Therapeutic potential of glutamatergic N-methyl-D-aspartate (NMDA) receptors-mediated molecules for autism spectrum disorders

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder with marked variability in severity and phenotypic manifestation clinically. Abnormal synaptic connectivity is implicated as an important contributor to the pathogenesis of ASD because all the ASD related genes share deficits in synaptic function. In addition, previous human and animal model studies indicate that the expression, trafficking and function of ionotropic glutamate receptors are altered, which result in altered synapse development and plasticity in a ASD-specific manner. To date, placebo-controlled clinical drug trials targeting the core social impairment of ASD have had negative results and the search for potentially novel agents targeting the core social impairment of autism continues. Since several recent findings provide evidence that the pathophysiology of ASD may include a component of glutamate receptor disruption, this is a line of thinking worthy of pursuit. In this review, we reported the evidences of abnormal glutamate metabolism in patients with ASD; brief introduction of the ionotropic N-methyl-D-aspartate (NMDA) glutamate receptors; genetic animal models of NMDA receptor hypofunction and hyperfunction; the preclinical studies and the human clinical trials exploring the effects of the glutamatergic NMDA receptors modulating agents in the treatment of ASD which were identified on the Medline and Clinical Drug Trial registry system. In conclusion, the positive preclinical data with drugs believed to act via modulation of NMDA-mediated signaling encourage further exploration of this mechanism as a targeted approach for ASD. However, up to now, only a few glutamatergic compounds have been studied in clinical trials of ASD, and the results are inconclusive. The data suggest that targeting the NMDA receptor can have promising therapeutic potentials in ASDs. The complex relationship between glutamate-mediated signaling and the behavioral-cognitive phenotype of ASD may be better elucidated in the future by combining the new cell-based models with well characterized subtype of ASD patients.


Autism spectrum disorders (ASD) are developmental disorders characterized by dysfunction in several core behavioral dimensions: social communication/interaction deficits and specific behavior characteristics. The social deficit dimension involves deficits in reciprocal social interactions, diminished ability to carry on conversation, and impaired daily interaction skills. The communication domain includes problems ranging from lack of verbal language to...
fluent but odd speech with little comprehension of pragmatics. The behavioral domain involves compulsive behaviors, unusual preoccupation with certain objects, rigid adherence to routines or rituals, repetitive motor mannerisms and unusual reactions to sensory stimuli [1]. ASD are disorders of male preponderance (sex ratio of 4:1), with an estimated worldwide prevalence of 1%-2.6% [2] and the heritability indices are estimated at 85%-92% [3]. The genetic studies revealed a remarkable heterogeneity, amounting to hundreds of different genes that may contribute to ASD [4]. These genes involve de novo mutations, common and rare variants, chromosome abnormalities, copy number variations, as well as the monogenic syndromic disorders that display autistic features [5, 6]. The heterogeneous nature of these genetic abnormalities is consistent with the severity and phenotypic variability observed clinically in ASD. Current findings are that all the ASD related genes shared deficits in synaptic function contribute to the pathogenesis despite the complexity of genes associated with it [7]. These evidences lead to the proposition that ASD is a neurodevelopmental disorder of the synapse with abnormal synaptic connectivity, and that there is imbalance in excitatory and inhibitory neurotransmission [8, 9]. Hence, glutamate, the major excitatory neurotransmitter in the human brain synaptic transmission with roles in learning, memory and synaptic plasticity, is hypothesized to play an important role in the pathophysiology of ASD.

Proposed abnormal glutamate metabolism in patients with ASD is supported by several lines of evidence as listed below. Glutamatergic dysregulation has been consistently found in patients with autism [10]. Peripheral glutamate has been shown to be increased in youth [11] and adults [12] with autism compared with controls. Post-mortem neuropathology in ASD has found elevated mRNA or protein levels of glutamatergic transporters and neurotransmitter receptors [13]. In vivo single-voxel proton magnetic resonance spectroscopy has noted increased glutamate and glutamine concentrations in the pregenual anterior cingulate cortex (with possible right-lateralization) in children with autism compared with controls [14]. Alternatively, one study using proton magnetic resonance spectroscopy reported adults with ASD had a significant decrease in concentration of combined glutamate and glutamine signal in the basal ganglia which were associated with variation in social development [15]. Genetic studies in autism have noted a number of rare gene alterations implicated in glutamatergic function. Those include neurexins, which via neureligans induce presynaptic differentiation of glutamatergic neurons and glutamate receptors [10].

Glutamate receptors are highly complex and fall into two main categories, ionotropic (voltage sensitive) and metabotropic (ligand sensitive) [16]. Each ionotropic or metabotropic receptor has three types, depending on binding specificity, ion permeability, conductance properties and other factors. The metabotropic glutamate receptors (mGluR) are a family of receptors classified into three groups, based on their sequence homology, agonist and antagonist pharmacology, and coupling to signal transduction pathways [16]. Reviews regarding molecules modulating the mGluR function and its implication in ASD were reported before (see [17, 18]) and will be omitted in this review.

The ionotropic glutamate receptors include kainate, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and N-methyl-D-aspartate (NMDA) receptors [19]. The NMDA receptors mediate a slower component of excitatory transmission and are critical postsynaptic mediators of activity-dependent synaptic plasticity [20]. Activation of NMDA receptors requires postsynaptic depolarization and the binding of two agonists, glutamate, and either glycine or D-serine at the glycine modulatory site [21]. Glycine is considered the main coagonist in the spinal cord and the hindbrain, and it has a high affinity for extrasynaptic NMDA receptors. D-serine is the main coagonist in the forebrain [22], and it is characterized by a high affinity for synaptic NMDA receptors. In hippocampus, thalamus, and neocortex, NMDA receptor glycine/D-serine site is normally not saturated [23, 24]. The influx of Ca2+ through the NMDA receptors triggers a cascade of intracellular events that regulate many types of neuroplasticity [25]. Genetic animal models of NMDA receptor hypofunction (serine racemase knockout (SR−/−) mouse) [23] and hyperfunction (glycine transporter 1 heterozygote (GlyT1+/−) mouse) [26] have been generated to determine how constitutive NMDA receptor hypofunction and hyperfunction would affect the glutamate receptor composition of the postsynaptic density (PSD) in the hippocampus and prefrontal cortex (PFC) [27]. NMDA receptor hypofunction mice demonstrated elevated protein levels of NMDA receptor subunits specifically in the PSD-enriched hippocampal fraction, but not in the PFC. NMDA receptor hypofunction mice showed no changes in the amounts of AMPA receptor subunits or PSD protein in either brain region. Conversely, NMDA receptor hyperfunction mice demonstrated elevated protein expression of NMDA receptor subunits and elevated amounts of AMPA receptor subunits (GluR1 and GluR2) in the PSD, and higher total amounts of GluR1. Similar to the NMDA receptor hypofunction mouse model, there were no protein changes observed in the PFC. These studies demonstrated that both negative and positive modulation of NMDA receptor function could result in the altered protein expression of particular ionotropic glutamate receptor subunits, and
illustrated the complexity of synaptic adaptation to altered NMDA receptor function.

To date, placebo-controlled clinical drug trials targeting the core social impairment of ASD have had uniformly negative results and the search for potentially novel agents targeting the core social impairment of autism continues. Since several recent findings provide evidence that the pathophysiology of ASD may include a component of NMDA receptor disruption [28], this is a line of thinking worthy of pursuit. Early studies indicated that direct targeting of glutamate receptors was associated with undesired side effects on cognition and neurotoxicity, hence it might be possible to target the functions of ionotropic glutamate receptors in synaptic plasticity while considering modulation of glutamatergic function. One of the therapeutic candidates is through agonist at glycine site. When partial agonist acted as weak agonists, they would facilitate receptor activation without creating the risk of overactivating the receptors and when it acted as antagonists, they would allow normal synaptic transmission to take place while simultaneously suppressing receptor hyperactivity, through the NMDA signaling. In this article, we reviewed molecules involved in NMDA receptors-mediated signaling as the potential pharmacological treatment of ASDs

PRECLINICAL STUDIES

D-cycloserine (partial NMDA agonist)

D-cycloserine (DCS) is a partial glycine B site agonist that targets the NMDA receptor. Low dose DCS administered peripherally was reported to enhance partner preference formation in animal models of autism (prairie voles) after microinfusions of DCS into the nucleus accumbens and the amygdale [29]. Balb/c mice strain is a genetic inbred model of impaired sociability and social motivation relevant to ASD. The Balb/c mouse strain is known to have functional alteration of its endogenous tone of NMDA receptor-mediated neurotransmission than other inbred and outbred comparator strains [30]. Deutsch el at demonstrated that DCS (given intraperitoneally) could improve measures of impaired sociability and spontaneous stereotypic behaviors during social interaction in both 8-week and 4-week old (i.e., one-week post-weanling) Balb/c mice [31, 32].

In addition, Shank2 is a multi-domain scaffolding protein and signalling adaptor enriched at excitatory neuronal synapses. Mutations in the human SHANK2 gene have been associated with ASD and intellectual disability. Shank2-mutant (Shank2(-/-)) mice carrying a mutation identical to the ASD-associated microdeletion in the human SHANK2 gene were shown to exhibit ASD-like behaviors and marked decrease in NMDA receptor function. After treatment with DCS, these mice were demonstrated to have improvement in social interaction [33]. All of the above preclinical works led to a proposition that DCS might be a therapeutic option for the social deficits of humans with ASD.

D-serine (partial NMDA agonist)

D-serine is an agonist of the glycine modulatory site which has a partial agonist effect on NMDA receptors and has been proved to increase social recognition and social memory in rats at high doses [34].

GLYX-13 (partial NMDA agonist)

GLYX-13 is a monoclonal antibody fragment with partial agonist effects that exceed that of DCS at the glycine modulatory site. In an inbred mouse model of ASD (bred for lower levels of play with conspecifics and reduced prosocial vocalizations), GLYX-13 was shown to “reverse” these ASD-analogous behavioral deficits [35].

CLINICAL PHARMACOLOGICAL TRIALS

As for human, currently there are no validated biological markers to measure glutamate pathology in CNS disorders or injuries [36]. Glutamate target modulation is further complicated by the large number of different receptors and transporters, the wide range of cell types expressing them, multiple regulatory controls, and a narrow concentration difference between normal synaptic function and excite-toxicity. All of the above make clinical assessment difficult and design of novel treatment a daunting task.

In the following section, we reviewed some of the completed or ongoing human clinical trials exploring the effects of the glutamatergic NMDA receptors modulating agents in the treatment of ASD which were identified on the Medline and Clinical Drug Trial registry system.

D-cycloserine (partial NMDA agonist)

D-cycloserine (DCS) has been proved to be effective in the treatment of various psychiatric disorders. Previous research has demonstrated that DCS can improve cognition in Alzheimer’s disease [37, 38]. DCS (as an add-on therapy with antipsychotics) can decreases the negative symptoms of patients with schizophrenia in some [39], but not all studies [40]. Studies also have supported the use of DCS to augment exposure therapy in adult anxiety disorders such as panic disorder, obsessive-compulsive disorder, social anxiety disorder, and specific phobias [41-43]. Other preliminary data also showed that while DCS combined with psychotherapy did not yield significant treatment-enhancing effects on
pediatric obsessive-compulsive disorder, trends for greater improvement in DCS-augmentation group were noted. A preliminary, open-label study of DCS in a sample of children with ASD appeared to reduce measures of social withdrawal over a 12 week trial. However, placebo-controlled DCS trials targeting the core social deficits of children with autism have yielded negative results.

**D-serine and GLYX-13 (NMDA partial agonists)**

While D-serine has been shown to be effective in clinical trials for patients with schizophrenia, it has not been reported in clinical trials for humans with ASD. GLYX-13 has been shown to be promising in animal models but there has not been clinical trials on human with ASD reported yet.

**Sarcosine (glycine transporter I inhibitor)**

An alternative method to indirectly increase the NMDA transmission is by the attenuation of the glycine reuptake through glycine transporter 1 (GlyT-1). Sarcosine, also known as N-methylglycine, is a potent endogenous inhibitor of GlyT-1 and can enhance NMDA neurotransmission. This molecule has been shown to be effective in clinical trials involving the negative and cognitive symptoms of chronic patients with schizophrenia, in acutely ill persons with schizophrenia, in patients with obsessive–compulsive disorders, to temporarily relieve the depressive and neuropsychiatric symptoms of patients with Parkinson-related dementia and to improve depressive-like behavior in a rodent model and inhuman depression.

Yang et al is the only group that has examined the efficacy and safety of a glycine transporter I inhibitor, sarcosine, in the 24-week open-label treatment of four high-functioning children with autistic disorder. They observed an activation effect clinically but it was not able to be reflected by the primary outcome measures tapping social reciprocity. Though the data are too preliminary to draw any definite conclusions about efficacy, they do suggest this therapy to be safe, and worthy of a double-blind placebo-controlled study with a focus on a certain subgroup of children with ASD.

**Memantine (NMDA antagonist)**

Memantine, a low affinity nonselective NMDA receptor antagonist, is hypothesized to block excessive glutamate effects that can induce neuroinflammatory activity and influence neuroglial activity. Previous study has demonstrated that memantine can be beneficial for Alzheimer's disease, can be used for the improvement of negative and positive symptoms of refractory schizophrenia patients when added to clozapine therapy, and for the attenuation of opioid physical dependence symptoms in humans.

Open-label studies suggest that memantine may be useful in the treatment of memory functioning and some behavioral symptoms for children with ASD. One recent published studies of double-blind, placebo controlled trial reported that memantine as adjunctive therapy to risperidone had decreased symptoms of irritability in the treatment of children with autism. Further double-blind placebo-controlled clinical trials will be needed. The team led by Dr Joshi from the Massachusetts General Hospital has initiated a 12-week placebo-controlled pilot study in an attempt to determine the effect and safety of memantine in the treatment of adults with ASD. The study is currently still ongoing.

**Riluzole**

Riluzole is approved for the treatment of amyotrophic lateral sclerosis in adults by the U.S. Food and Drug Administration. Although the exact mechanism of riluzole is unknown, it is thought to inhibit the release of glutamate at the presynaptic nerve cell terminal and enhance glutamate reuptake. Grant et al reported the first open-label trial showing significant response of riluzole in children with treatment refractory obsessive-compulsive disorder (OCD). Wink et al reported much improvement in riluzole targeting severe repetitive behavior in three persons with ASD. These preliminary results suggest that riluzole could be investigated for the treatment of repetitive behavior in individuals with ASD.

**Oxytocin**

Oxytocin is a nine-amino-acid peptide that is synthesized in the paraventricular and supraoptic nucleus of the hypothalamus and the best known effect is in facilitating uterine contractions during labor and promotes lactation. Oxytocin has been known to play a key role in regulating affiliative behaviors, the mother and infant bond and social recognition. Recently, oxytocin and oxytocin receptors are implicated in neuropsychiatric disorders, particularly ASD which involves deficits in social cognition. The detailed mechanism of oxytocin’s effect remains unclear. It has been reported that the suppression of basal glutamatergic neurotransmission and facilitation of activity-dependent synaptic plasticity in the infralimbic medial prefrontal cortex might be critical for the effect of oxytocin on social cognition.

Oxytocin administration was shown to enhance the ability
of adults with autism to understand emotions in speech, to improve in measures of social cognition and quality of life, and to help children with autism to better recognize people's intentions by reading their eyes. Recently, oxytocin administration in adults with ASD has been proved to increase the saliency of social stimuli as demonstrated in functional magnetic imaging, which suggest that oxytocin might promote face processing and eye contact in individuals with ASD as prerequisites for social interaction.

Acamprosate

Acamprosate is a U.S. Food and Drug Administration-approved drug to treat alcohol dependence. Acamprosate has been demonstrated to bind at a specific spermidine-sensitive site at the NMDA receptor. It may enhance receptor activation at low glutamate concentrations and inhibit activation at high glutamate concentrations. It has also been shown to act as an antagonist at metabotropic glutamate receptors. In addition, the drug likely impacts both gamma-aminobutyric acid and glutamate neurotransmission.

Erickson et al reported an open-label experience with acamprosate targeting social impairment in six children with autism. Five of them were judged treatment responders over 10 to 30 weeks (mean duration, 20 weeks) of treatment. Researchers in Children’s Hospital Medical Center, Cincinnati in collaborating with Autism Speaks are currently recruiting 5 years to 17 years subjects for a double-blind placebo-controlled study of acamprosate in autism (NCT01813318)

FUTURE PERSPECTIVES

Human and animal model studies indicate that the expression, trafficking and function of ionotropic glutamate receptors are altered, resulting in altered synapse development and plasticity in a ASD-specific manner. However, up to now, only a few glutamatergic compounds have been studied in clinical trials of ASD, and the results are inconclusive. This highlights the need for clinical assay of NMDA function in individuals with ASD, which is not feasible at this point. Taken together, the positive preclinical data with drugs believed to act via modulation of NMDA-mediated signaling encourage further exploration of this mechanism as a targeted approach for ASD. However, suitable clinical measures of “target engagement” are in need of clarification and further studies are necessary to understand in greater detail the changes of NMDA receptors in various ASD subtypes. In addition, since NMDA receptors are heavily regulated by experience, many environmental factors may modulate their functions. It will be necessary to characterize the timing of the changes affecting NMDA receptors during development which will be useful for selection of optimal therapeutic window.

Even though animal models and in vitro cellular models are very useful for understanding neurobiological pathways affecting glutamate receptors in ASD, they cannot reproduce all characteristics of the human disorders. In humans, ASD may be the result of defects in many genes, which interact with environmental factors, including experience, nutrition, toxins and infections to regulate the expression and functions of genes. Some of these barriers may be reduced by new techniques for using the induced pluripotent stem cell (iPSC) technique. Fibroblasts from patients with a specific disorder (e.g., ASD) can be programmed to differentiate disorder-specific human-induced pluripotent stem cells into brain cells by the iPSC technique. The implication is that live human neurons can be derived from humans with an ASD phenotype without identifying which genes are interacting to produce the disease state in each patient. Even though human iPSC-derived neuron models from non-syndromic ASD has not yet been produced, with the huge advancements in stem cell research, the establishment of such complex-gene ASD model as a platform for drug development will be on the horizon.

In summary, the data suggest that targeting the NMDA receptor can have promising therapeutic potentials in ASDs. The complex relationship between glutamate-mediated signaling and the behavioral-cognitive phenotype of ASD may be better elucidated in the future by combining the new cell-based models with well characterized subtype of ASD patients.

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References


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67. Frizzo ME, Dell'Onder LP, Dalcin KB, Souza DO. Riluzole enhances glutamate uptake in rat astrocyte cultures. Cell Mol


