic treatment (79). Similar reductions in NO metabolites have also been reported in CSF of patients with schizophrenia as compared to controls (82). Reduced NO production in schizophrenia is also thought to be related to reduced cGMP levels in CSF (83), an effect that has also been reversed with antipsychotic treatment (84,85).

A recent study implicated the glutamate-NO-cGMP pathway in schizophrenia via the postsynaptic density (PSD)-95 protein, the protein that directly links the NR2 subunit of the NMDA receptor to the nNOS enzyme (86). In this study, fibroblast cells taken from 6 patients with schizophrenia were reprogrammed into human induced pluripotent stem cells and then further differentiated into disease-specific neurons for the illness. These neurons had reduced neuronal connectivity, reduced neurite numbers, and reduced glutamate receptor expression, also suggesting that several neuronal components that are necessary to produce adequate levels of NO in the brain are diminished in the illness.

Studies that have measured clinical response to pharmacologically-mediated NO neurotransmission in schizophrenia have been limited, and as a result, drug development directed toward this particular molecular target has yet to be adequately pursued. Phosphodiesterase-5 (PDE-5) inhibitor drugs like sildenafil have been used as an augmenting strategy in schizophrenia to enhance cGMP signaling mechanisms downstream of NMDA receptors (87). Phosphodiesterase-5 specifically degrades cGMP. By inhibiting the PDE-5 enzyme, the degradation of cGMP is protected and downstream effects of this second messenger are achieved. In schizophrenia, this strategy acts to increase cGMP levels without directly affecting the NMDA receptors, bypassing the NMDA receptor dysfunction. As an adjunctive treatment, sildenafil combined with risperidone significantly improved the negative symptoms of the illness (87).

An alternative approach to improve NMDA receptor functioning by increasing NO production has recently been investigated in a randomized clinical trial in schizophrenia. In this study, an intravenous infusion of sodium nitroprusside (SNP) in patients already on antipsychotics and in the early stages of schizophrenia, produced rapid (within 4 hours of a single infusion) improvements of positive, negative, anxiety and depressive symptoms of the illness as compared to those patients receiving a placebo infusion (88). Following a single infusion of SNP at a dosage of 0.5µg/kg/min, significant effects were found on the total scores and on each of the 4 subscale scores (thinking disorder, withdrawal-retardation, anxiety-depression, and activation subscales) of the 18-item Brief Psychiatric Rating Scale (BPRS) and also on the PANSS negative subscale scores, with no significant cardiovascular side-effects, SNP toxicity, or exacerbation of symptoms (88). Symptom improvement also persisted for 4 weeks after the infusion (although adjustment of the doses of the antipsychotics was allowed 7 days after the infusion). Rapid-acting therapies that can control acute psychotic episodes within emergency and acute care settings are needed as existing antipsychotic medications often require significant time to produce an effect that will adequately reduce psychotic symptoms. Control of acute psychosis by the intravenous administration of NO donors may be a promising option for quick and efficient symptom relief within a reasonable time frame. Future development of SNP analogs or alternative drug delivery formulations to improve compliance and suitability for long-term use may ultimately be an effective treatment option for schizophrenia. The success of SNP administration has stimulated research on other NO donors, and in a preliminary study with L-arginine, we have found improvement in some symptoms of schizophrenics currently on antipsychotics (see this website for design details: http://clinicaltrials.gov/ct2/show/NCT00718510). Thus, administering NO donors may represent an exciting new option for treating schizophrenics. However, it is not yet certain that increasing NO levels is the primary mechanism of action of SNP, and there is considerable contradictory literature regarding whether or not NO and NOS are decreased in schizophrenia (see reference 16 for a comprehensive review).

In summary, research studies in recent years on glutamate and NO have enhanced our understanding of the etiology of schizophrenia, stimulated further investigation and provided much-needed insights into potential new targets for drug development.

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