The Hypothesis of NMDA Receptor Hypofunction for Schizophrenia

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Schizophrenia, a multifactorial mental disorder with polygenic inheritance as well as environmental influences, encompasses a characteristic group of symptoms and neurocognitive deficits. Cognitive function, a major determinant of quality of life and overall function in schizophrenia, contributes more to the prognosis of the disease than positive symptoms, such as delusions or hallucinations. Although its exact etiological mechanisms remain relatively unknown, extensive studies are ongoing to explore. Among them, one of the primary causal factors is dysfunction of the N-methyl-D-aspartate (NMDA)-type glutamate receptors. This article reviews the clinical limitations of current antipsychotics in treating the core symptoms of schizophrenia and the trend in the reconceptualization of the disease nature and treatment modalities. The NMDA receptor model plays a critical role in the revolution of pharmaceutical industry as a new set of drug targets in addition to those based on the traditional monoaminergic models is proposed. The evidence of NMDA receptor hypofunction in schizophrenia is accumulating from the investigations on the modulation of glutamatergic system, particularly the intrinsic NMDA/glycine site, through genetic research and various clinical trials. A group of “NMDA-enhancing agents,” being used either as adjuncts to typical/atypical antipsychotics or as monotherapy, in schizophrenic patients, particularly those with refractory negative and cognitive symptoms, offer efficacy in preclinical and early clinical trials. Novel therapeutic agents acting as NMDA enhancers show promise as the next wave of drug development for schizophrenia.

Key words: schizophrenia, NMDA receptor hypofunction, negative symptoms, cognitive function

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Introduction

Schizophrenia, affecting about 1% of the population worldwide, is a devastating and costly illness due to its resistance to treatment, the consequences of relapse, and substantial economic burden. Clinical symptoms of schizophrenia have three (positive symptoms, negative symptoms, and neurocognitive deficits) main categories. The latter two possess high predictive value for clinical outcomes [1] and account for much of the long-term morbidity of this illness. Optimizing the treatment of schizophrenia will be an important goal in the early era of 21st century, particularly on negative symptoms, and neurocognitive deficits.

Drug models have been applied extensively to study the pathophysiology of schizophrenia and thus provide a better insight into the neurobiology of the disorder. Hypofunction of N-methyl-D-aspartate receptor (NMDAR) mediated neurotransmission is implicated in the critical deficits associated with many brain disorders, especially schizophrenia [2]. This is evidenced by observations of the clinical simulation exerted by the non-competitive antagonists of NMDAR, phencyclidine (PCP) and ketamine on nonpsychotic individuals and schizophrenic patients [3]. Therefore, in addition to the dopamine and serotonin hypotheses, the NMDA hypofunction model of schizophrenia has recently gained extensive attention.

Enhancing NMDAR neurotransmission has been considered as a novel treatment approach, in particular through the glycine “modulatory” component (the coagonist site) at these receptors to avoid the excitotoxicity mediated through the glutamate binding site [4]. Recent advances in understanding the function, pharmacology, genetics and structure of NMDAR have promoted a search for new compounds that could be therapeutically beneficial. These compounds act on the coagonist binding sites, either directly or indirectly (Figure 1). Various NMDA-enhancing agents have been proposed and they have been or currently are under extensive studies. Many clinical trials on NMDA-enhancing agents have revealed encouraging results.

Glutamatergic Hypothesis

The classical dopamine hypothesis of schizophrenia [5] postulates that dopaminergic hyperactivity is responsible for the psychotic symptoms of this disorder. First-generation antipsychotic drugs ([FGAs], typical antipsychotics), which were developed in the 1950s with blockade of D2 receptor as a necessary therapeutic action [5], treat positive symptoms of schizophrenia effectively. In addition to D2 receptor blockade, the 5-HT2A receptor blockade plays a contributory role in the actions of the second-generation antipsychotic drugs ([SGAs], atypical antipsychotics), thus has advantages over typical antipsychotics in terms of greater efficacy for improving positive and negative symptoms, equivocal beneficial effects on cognitive function, and less extrapyramidal side effects and tardive dyskinesia. SGAs have been gradually replacing the FGAs since 1990s and became the first line treatments for schizophrenia. Nevertheless, these medications still show limited efficacy on negative and cognitive symptoms of schizophrenia as well as qualities of life, and they are associated with severe side effects, including agranulocytosis, sudden cardiac death, stroke, diabetes mellitus, hypercholesterolemia and significant weight gain.

Carlsson et al. [6] have pointed out that it is likely the dopaminergic system is not the only
dysfunctional system in schizophrenia as post-mortem examination of the brains of patients with schizophrenia did not reveal alterations in the levels of dopamine or dopamine receptors. Interactions between dopamine and several other neurotransmitters in complex neural networks have been revealed, thus other neurotransmitter systems are likely involved in the pathophysiology of schizophrenia.

Several studies have provided evidence that a dysfunction in glutamatergic neurotransmission might be involved in the pathophysiology of schizophrenia. Kim et al. [7] were among the pioneers in proposing a glutamatergic hypothesis of schizophrenia, based on findings of significantly reduced cerebrospinal fluid (CSF) levels of glutamate in patients with schizophrenia compared with normal controls. Studies of post-mortem brain and CSF have revealed a lower density of glutamatergic receptors and lower level of glutamate in schizophrenic patients than in healthy comparison subjects [8]. Lower indices of glut-
matergic neurotransmission are in correlation to more prominent thought disorder as well as ventricular enlargement [9]. Among these findings, the most compelling and more direct evidence is provided by the psychomimetic effects of the NMDA antagonists, phencyclidine (PCP) and ketamine [8].

Glutamate and dopamine have been reported to exhibit reciprocal actions at subcortical structures. The mechanisms underlying hyperdopaminergic function in schizophrenia may involve cortical glutamatergic projections to dopamine neurons in the midbrain [6]. Investigators further raised the hypothesis that NMDARs which regulate mesolimbic and mesocortical dopamine pathways may be hypoactive in schizophrenia [10].

Dopaminergic neurons are mastered either directly by corticofugal glutamatergic neurons or indirectly through γ-aminobutyric acid (GABA) interneurons, which act as accelerators or brakes, respectively [11]. A descending glutamatergic pathway projecting from cortical pyramidal neurons to dopaminergic neurons in the ventral tegmental area (VTA) normally functions as a brake on the mesolimbic dopaminergic pathway through an inhibitory GABAergic interneuron in the VTA, resulting in tonic inhibition of dopamine release from the mesolimbic pathway. Hypoactive NMDAR, as in schizophrenics or induced by ketamine, in the VTA may fail to tonically inhibit mesolimbic dopaminergic neurons; this would cause mesolimbic dopamine hyperactivity and thus the positive symptoms. Different from the actions of cortico-brainstem glutamatergic neurons on mesolimbic dopaminergic neurons, separate cortico-brainstem glutamatergic neurons synapse directly upon those dopaminergic neurons in the VTA that project to the cortex, so-called mesocortical dopaminergic neurons, and normally act to tonically excite them [10]. Therefore, hypoactive NMDAR in the VTA would cause mesocortical dopamine hypoactivity and thus the cognitive deficits and/or negative symptoms.

Although a focus on cognitive deficits and/or negative symptoms has come up with a more recent hypothesis of schizophrenia as a “glutamate disorder” [12], the glutamatergic hypofunction hypothesis is not in conflict with a role for dopamine in the pathogenesis of schizophrenia or with the action of currently available antipsychotics. This notion is supported by a rodent study which demonstrated that persistent NMDAR blockade produced a rapid and profound decrease in the levels of D2 receptor mRNA and receptor density, which suggests that NMDAR play an important role in the expression of D2 receptors in basal ganglia. Therefore, the interaction between glutamate and dopamine regulate the functions of the basal ganglia [13]. It was further proposed that dopamine receptor blockade might act secondarily to balance glutamatergic insufficiency [6]. Consequently, enhancement of NMDAR-mediated neurotransmission has been proposed as having the therapeutic potential at a fundamental pathophysiological level. Supporting the hypothesis, accumulating evidences from ongoing and forthcoming clinical trials, using drugs acting on NMDAR, give rise to optimism.

NMDA hypofunction model of schizophrenia complements the dopamine and serotonin hypotheses and its role has gained extensive attention. Over the last two decades, the relationship of NMDA function and schizophrenia is supported by the evidences of the effects of the noncompetitive antagonists of NMDAR. Anis et al. [14] firstly reported that PCP and ketamine blocked the action of NMDA in ion flow through the NMDAR in the brain. A PCP receptor site was soon identified and characterized [15], where non-competitive antagonists, such as PCP, ketamine and MK-
801 bind, within the NMDA ion channel which is composed of both NR1 and NR2 subunits. These NMDA antagonists induce psychiatric and physiological changes that closely resemble schizophrenia [3]. In contrast to amphetamine/dopamine model, which implies increased dopaminergic activity in the brain, PCP induces not only positive symptoms similar to amphetamine, but also negative symptoms and cognitive deficits seen in schizophrenia [3]. The physiologic manifestations of schizophrenia such as hypofrontality, disruption of prepulse inhibition (PPI), enhanced subcortical dopamine release, and increased metabolism and extracellular glutamate levels in defined limbic circuits [16] are demonstrated by these antagonists as well. These findings suggest that schizophrenic symptoms could possibly arise from attenuated NMDAR-mediated neurotransmission. It was further postulated that the psychotomimetic effects may not be exerted by noncompetitive antagonists alone but could be an outcome of any dysfunctional attenuation of the NMDAR-mediated neurotransmission [17].

NMDAR, a subtype of the ionotropic glutamate receptor with a family of subunits identified thus far including NR1, NR2A, NR2B, NR2C, and NR2D [18], plays an important role in neurodevelopment and cognition. A functional NMDAR is composed of multiple subunits including NR1 and one of four NR2 subunits (A-D), which contain binding sites for glycine and glutamate, respectively, to form heteromeric receptor-ion channels [18] (Figure 1). Some studies offer the evidence of developmental regulation of specific components of the NMDAR unit [18]. An animal study of mRNAs encoding NMDA receptor subunits in the developing rat CNS provided evidence that the NR1 gene is expressed in virtually all neocortical neurons at all stages, whereas the four NR2 transcripts display dissimilar developmental expression pattern [18]. Therefore, deficits in NMDA neurotransmission can potentially account for developmental risk factors and cognitive impairments in schizophrenia.

**Evidence of NMDA Receptor Hypofunction in Schizophrenia**

**Challenging tests with NMDA receptor antagonists**

PCP and ketamine function primarily by binding to a site within the ion channel of the NMDAR that blocks cations influx, thereby acting as noncompetitive antagonist [19] (Figure 1). Binding studies in brain tissue from schizophrenics on PCP sites with PCP ligands (3H-MK-801, 3H-TCP) show significant differences between schizophrenics and healthy comparisons. The regional distribution of PCP sites in controls is the highest in frontal cortex, followed by entorhinal cortex, hippocampus and amygdale, while the fewest number of receptors is in the substantia nigra and the nucleus dentatus [20]. In the postmortem brains of schizophrenia, PCP sites are more abundant in regions including orbital frontal cortex, amygdala, entorhinal area, hippocampus and putamen [20].

**Nonpsychotic volunteers**

Studies have been conducted to demonstrate that the administration of subanesthetic doses of PCP to nonpsychotic subjects induced neuropsychological and behavioral psychopathology similar to that associated with schizophrenia while acutely PCP-intoxicated individuals were almost indistinguishable from symptomatic schizophrenic patients [21]. But placebo-controlled, double-blind studies of the effects of NMDA antagonists in humans have been limited for safety concern as highly potent PCP can induce pathomorphologi-
cal changes in rat brain as well as prolonged toxic psychosis and severe medical problems in humans [21]. Ketamine, with lower binding affinity to NMDAR and lower potency than PCP, produces minimal cardiac and respiratory adverse effects and its anesthetic and behavioral effects mitigate soon after administration [22]. When subanesthetic dose (0.3-0.5 mg/kg) was infused intravenously to nonpsychotic subjects, ketamine produces an avolitional state characterized by blunted affect, withdrawal, and psychomotor retardation [23], psychotic symptoms in the form of suspiciousness, disorganization, and perceptual alterations [24], and cognition deficits demonstrated by impaired performance on tests of vigilance, verbal memory, verbal fluency, and the Wisconsin Card Sorting Test [3, 23]. Significant increase in the scores of Brief Psychiatric Rating Scale (BPRS), Scale for the Assessment of Negative Symptoms (SANS) and Scale for the Assessment of Positive Symptoms (SAPS) were observed [24]. Dissociative symptoms were prominent, with de-personalization in particular similar to the early feature of the schizophrenia prodrome [3]. In addition, during smooth pursuit eye tracking task, ketamine induces nystagmus and oculomotor dysfunction that were similar to some of the abnormalities seen in schizophrenia [8].

**Patients with schizophrenia**

Further studies were conducted in patients with schizophrenia to elucidate the possible underlying etiology of schizophrenia. Chronic PCP abusers have commonly been misdiagnosed as schizophrenics, whereas PCP administration exacerbates symptoms in chronic stabilized schizophrenic patients [3, 21]. Dramatic exacerbation of psychotic symptoms was observed in chronic schizophrenics under administration of subanesthetic doses of PCP (0.1 mg/kg) [25]. These patients became more assertive, hostile, and unmanageable, and these changes lasted not only a few hours as in nonpsychotic volunteers but around four to six weeks [25], suggesting the substantial NMDA vulnerability of this population that is easily to deteriorate further. Similar to PCP findings, overall, patients with schizophrenia receiving subanesthetic dose of ketamine experienced a brief worsening of positive and negative symptoms as well as further impairment in recall and recognition memory [8].

**Novel Therapy: Modulation of NMDA Receptors**

**NMDA glycine-site agonists**

NMDAR is unique as it consists of a co-agonist site that binds the endogenous full agonists, in addition to the glutamate recognition site. Glycine, D-serine, or D-alanine acts as an obligatory endogenous co-agonist for activation of the NMDAR complex through a strychnine-insensitive site on the NR1 subunit [26] (Figure 1). Several approaches have emerged aiming towards modulating this co-agonist site. D-serine is a more potent agonist than glycine at the coagonist site and has a greater ability to penetrate the blood brain barrier [27], indicating that D-serine administration may be more efficacious for the symptoms of schizophrenia. D-serine is synthesized in protoplasmic astrocytes by SR that reversibly converts L-to D-serine and is degraded by D-amino acid oxidase (DAAO) into hydroxylpyruvate [27]. Reduction of central and peripheral D-serine levels in schizophrenic patients resulting in impaired D-serine function could contribute to NMDAR hypofunction [27]. Several controlled clinical trials have shown that co-administration of D-serine or glycine with antipsychotics can ameliorate...
some symptoms of the disorder [9, 10, 28]. D-alanine, another endogenous full agonist of the coagonist site, might also have beneficial effects on schizophrenia [26]. D-cycloserine, an anti-tuberculosis drug, is a partial agonist at the coagonist site. Its clinical efficacy is less than the full agonists [29] as the intrinsic activity of D-cycloserine is only about half that of full agonists (i.e., glycine, D-serine) in potentiating NMDAR activation. D-cycloserine may even function as a partial antagonist in the presence of high levels of glycine and D-serine, leading to decreased NMDA neurotransmission [29].

**Glycine transport inhibitors**

Modulation of the NMDAR function can be done through a number of sites other than the coagonist site. Extracellular glycine levels are regulated through uptake by two types of high affinity glycine transporters, GlyT-1 and GlyT-2. GlyT-2 has a more limited distribution, predominantly in brain stem and spinal cord neurons, and is thought to provide the principal glycine uptake mechanism at inhibitory glycinergic synapses [30], whereas GlyT-1 is widely expressed in glial cells of the hippocampus, cortex, and cerebellum, as well as the brain stem and spinal cord in association with NMDAR [30] and it has been proposed to function mainly at excitatory synapses by regulating glycine levels at the coagonist binding site of NMDAR.

As evidence suggests that synaptic glycine may be efficiently regulated at a subsaturating level by the GlyT-1, a rational approach to enhance NMDA neurotransmission might be through blocking the reuptake of glycine by GlyT-1. This mechanism is analogous to that of using a serotonin reuptake inhibitor to potentiate serotonergic neurotransmission [17]. In support of this hypothesis, it was demonstrated that N-[3-(4'-fluoro-phenyl)-3-(4'-phenylphenoxy) propyl] sarcosine (NFPS), a specific inhibitor of GlyT-1, potentiated NMDAR-mediated responses, such as increased LTP in the dentate gyrus and enhanced PPI of acoustic startle, in vivo [31]. GlyT-1 knockdown mutation further demonstrated that the effects exerted by NFPS were indeed mediated by GlyT-1 [32]. Sarcosine, which is an endogenous inhibitor of GlyT-1, has shown clinical efficacy while being administered as add-on therapy to FGAs and SGAs or as monotherapy, thereby supporting its NMDA-enhancing and antipsychotic functions [4, 33, 34] (Figure 2).

**D-amino acid oxidase inhibitors**

DAO is a flavoenzyme that catalyzes the oxidative deamination of D-amino acids. D-serine, the endogenous NMDAR co-agonist, is the predominant D-amino acid in mammalian CNS. DAO might have the potential in modulating NMDAR function via D-serine degradation [35], contributing to the reduction in NMDAR-mediated neurotransmission, thus increased DAO is proposed to be in association with susceptibility to schizophrenia. Consistent with the altered D-serine metabolism in schizophrenia, lower serum levels of D-serine were found in schizophrenic patients as compared to healthy controls [36].

Several lines of evidence support the above hypothesis: in postmortem studies, the mean DAO activity is two-fold higher in the schizophrenia group compared with the control group and there is increased DAO expression in the bilateral hippocampal CA4 of the schizophrenia group [37]; in genetic studies, DAO shows associations to schizophrenia in several, though not all, studies [35]. Collectively, DAO inhibition and D-serine elevation in combination is suggestive of potential therapeutic benefits.
Figure 2. Schematic illustration of glutamatergic system. The ionotropic glutamate receptors, including N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate subtypes, are located in the postsynaptic membrane. They participate in synaptic transmission by directly opening ion channels upon glutamate binding, allowing ion influx (Na⁺, Ca²⁺) and causing excitatory post-synaptic current (EPSC) and mainly function to mediate fast synaptic transmission. Among them, NMDARs are the subtype with strongest implication in the pathophysiology of schizophrenia. Glutamate is agonist, which is synthesized and stored in high concentration within presynaptic nerve terminals and released from the nerve terminal into the synaptic cleft; glycine and D-serine are co-agonists of the NMDAR.

D-serine is synthesized by serine racemase from L-serine. D-serine is localized to both neurons and astrocytes and is uptaken by ASC-1 in the presynaptic membrane. D-serine is metabolized by DAAO into hydroxyl pyruvate. Role of DAOA (G72) as an activator or inhibitor is unclear. Glycine is uptaken by GlyT-1 and metabolized to L-serine by glycine cleavage system (GCS).

Sarcosine inhibits glycine uptake through GlyT-1. DAAO inhibitors act to reduce D-amino acids degradation. Other potential regulators and drug targets of NMDA synapse include the “glycine” co-agonist site, serine racemase, D-amino acid oxidase activator (DAOA, G72), and arginine-serine-cysteine transporter-1(ASC-1).

AMPA receptors have the characteristic of being mobile and they function cooperatively with NMDARs to maintain overall integrity of glutamatergic synapses. Activation of AMPA receptor depolarizes the synaptic membrane allowing Ca²⁺ influx through unblocked NMDA channels in a voltage-dependent manner.
Therapeutic Effects Exerted by NMDA-enhancing Agents

Tsai and Lin [38] performed a meta-analysis which is the most comprehensive review of NMDA-enhancing agents. It included all published, randomized, double-blind trials of the NMDA-enhancing agents and reviewed 26 studies with about 800 patients with schizophrenia. The clinical efficacy among different NMDA-enhancing agents as adjuncts to different concomitant antipsychotic agents on different symptom domains, efficacy, the dose-response and the side effects were examined.

Glycine
Glycine added to FGAs

The efficacy of glycine as adjuvant therapy to FGAs in treating negative symptoms and cognitive deficits has been investigated in several small-scale double-blind and open-label clinical trials since 1990. One study [39] with 6 patients found no beneficial effects regarding the negative symptoms of schizophrenia. However, the limited penetrability of glycine across the blood-brain barrier and a relative low dose of glycine (10.8 g/day) administered orally might explain the lack of effectiveness in this study. Three studies revealed significant improvement in negative symptoms [40-42]. In 2007, the Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST), a randomized double-blind study with a duration of 16 weeks and with the participation of 4 sites in the United States and one site in Israel, was published [43]. A total of 157 patients were randomly assigned to glycine, D-cycloserine and placebo groups. This study suggested that glycine is not an effective therapeutic option for treating negative symptoms or cognitive deficits as there was no significant SANS total score differences between glycine and placebo groups. Moreover, greater reductions in negative symptoms for both the glycine and D-cycloserine groups in comparison to the placebo group were detected in inpatients, but not in outpatients. Furthermore, there was no significant glycine/placebo between-group difference on the cognitive measure. In terms of adverse effect, more new or worsened nausea was observed in glycine subjects than in placebo subjects.

Three of the above mentioned trials [41, 42, 44] also evaluated the cognitive effects of glycine based on PANSS cognitive subscale, showing a beneficial effect in cognitive function. But it is noteworthy that these studies were conducted on relatively small sample sizes and over short-term periods.

Glycine added to SGAs

A 6-week double-blind placebo-controlled crossover trial with high-dose glycine (0.8 g/kg body weight per day) added to olanzapine and risperidone [45] revealed significant improvements in negative symptoms and cognitive function. Another short-term trial [42] also showed a significant reduction in negative symptoms and an improvement in cognitive function based on PANSS cognitive subscale. However, these clinical trials were conducted on small samples under short-term treatment. In 2007, the CONSIST [43] suggested that glycine is not an effective therapeutic option for patients with negative symptoms or cognitive impairments not benefited by SGAs as no significant difference in change in the SANS total score and in the average cognitive domain Z scores between glycine and placebo groups was observed.
Glycine added to clozapine

About one third to two thirds of treatment-resistant schizophrenic patients fail to benefit from clozapine therapy or are partial responders despite adequate dosage and duration. During the last two decades, several clozapine adjunctive agents have come into clinical practice in order to maximize the efficacy of clozapine, including FGAs, SGAs, mood stabilizers, other anticonvulsants, selective serotonin reuptake inhibitors (SSRI), and glycineric agents. Since 1990’s the efficacy of glycine as clozapine adjuncts has been evaluated in several clinical trials. Seven trials [41, 42, 44, 46-49] were published. Five of them [42, 44, 46, 48, 49] did not find a significant improvement in positive symptoms. One study [47] found worsening of positive symptoms. Four trials [41, 42, 44, 46] showed significant reductions in negative symptoms and the improvement persisted after discontinuation of glycine. Cognitive functioning was assessed in only four trials [41, 42, 44, 48] and three of them found positive change based on cognitive subscale of PANSS.

D-serine

Five clinical trials with D-serine as adjuvant therapy to antipsychotic treatment [9, 28, 34, 50, 51] were reported. First trial evaluated the addition of D-serine to either FGAs or SGAs, with significant improvements in positive, negative and cognitive symptoms [9]. Five trials [4, 28, 34, 50, 51] evaluated the addition of D-serine to SGAs with three trials showed encouraging results. One study [51] published in 2010 performed a 4-week, double-blind investigation of adjunctive D-serine at dose-escalation (30, 60 or 120 mg/kg body weight/day) and the findings suggested beneficial effects in treatment of positive symptoms, negative symptoms, and cognitive deficits at doses of 60 and 120 mg/kg/day, and significant dose-dependent increase of plasma D-serine levels correlated with improved symptomatic and neuropsychological function. Renal side effect was observed at high D-serine dosage, which resolved upon D-serine discontinuation. The other study [28] also demonstrated significant improvements in positive, negative and cognitive symptoms. But one study [34] with D-serine and risperidone co-treatment in patients with acute exacerbation of symptoms and another study [50] with addition of D-serine to clozapine did not find beneficial effects in any of the three core symptoms of schizophrenia.

D-alanine

There is only one clinical trial [26] examining the efficacy of D-alanine as adjuncts to antipsychotics. Thirty-two schizophrenic patients were enrolled in this 6-week double-blind, placebo-controlled trial, in which D-alanine (100 mg/kg/day) was added to their stable antipsychotic regimens, including various typical antipsychotics and risperidone. Significant improvements in positive and negative symptoms were the findings. Cognitive symptoms assessed by the cognitive subscale of PANSS also revealed improvement. D-alanine was, moreover, a well-tolerated compound, and no significant side effect was noted [26]. The positive findings of D-alanine as add-on therapy for schizophrenia, particularly the negative and cognitive symptoms, further support the hypothesis that augmentation of NMDA neurotransmission through the NMDA coagonist site is a promising approach for schizophrenia.

D-cycloserine

D-cycloserine added to FGAs

Nine trials evaluated the addition of D-cycloserine to FGAs in chronic schizophrenic patients [29, 43, 55-58]. The first study [52] re-
ported that an exacerbation of positive symptoms was observed in about 70% of the patients receiving adjunctive high-dose D-cycloserine (dosages between 500 mg/day and 3,000 mg/day). The second study [53] reported that 250 mg D-cycloserine daily aggravated psychotic symptoms in four of seven patients and only one patient exhibited a slight improvement. Three trials [55-57] exhibit significant reductions in negative symptoms, while three others [29, 43, 58] did not find significant changes in negative symptoms. In addition, one trial [55] demonstrated equivocal improvement of one cognitive task at the dose of 50 mg/day, while four other trials [29, 43, 56, 58] did not find significant changes in cognitive symptoms. One study [54] with doses of 100 mg/day showed worsening of positive symptoms and failed to improve negative symptoms. These unexpected findings may be explained by the antagonistic effects of higher-dose D-cycloserine at the coagonist site of the NMDAR due to competition with the endogenous agonist glycine and/or D-serine or its interaction with antipsychotics which have endogenous activity on NMDAR-mediated neurotransmission [54].

**D-cycloserine added to SGAs**

Four trials evaluated the effect of D-cycloserine added to SGAs [43, 57, 59, 60]. None of them detected any significant change in positive symptoms. Three of these studies [57, 59, 60] demonstrated a significant improvement in negative symptoms at the dose of 50 mg/day. CONSIST trial [43] showed no effect of D-cycloserine at 50 mg/day on negative symptoms. Two trials [43, 60] examined the effects on cognitive function and revealed no significant impact.

**D-cycloserine added to clozapine**

Three trials evaluated the effect of D-cycloserine as add-on therapy to clozapine [56, 59, 61]. No significant effect on cognitive or positive symptoms was observed in any of the trial. One trial with D-cycloserine given at a dose of 50 mg/day [59] found improvement in negative symptoms. However, the other two trials [56, 61] with D-cycloserine given at the same dose showed worsened negative symptoms.

**Sarcosine**

Four double-blind placebo-controlled clinical trials investigated the effects of sarcosine as adjuncts to stable antipsychotic regimens. The first trial [33] evaluated the addition of sarcosine (2 g/day) to typical antipsychotics and to risperidone, revealing significant improvements in the positive, negative and cognitive symptoms. The second trial [34] evaluated the addition of sarcosine (2 g/day) or D-serine (2 g/day) to risperidone in patients with acute exacerbation of schizophrenia, revealing significant improvements in positive, negative, and cognitive symptoms and the therapeutic effects of sarcosine are superior to those of D-serine. Previous studies found no beneficial effects of glycine, D-serine, or D-cycloserine as add-on therapy to clozapine, Lane et al. [62] thus further examined the combined effect of sarcosine (2 g/day) and clozapine, which exhibited no improvement in the core symptoms.

Addition of sarcosine to an existing antipsychotic regimen other than clozapine has shown its efficacy for both chronically stable and acutely ill patients. However, the efficacy of NMDA agents as a primary antipsychotic agent has not yet been demonstrated. Therefore, Lane et al. [63] evaluated the effect of sarcosine monotherapy on 20 acutely symptomatic schizophrenic patients, who
were randomly assigned to receive either 1 or 2 g of sarcosine daily. Patients receiving the 2 g daily dose were more likely to respond, particularly the antipsychotic-naïve patients. However, in order to fully assess the therapeutic effect of sarcosine, placebo-or active-controlled, larger-sized studies are needed. Recently, Lane et al. [4] conducted a 6-week double-blind, placebo-controlled trial with enrollment of 60 chronic schizophrenic patients comparing the effects of sarcosine (2 g/day) and D-serine as adjuncts to existing stable antipsychotics. Greater efficacy of sarcosine therapy than D-serine for all outcome measures was the finding whereas D-serine treatment has better efficacy than placebo group.

Perspectives

**D-amino acid oxidase inhibitors**

Experimental evidence supports that D-serine, D-cycloserine, and D-alanine as adjuncts to FGAs or SGAs are efficacious approaches in treating negative symptoms and cognitive impairments. Findings have been most encouraging in studies that have used full agonists of the NMDA receptor (i.e., glycine, D-serine, D-alanine) as opposed to the partial antagonist D-cycloserine. Furthermore, effects have generally been more powerful when these agents were used in combination with antipsychotics other than clozapine.

In addition to the coagonist site and the GlyT-1, another novel drug target to enhance NMDA function is DAAO which degrades D-amino acids including D-serine, D-alanine and other D-amino acids. Overactive DAAO may contribute to NMDA hypofunction, thus inhibition of DAAO is a plausible approach to enhance NMDAR function in schizophrenia. The therapeutic value of DAAO inhibitors is relatively unexplored and remains at preclinical stage, therefore future extensive study is expected to establish their efficacy, tolerability, and mechanism. Nevertheless, our recent findings revealed that a DAAO inhibitor is much more efficacious than other NMDA-enhancing agents in improving the symptoms of schizophrenia. Furthermore, it improves the cognition as measured by a comprehensive MATRICS-like cognitive battery.

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