Review

Glutamate mediated signaling in the pathophysiology of autism spectrum disorders

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ABSTRACT

Autism spectrum disorder (ASD) is a childhood neurodevelopmental disorder. During fetal and neonatal brain development, the cues for neurodevelopment are regulated in a well orchestrated manner. Generally, neurotransmitters play a major role in the formation of central nervous system (CNS) and peripheral nervous system (PNS). Glutamate, the excitatory neurotransmitter actively participates in various neurodevelopmental processes through complex regulatory events. Excitatory neurotransmitter signaling via glutamate receptors modulates cognitive functions such as memory and learning, which are usually impaired in ASD. Therefore, glutamate and its regulatory molecules are considered as potential targets for these disorders. Pharmacological, biochemical and behavioral studies reveal possible involvement of glutamatergic system in ASD pathology. An abnormal increase in electrical activity resulting from excessive glutamate signaling causes prolonged alterations in behavior, as commonly seen in ASDs. On the contrary, reports on animal models of hypoglutamatergia demonstrate phenotypes that overlap with features seen in autism. So controversies prevail whether to regard autism as hyper- or hypo-glutamatergic disorder. This paper reviews the role of glutamate and its regulatory proteins such as different receptors, transporters and metabolizing enzymes in the pathophysiology of ASD based on evidences gathered through multidisciplinary approaches. All these information raise the possibility of exploiting glutamatergic neurotransmitter system for future therapeutic interventions for ASD.

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Contents

1. Introduction

Autism is the prototypical form of pervasive developmental disorders (PDDs), characterized by complex behavioral impairments detectable as early as 18–36 months of age. Deficits are often manifested in domains such as social interaction, communication, and are also associated with stereotypic, repetitive and restrictive behavior and interests. Besides autism, PDDs include disorders such as Asperger's syndrome, pervasive developmental disorder-not-otherwise specified (PDD-NOS), Rett's disorder and childhood disintegrative disorder (American Psychiatric Association, 1994). Asperger's syndrome and PDD-NOS are phenotypically related to autistic disorder and have

varying degrees of severity. Therefore, they are classified under the broad definition of autism and are collectively known as autism spectrum disorders or ASDs.

Autism has been considered to be a rare disorder till two decades back, but the recent epidemiological studies on American and European populations show a steep increase in its prevalence (Chakrabarti and Fombonne, 2001; Fombonne, 2003). A recent study on school-aged children in Canadian population reveal the prevalence rate of PDD and narrow diagnosis of autism to be close to 1% and 0.25% respectively (Lazoff et al., 2010). The increased prevalence rate possibly reflects broadening of the autism concept and improved diagnostic as well as detection criteria (Fombonne, 2003). There exists a sex bias in the incidence of autism with four times more males affected than the females (Abrahams and Geschwind, 2008), which raises the possibility of major involvement of X-chromosomal genes in its etiology. Two previous genome scan studies provided a moderate evidence of linkage to X chromosome (Liu et al., 2001); (Shao et al., 2002). But, the results of multivariate analysis of phenotypic expression that has been conducted within families do not support any linkage to X-chromosome under a multifactorial model (Pickles et al., 2000). Mutations in two X-linked neuroligin genes, *NLGN3* and *NLGN4* have been highlighted as autism risk genes, but the low frequency of the mutations in these genes does not support them to be major contributors for the sex bias in autism etiopathogenesis (Jamain et al., 2003; Vincent et al., 2004). Furthermore, there are reports that suggest lack of any major effects of genes on the X-chromosome (Schultz and Klin, 2002). Even though, the disease mechanism has not yet been elucidated, the family and twin studies demonstrate that ASD is a complex multifactorial disorder involving many genes.

Approximately 6% of the cases with autistic traits have known etiologies that include fragile-X syndrome, neurofibromatosis, and tuberous sclerosis, etc. (Fombonne, 2002). But in majority of the cases the pathology is still a mystery. Different statistical models based on family studies indicate that at least fifteen genes with epistatic interaction are involved as predisposing factors (Jones and Szatmari, 2002; Pickles et al., 1995) besides unidentified non-genetic elements (Muhle et al., 2004). Concurrently, genomewide mapping studies identified several autism susceptibility regions throughout the genome (Gupta and State, 2007; Muhle et al., 2004; Weiss et al., 2009). Some chromosomal regions like 7q and 17q have been replicated in many studies as autism susceptibility loci (Benayed et al., 2005; Campbell et al., 2006; Devlin et al., 2005; Philippe et al., 1999; Yonan et al., 2003).

Biochemical and pharmacological studies have unveiled dysfunction of various neurotransmitter systems as causing autism. Major neurotransmitters that are implicated in autism include serotonin, dopamine, glutamate, GABA, etc. (Burgess et al., 2006; Lam et al., 2006; Rolf et al., 1993). Among these, glutamate has been shown to be directly involved in general cognitive functions such as memory and learning (Manent and Represa, 2007). Receptors of glutamate have also been extensively studied as potential candidates for several neurological and psychiatric diseases (Blandini, 2010; Moldrich et al., 2001; Nicoletti et al., 2011; Richards et al., 2010). In the case of autism, it has been proposed as a hypoglutamatergic disorder based on the neuroanatomical studies and the similarities between symptoms produced by glutamate antagonists in healthy and autistic subjects (Carlsson, 1998). Furthermore, animal models of hypoglutamatergia exhibit behavioral phenotypes as seen in autism, such as defective habituation, restricted behavioral repertoire and inability to change behavioral paradigm (Nilsson et al., 2004). Moreover, changes in the glutamate concentration have been demonstrated in autistic patients in comparison to controls, with high levels in plasma and low levels in platelets (Aldred et al., 2003; Fatemi, 2008). Information on direct involvement of abnormalities in glutamate receptor genes and deregulation of glutamatergic pathway in ASD pathophysiology are also available (Gupta and State, 2007; Jamain et al., 2002; Purcell et al., 2001; Serajee et al., 2003).

In this article we review the importance of glutamate in CNS development and the ways by which the perturbations in the functioning of glutamatergic system leads to autistic pathology. We also briefly review current evidences that substantiate participation of glutamate signaling in the pathophysiology of ASD and discuss whether the existing knowledge on glutamate mediated signaling and its regulation can be translated to design therapeutic strategies for ASD.

2. Involvement of neurotransmitters in neurodevelopment

Defects in neurodevelopment have an adverse effect on various brain functions. Neurotransmitters, the neuronal signaling molecules play an important role in the normal development of the brain, and are also important for maintaining functions such as memory, learning, behavior, motor activity, etc. Neurotransmitter mediated signaling is initiated by the binding of specific neurotransmitters to its receptor proteins on the post-synaptic membrane, the number of which varies for each neurotransmitter. The binding culminates in a change in the electrical potential of the post-synaptic neurons either through its excitation or inhibition. Action of neurotransmitters at the synapse is regulated through a number of mechanisms that include removal of neurotransmitters from the synaptic cleft by glial cells, reuptake of neurotransmitters by the neurons that have released it and by blocking the flow of ligands to specific receptors. Neurotransmitters are mainly of three major categories such as (1) amino acids (primarily glutamic acid, gamma amino butyric acid GABA, aspartic acid and glycine), (2) peptides (vasopressin, somatostatin, neurotensin, etc.) and (3) monoamines (norepinephrine, dopamine and serotonin) and acetylcholine. Neurotransmitters and their receptors make their appearance during early developmental stages and this has been suggested to aid in modulating developmental processes such as neuronal migration, differentiation, growth cone motility and synaptogenesis (Haydon et al., 1987; Kwong et al., 2000). The morphogenic property attributed to these neurotransmitters is transient and is effective only during specific stages of development (Herlenius and Lagercrantz, 2001). Imbalances in neurotransmitter level and its regulation result in various neurological disorders that include developmental disorders also. A list of different neurotransmitters and the disorders that are associated with them are given in Table 1. The table also depicts whether these neurotransmitters are implicated in autism.

3. Overview of glutamate receptors

Glutamate is the principal excitatory neurotransmitter in brain and acts via two types of receptors: metabotropic and ionotropic receptors. Metabotropic glutamate receptors (GRM) are G-protein coupled receptors. These are membrane bound receptors, activated by the ligand and are involved in intracellular signal transduction, mediated via interaction with G-proteins (Kew and Kemp, 2005). There are three groups of metabotropic glutamate receptors, which are classified as: Groups I (GRM or mGluR 1 and 5), II (GRM or mGluR 2 and 3) and III (GRM or mGluR 4 and 6–8). Furthermore, most of these metabotropic glutamate receptors have splice variants also (Ferraguti and Shigemoto, 2006). Each of these seven transmembrane receptors have an extracellular domain which has the glutamate binding region and C-terminal end facing intracellularly, through which it modulates G-protein coupling (Conn and Pin, 1997). Group I that includes GRM1 and GRM5 receptors activates phospholipase C. Group II and Group III receptors are negatively coupled to cyclic AMP production, but differ in their agonist selectivity. GRM7 and GRM8 are located within the pre-synaptic grid (Ferraguti and Shigemoto, 2006; Pin and Duvoisin, 1995), whereas GRM3 and GRM2 are located on the pre-terminal axons. Group I GRMs are predominantly positioned perisynaptically on the post-synaptic membrane (Conn and Pin, 1997).

Table 1

The list of various neurotransmitters with associated clinical conditions and their possibility of involvement in autism.

Neurotransmitters	Clinical phenotypes that are associated with the neurotransmitters	References	Whether implicated in autism or not
Glutamate is a major excitatory neurotransmitter associated with learning and memory. It acts on the N-methyl-D-aspartate (NMDA) receptors and the non-NMDA amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and kainate receptors.	Dysfunction in glutamate levels are involved in many neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's, disease. High levels also contribute to depression, obsessive compulsive disorder, epilepsy and autism.	(Francis, 2003; Nicoletti et al., 2011)	Involved in autism.
GABA (gamma-aminobutyric acid) is an inhibitory neurotransmitter and is very widely distributed in the neurons of the cortex. GABA contributes to motor control, vision, and many other cortical functions. It also regulates anxiety.	GABAergic dysfunction is associated with mood disorder and depression. Some drugs that increase the level of GABA in the brain are used to treat epilepsy and to calm the trembling of people suffering from Huntington's disease.	(Gajcy et al., 2010; SidAhmed-Mezi et al., 2010)	Involved in autism.
Glycine is an inhibitory neurotransmitter. It is de-activated in the synapse by a simple process of reabsorption by active transport back into the pre-synaptic membrane.	Mutation of glycine receptors in humans causes the rare but potentially fatal disorder of hyperekplexia or human startle disease involves the mutation.	(Gregory et al., 2008)	Involved in autism.
Norepinephrine is a neurotransmitter that is important for attentiveness, emotions, sleeping, dreaming, and learning. Norepinephrine is also released as a hormone into the blood, where it causes blood vessels to contract and heart rate to increase.	Norepinephrine plays a role in mood disorders such as manic depression.	(Ressler and Nemeroff, 1999)	Involved in autism.
Dopamine is an inhibitory neurotransmitter involved in controlling movement and posture. It also modulates mood and plays a central role in positive reinforcement and dependency.	The loss of dopamine in certain parts of the brain causes the muscle rigidity typical of Parkinson's disease. Dopamine is also implicated in ADHD.	(Cadet et al., 2010)	Involved in autism.
Serotonin contributes to various functions, such as regulating body temperature, sleep, mood, appetite, and pain.	The conditions that arise as a result of certain imbalances in serotonin include depression, suicide, repetitive behavior, and aggressiveness.	(Arango et al., 2003; Oquendo and Mann, 2000)	Involved in autism.
Acetylcholine is widely distributed excitatory neurotransmitter that triggers muscle contraction and stimulates the excretion of certain hormones. In the central nervous system, it is involved in wakefulness, attentiveness, anger, aggression, sexuality, and thirst, among other things.	Alzheimer's disease is associated with a lack of acetylcholine in certain regions of the brain.	Greenberg et al., 2010)	Involved in autism.

Ionotropic glutamate receptors on the other hand form ligand-gated ion channels and are named after their protypical agonists: NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazolepropionate) and kainate. Six gene families encode all these receptors, of which AMPA receptors (GluR1–4), kainite receptors (GluR5–GluR7, KA-1 and KA-2) and NMDA receptors (NR1, NR2A-D and NR3A) are encoded by one, two and three gene families respectively. AMPA and NMDA receptors are responsible for the post-synaptic density (Monaghan et al., 1989; Whitlock et al., 2006). Kainate ionotropic receptor acts pre-synaptically to decrease glutamatergic transmission in the hippocampus (Pinheiro and Mulle, 2006). The pre-synaptic localization of these receptors are functionally important in γ-aminobutyric acid(GABA)ergic synapses (Manent and Represa, 2007). It also generates post-synaptic currents at specific sites in the hippocampus and amygdala (Huettner, 2003).

Glutamate receptor 6 (GluR6), a kainate receptor (KARs) is expressed during brain development and is post-transcriptionally modified by editing (Gregor et al., 1993). Glutamate interacts continuously with the receptors until it gets diffused or is removed from the extracellular fluid. The temporal and spatial regulation of glutamate in the synapse is effectively brought about by excitatory amino acid transporters (Kim et al., 2010).

4. Glutamate transporters

Glutamate transporters are essential component of the glutamate mediated neurotransmitter signaling and they regulate the synaptic level of glutamate, which helps in preventing excitotoxicity. Excitotoxicity results from persistent and uncontrolled neuronal activation causing damage to target neurons. Five types of glutamate transporters are known to occur, which differ slightly in their function and localization. Two types (glial glutamate and aspartate transporter, GLAST/EAAT1 and glial glutamate transporter, GLT/EAAT2) are expressed predominantly in astroglia and the other three are in neurons (excitatory amino acid carrier, EAAC1/EAAT3 and excitatory

amino acid transporters 4 and 5, EAAT 4 and EAAT 5) (Maragakis and Rothstein, 2004). Initial cloning of the first three transporters was carried out in 1992 from rat brain (Pines et al., 1992; Storck et al., 1992), which has provided tremendous insight into the physiology and mechanism of action of excitatory amino acid transporters. Subsequently, through homology screening, the other two transporters EAAT4 and EAAT5 have been identified from cerebellum and retina respectively (Furuta et al., 1997; Pow and Barnett, 2000). Additional splice variants through mRNA splicing have also been identified for glutamate transporters (Lauriat et al., 2007; Macnab et al., 2006). They are all Na^+/K^+ dependent proteins and the transmembrane gradients of Na^+ and K^+ is the driving force for transport (Massie et al., 2008; Rose et al., 2009). The neuronal transporters seem to be linked to glutamate gated Cl^- channel and it opens up when glutamate binds to it. Subsequently it leads to the hyperpolarization of post-synaptic membranes, thereby diminishing synaptic activity. This phenomenon is functionally relevant in Purkinje cells of the cerebellum, which shows predominant expression of EAAT4 (Takayasu et al., 2009). Glial glutamate transporters (GLTs) have a marked differential regional distribution. GLT is predominant in the hippocampus, whereas GLAST is predominant in the cerebellum (Maragakis and Rothstein, 2004). Differences are also observed in the proximity of astrocytic processes to glutamatergic synapses, such that synaptic cross-talk may be possible at certain sites in the hippocampus. High affinity glutamate transport and synaptic inactivation are mainly achieved through astroglial transporters. Rat neuronal transporter EAAC (equivalent to the human EAAT3 transporter) is generally expressed in the post-synaptic neuronal membrane (with up to 15 times the density of AMPA receptors) and the pre-synaptic localization is limited to GABAergic terminals. Upon binding of glutamate to this transporter, it contributes to physiological termination of the excitatory post-synaptic current. Plasma membrane glutamate transporters also transport D-aspartate and L-aspartate. The vesicular glutamate transporter has very different properties. It is driven by the proton gradient and appears to be selective for L-glutamate.

5. Glutamate signaling in CNS

Upon excitation of the pre-synaptic neuron, glutamate is released from the synaptic vesicle into the synaptic cleft when the vesicles are fused with the membrane of pre-synaptic neurons. The released glutamate binds to ionotropic (NMDAR, AMPAR) or metabotropic receptors (mGluR) expressed on the post-synaptic neurons. Binding of glutamate to mGluRs triggers activation of G-protein-dependent intracellular signaling cascade. Activation of ionotropic glutamate receptors AMPA, kainate, and NMDA by glutamate opens ion channels for Na^+ and Ca^{2+}. Overstimulation of glutamate receptors results in neuronal damage and neurodegeneration as a result of excitotoxicity. Excessive glutamate, acting on the same glutamate receptors, over-activate glutamate receptors (specifically NMDARs), causing high levels of calcium ions (Ca^{2+}) influx into the post-synaptic cells which in turn activate a cascade of cell degradation processes that involve proteases, lipases, nitric oxide synthase and a number of enzymes that damage cell structures leading to cell death. As mentioned earlier, glutamate mediated excitotoxicity in neurons is prevented by transporting it back into cells from the synaptic region by excitatory amino acid transporters (EAATs) mostly present in astrocytes.

6. Glutamate and GABAergic system in CNS

While glutamate regulates excitatory synaptic transmission, γ-aminobutyric acid (GABA) is involved in inhibitory neurotransmission. Equilibrium between excitatory and inhibitory neurotransmissions is always maintained in the brain to avoid many of the pathological conditions. Glutamatergic synapses release glutamate, which is predominantly taken up by astrocytes, where it gets amidated to glutamine by astrocyte-specific enzyme glutamine synthetase. It has been shown that transgenic mice lacking glial specific transporter are more susceptible to excitotoxic damage. The glutamine is then released into the neurons and the phosphate-activated glutaminase catalyzes the production of glutamate and ammonia from glutamine. To some extent, glutamate might also be metabolized within the TCA cycle of both neurons and astrocytes. Glutamate formed by phosphate-activated glutaminase in the GABAergic neurons is converted by glutamate decarboxylase (GAD) enzyme to GABA. Two genes for GAD have been cloned and the two isoforms differ in their affinity for the cofactor pyridoxal phosphate and their subcellular localization (Erlander et al., 1991). Supply of glutamine to GABAergic neurons might be quantitatively less significant, as these neurons most likely exhibit a larger proportion of reuptake of released neurotransmitter compared to their glutamatergic counterpart (Bak et al., 2006). In the GABAergic synapse the released neurotransmitter GABA is taken up into both the surrounding astrocytes and the pre-synaptic terminal. In the astrocytes, GABA is metabolized in two steps to succinate, which is further metabolized in the TCA cycle to α-ketoglutarate and then glutamate.

7. Glutamate signaling in neurodevelopment

Glutamatergic signaling plays an important role in regulating neuronal migration, differentiation, neurite outgrowth, synaptogenesis and neuron survival in the developing brain (Elias et al., 2008; Georgiev et al., 2008; Komuro and Rakic, 1993; Manent and Represa, 2007). It is largely facilitated through Ca^{2+} gating. Blockade of NMDA receptors during the prenatal period using dizocilpine, phencyclidine or ethanol can induce apoptosis in vulnerable neurons. Glutamate also plays essential roles in various brain activities in mammals, such as motor control, synaptic plasticity, learning and memory, cognition, etc. (Francis, 2003; Manent and Represa, 2007). Disruption of Glutamate signaling is central to major neurological and psychiatric disorders, including Alzheimer's and Parkinson's diseases, schizophrenia, mood disorders, depression, epilepsy, anxiety, and stress- and trauma-related disorders (Francis, 2003; Nicoletti et al., 2011).

Some of these conditions are co-morbid symptoms of autism. Glutamate receptors are implicated in the processes of learning and memory through long-term potentiation, a form of synaptic strengthening that follows brief, high frequency stimulation and long-term depression, a long lasting reduction in synaptic transmission (Ge et al., 2010).

Apart from the release of glutamate from nerve endings or astrocytes regulated by vesicular glutamate transporters (Moriyama and Omote, 2008), glutamate is also released by non-vesicular mechanisms, which exerts a paracrine action on neuronal migration. Glutamate and their receptors play a crucial role in the formation of brain cyto-architecture, via its paracrine signaling action in the immature brain. It is mainly achieved by its controlling mechanisms that govern neuronal migration, outgrowth, positioning and synaptogenesis (Johnston, 1995; Manent et al., 2005; Mattson, 2008). Activation of specific GABA and glutamate receptors (GluRs) regulates cell migration, leading to radial and tangential neuronal migration (Manent and Represa, 2007). The distribution, electrophysiology, and molecular characteristics of glutamate receptors change markedly during brain development, making the developing brain vulnerable to variations in glutamate neurotransmission.

8. Glutamate signaling in autism

Autism is a neurodevelopmental disorder with severe social deficits. Several lines of evidence suggest that abnormalities in glutamatergic signaling pathways occur in autism. Glutamate function in the CNS is linked not only to synaptic neuronal interactions, but also to other roles including brain maturation and cortical organization (Manent and Represa, 2007; Wijetunge et al., 2008). Glutamate receptors are mainly concentrated in regions that have been repeatedly implicated in autism, including the cerebellum and hippocampus (Ozawa et al., 1998). Cerebellum is involved in several processes that include cognitive, affective and sensory functions in addition to motor tasks. Attention related cerebellar function is usually reduced in autistic individuals (Allen and Courchesne, 2003). The evidences from cerebellar lesions in songbirds have suggested that these lesions resulted in deficits in cognitive and motor aspects of working memory task (Spence et al., 2009). In a study involving gene expression patterns in the post-mortem cerebellar sample, Purcel et al. have suggested specific abnormalities in AMPA type receptors and glutamate transporters in this region (Purcell et al., 2001). These reports indicate that neuroanatomical alterations, possibly generated through aberrant glutamate signaling result in cognitive deficits as observed in autistic phenotype. Excitatory glutamate signaling via glutamate receptors (GluRs) plays a role in cortical development (Manent and Represa, 2007), which also indicate its involvement in the pathogenesis of ASD. In the adult brain, NMDA-type glutamate receptor is required for long-term potentiation, the physiologic process underlying learning and memory (Malenka and Nicoll, 1999). It has been speculated that at least some of the behavioral abnormalities of autism can be attributed to memory deficit (Rapin and Tuchman, 2008). Furthermore, it has been indicated that glutamate may be important in the acquisition of emotional behavior (Hoehn-Saric et al., 1991). Because of these diverse functions, glutamate and its receptors are indicated in a wide variety of neurobiological and behavioral alterations associated with ASD.

Candidate gene screening and genetic association studies have shown that kainate receptor GluR6 (Jamain et al., 2002), metabotropic GluR8 (GRM8) (Serajee et al., 2003), GRIN2A (Barnby et al., 2005) are associated with ASD. Interestingly, cDNA micro-array techniques along with other mRNA and protein expression studies of brain tissues of persons with autism identified significant increases in the expression of several genes associated with glutamatergic pathways, including EAAT1 and AMPA glutamate receptor (Purcell et al., 2001). Such disturbances of the glutamatergic system might as well affect

cortical development and plasticity, as experimental evidence suggests that GluRs plays a role in the activity dependent refinement of synaptic connectivity (Derkach et al., 2007). Cortical developmental problems are relevant in the neuropathology of ASD.

9. Involvement of glutamate signaling molecules in ASD pathology

Genome wide scan studies have shown evidences of linkage of autism to the chromosome 7q21–32 region (IMGSAC, 2001). Chromosome 7q31 region contains metabotropic glutamate receptor 8 (GRM8) gene, which is a good positional and functional candidate for susceptibility to autism. Glutamate mediated signaling is negatively regulated by GRM8 through inhibition of glutamate release at the synapses. Therefore, this receptor indeed plays a role in preventing neuronal hyperexcitability and maintaining the homeostasis (Cartmell and Schoepp, 2000). SNPs in GRM8 gene has been examined for genetic correlation with the disorder by Serajee et al. in 196 multiplex families (Serajee et al., 2003). TDT results indicate suggestive evidences for linkage disequilibrium between autistic disorder and variants of GRM8 gene. A study by Li et al. (2008) revealed a reverse trend suggesting lack of association of 4 SNP markers (rs1800656, rs712723, rs2237731 and rs17862331) of GRM8 with autism (Li et al., 2008). Gene expression analysis has demonstrated that it is distributed in several brain regions and most of these regions show anatomical defects in autistic individuals (Malherbe et al., 1999); (Bailey et al., 1998). Fragile-X syndrome, caused by mutation in FMR1 gene is an identified cause of autism. The syndrome is characterized by decreased expression or absence of fragile X mental retardation protein (FMRP). Abnormal neuronal migration in hippocampus and cerebellum are noticed in fragile-X patients and this is a feature in autism also (Dolen et al., 2007). FMRP is a RNA binding protein and it negatively regulates the protein synthesis at the synapses. Huber et al., 2002 have shown that mGluRs trigger an increase of protein synthesis dependent long-term depression in the hippocampus of FMR-1 knockout mice suggesting involvement of mGluRs in the manifestation of behavioral phenotypes in fragile X syndrome (Huber et al., 2002). Epilepsy is one of the neurological abnormalities, which often occurs as a comorbid symptom in autism. mGluR agonists has been shown to be inducing convulsions in mice, where as the antagonists reduced the epileptic discharges in these animals (Chapman et al., 2000).

Expression of several ionotropic glutamate receptors have also been investigated in relation to etiology of autism. Microarray analysis of postmortem brain tissue revealed altered level of expression AMPA-type glutamate receptors 2 and 3 in the cerebellum of individuals with autism (Purcell et al., 2001). The expression of AMPA glutamate receptors with four subtypes, GluR1, GluR2, GluR3 and GluR4 are abundant in the pyramidal layer of the hippocampus and Purkinje cells in the cerebellum (Breese et al., 1996; Tanaka et al., 2000). These brain regions have been noted as having major anatomical deficits in autism (Ozawa et al., 1998). Hippocampus is one of the key sites for generation and integration of neurons (van Praag et al., 2002). Gould et al. (1999) reported that hippocampal neurogenesis is enhanced by learning new skills. Aylward et al. has pointed out a reduction in the volume of amygdala and hippocampus in autistic individuals, with respect to the total brain volume (Aylward et al., 1999). Collectively these studies provide evidences to support AMPA glutamate receptors as potential functional candidate gene for autism. Ramanathan et al. observed a 19 megabase deletion spanning 4q32 to 4q34 chromosomal region in an autistic patient and this deleted region contains AMPA 2 glutamate neurotransmitter receptor gene that encodes the glutamate receptor GluR2 subunit (Ramanathan et al., 2004). GluR2 is the major determinant of AMPA receptor structure. Therefore, the authors suggested that this hemizygous deletion of AMPA2 in the autistic subject might result in alteration of the structural and functional

plasticity of synapses. A recent study by Mejias et al. (2011) have shown a positive correlation of glutamate receptor interacting protein 1 (GRIP1) to be positively associated with autism and their finding support a role for GRIP in social behavior and thus in autistic phenotype (Mejias et al., 2011).

NMDA receptors occur as multiple subtypes which differ in their subunit composition and in their biophysical and pharmacological properties. Significant overexpression of NR2A and NR2B subunits of NMDA receptors was observed in the neocortex of 2 week old rats prenatally exposed to valproic acid (Rinaldi et al., 2007). Valproic acid (VPA) is a powerful teratogen causing birth defects in humans, including autism spectrum disorder (ASD), if exposure occurs during the first trimester of embryogenesis (Arndt et al., 2005; Moore et al., 2000). NMDA receptors influence not only the retraction of incorrectly placed axon arbors and synapses but also the elaboration of correctly positioned terminals. NMDA receptors also play significant roles in cortical development and activity-dependent plasticity (Groc et al., 2006; Manent and Represa, 2007). GRIN2A, which encodes one of the NMDA receptor subunits, has also been studied in relation to autism, as it plays a role in long term potentiation associated with learning and memory and is evident from mouse models of a complex behavioral phenotype (Bannerman et al., 2004). GRIN2A is located on chromosome 16p, a region identified as a possible autism susceptibility locus (IMGSAC, 2001; Lamb et al., 2005; Philippe et al., 1999). Studies by Barnby et al. (2005) have shown that variants within GRIN2A might increase the risk for autism. SNP in intron 10 of GRIN2A showed evidence of association with autism through family based analysis, whereas other two SNPs in exon 6 and the 3′ UTR revealed association in case–control analysis (Barnby et al., 2005). They have suggested through logistic regression analysis that the haplotypes of this gene significantly contribute towards risk for autism.

A histological study on autistic brains revealed small sized neurons and increased neuronal packing in the limbic region including hippocampus (Bauman and Kemper, 1985). A subsequent study that involved Golgi staining of hippocampal pyramidal neurons by Raymond et al. (1996) has shown reduced complexity in dendritic branching revealing curtailment in neuronal maturation (Raymond et al., 1996). On the basis of these findings Blatt et al. (2001) conducted quantitative receptor autoradiographic studies which suggested no changes in the density and distribution of MK801 binding NMDA receptor and kainate binding receptors (Blatt et al., 2001). However, it has been shown that kainate administration in rat brain and over expression of glutamate receptor 6 (GluR6), a kainite receptor leads to seizures. On the other hand, GluR6 deficient mice show reduced susceptibility to kainate induced seizure (Mulle et al., 1998). Post-synaptic kainate receptors have been reported to participate in the excitatory post-synaptic current in various synapses. Since autism is often associated with co-morbid epilepsy phenotype, the role of GluR6 in the etiology of autism cannot be ruled out. The gene coding for GluR6 has been studied in relation to autism by different research groups. The gene is localized in one of the so-called autism risk regions at chromosome 6q21 (Philippe et al., 1999). Jamain et al. (2002) found a significant maternal association between GluR6 gene with autism in affected sib-pair (ASP) method and transmission disequilibrium test (TDT). Significant transmission of maternal allele was observed in three different markers in intron 4, exon 15 and exon 16 of GluR6 gene. Later on, Shuang et al. (2004) also demonstrated suggestive linkage of this gene with autism. Our group has performed a genetic association analysis on the Indian autistic population and contrary to these earlier reports, we observed lack of association of markers of this gene with autism (Dutta et al., 2007). GluR6 have two major activities: i) they contribute to the excitatory post-synaptic current/potential in response to glutamate and ii) modulate the release of neurotransmitters (γ-aminobutyric acid-GABA and glutamate) through pre-synaptic action. Mutations in GluR6 could thus, have important consequences in the integration of excitatory synaptic signals controlling these aspects of behavior. GluR6

is abundantly expressed in brain regions involved in learning and memory (such as the hippocampus) as well as in motor and motivational aspects of behavior (such as basal ganglia and the cerebellum). Additionally, abnormal regulation of GluR6 expression could play a role in high occurrence of epileptic seizures in autistic subjects (Rapin and Katzman, 1998).

Regulation of extracellular glutamate concentration at the synapse partly depends on the function of sodium-dependent glutamate transporters present peri-synaptically on astrocytes or neurons. Dysfunction of these transporters may result in severe pathological problems including excitotoxicity (Blaylock and Strunecka, 2009). It is also true that the activity of glutamate transporters is regulated by extracellular concentration of glutamate. These molecules mediate the reuptake of glutamate from the synapses so that its level is maintained at extracellular site to prevent excitotoxicity (Maragakis and Rothstein, 2004). Recently, an animal model for mental illness has been generated, in which glutamate receptors are over stimulated by genetic down-regulation of glial glutamate transporters, GLT1 and GLAST (Tanaka, 2009). Downregulation of glial glutamate transporter results in decreased uptake of glutamate and an elevation of extracellular glutamate level. Resulting mutant mice replicated behavioral and neuroanatomical abnormalities often observed in autism, which include abnormal social interaction and selected phenotypic abnormalities with enlarged amygdala and hippocampus. Increased expression of EAAT 1 and 2 was observed in the cerebellum of autistic patients (Purcell et al., 2001). Upregulation of EAAT 1 and 2 in the brain samples of autistic individuals may be due to above-normal level of extracellular glutamate concentrations. Gegelashvili et al. (1996) reported that exposure of astrocyte cultures with glutamate for prolonged periods upregulated EAAT1 and increased the capacity for glutamate uptake. The glutamate transporter gene, *SLC1A1* encodes the neuronal glutamate excitatory amino acid carrier 1 and is highly expressed in brain regions implicated in the pathogenesis of ASD. A genetic association study on three markers of this gene was initially conducted by Brune et al. (2008) in strictly defined samples with autism. They have demonstrated that the G allele of SNP rs301979 has been under transmitted to autistic individuals. Recently the same group has done a follow-up study including more samples and markers of 4 different glutamate transporter genes (*SLC1A1*, *SLC1A2*, *SLC1A3*, *SLC1A6*) (Jacob et al., 2010). This study supported their earlier findings on SLC1A1, but failed to show association with the other four genes. Gadow et al. (2010) examined association of single nucleotide polymorphism (SNP) rs301430 which is a coding synonymous SNP in exon 10 in glutamate transporter gene (SLC1A1) with severity of repetitive behaviors and anxiety in children with autism spectrum disorder (ASD). They reported association of C/C genotype with separation anxiety and generalized anxiety in children with ASD, which suggests that *SLC1A1* rs301430 polymorphism may be a more general risk factor for trait anxiety in this clinical phenotype. Several genome scan studies have revealed that chromosome 2q shows significant linkage to autism. Therefore, Ramoz et al. (2004) initiated a study to identify the candidate gene located at chromosome 2q24-q33. Their investigation using TDT and TRASMIT demonstrated that two variants within SLC25A12 gene, the rs2056202 (A/G) and rs2292813(A/G) in flanking intronic sequences located 21 base pairs upstream of exon 4 and 70 base pairs downstream of exon 16 respectively were associated with the phenotype. The analysis revealed preferential transmission of G allele for both the markers suggesting their possible contribution as risk markers for ASD. A follow-up study including other candidate genes in this chromosomal region further replicated the biased transmission of rs2056202 to affected autistic probands (Ramoz et al., 2008). The encoded protein mitochondrial aspartate/glutamate carrier (AGC1) protein is localized on the mitochondrial inner membrane and is engaged in the malate/aspartate NADH shuttle and is involved in oxidative phosphorylation (Palmieri et al., 2001). Mitochondrial

aspartate/glutamate carriers are also expressed in neurons and neural stem cells (del Arco and Satrustegui, 1998). *SLC25A12* mRNA and the AGC1 protein are widely expressed in adult mouse CNS, particularly in neural nuclei in the brain stem (Ramos et al., 2003). Overexpression of mitochondrial aspartate/glutamate carrier protein can lead to increased mitochondrial ATP production (Lasorsa et al., 2003). Genetic variations in this gene leading to dysfunction or altered expression of this protein, may lead to an alteration in mitochondrial function and ATP synthesis. Since neurons are major energy users, any small changes in mitochondrial function and ATP synthesis may cause selective damage or changes in neurons. Mitochondrial hyper-proliferation and partial respiratory chain block were observed in few autistic individuals, which support the role of mitochondrial dysfunction leading to alteration in energy metabolism in autism (Filipek et al., 2003). Subsequent to the initial study conducted by Ramoz et al. (2004) several association analyses have been carried out by different groups. But these studies yielded conflicting results on the possibility of association of this gene with autism (Blasi et al., 2006; Correia et al., 2006; Rabionet et al., 2006; Sakurai et al., 2010; Segurado et al., 2005; Silverman et al., 2008). A recent postmortem study using temperocortical gray matter from six matched patient control pairs has demonstrated that all six patients had increased AGC transport irrespective of whether they had seizure and this increase has been blunted by Ca^{2+} chelator suggesting the involvement of excess Ca^{2+} level in the activation of the transport process (Palmieri et al., 2010).

Glutamic acid decarboxylase (GAD) is a rate limiting enzyme in glutamate/GABA cycle which converts glutamate to gamma amino butyric acid in the brain. GAD exists as two isoforms, GAD65 and GAD67 derived from two unlinked genes in the adult brain (Bu et al., 1992). Since, GAD67 is localized in chromosome 2q31.1 which is a susceptibility locus for autism, GAD67 gene might be a potential candidate marker for GABAergic abnormalities seen in autism (Rabionet et al., 2004). All cerebellar Purkinje cells abundantly express GAD67. Reductions in Purkinje cell number and size have also been widely reported in autism (Kern, 2003). A marked decrease of 40% mRNA expression for GAD67 was observed in Purkinje cells of autistic individuals compared to control brains (Yip et al., 2007). Their findings suggest that the reduction in the GABA input to cerebellar nuclei disrupts the output to cerebral cortex causing motor and cognitive behavioral problems. In a subsequent study they have quantitated the GAD65 mRNA in dentate gyrus subpopulations and found that its level is reduced in larger cells, but not in small cell populations of autistic children (Yip et al., 2009). It suggests that there may be a differential effect in the dentate gyrus subpopulations in the cerebellum which might account for the underlying defective circuitry, which leads to the cognitive deficits in autism. An earlier study also reported reduction of GAD65 and GAD67 protein in parietal cortex and in the cerebellum of autistic brains (Fatemi et al., 2002). There are also reports of increased glutamate level in the blood and platelets of autistic subjects (Aldred et al., 2003; Shinohe et al., 2006).

10. Glutamate based therapeutic intervention for autism

Many children with autism spectrum disorder have a hyperexcitability in the brain triggered by glutamate. This may be caused by higher level of glutamate in the synapses and/or by the overexpression of its receptors. A double-blind, placebo-controlled, parallel group study in 28 children with primary diagnosis of autism with lamotrigine, a drug that modulates glutamate release showed that the children exhibited improvements in severity and behavioral features of autistic disorder, language and communication, socialization, and daily living skills after a 4-week drug free period following the drug treatment (Belsito et al., 2001). However, they have failed to observe any significant differences in improvements between lamotrigine or placebo groups on the Autism Behavior Checklist, the Aberrant Behavior Checklist, the Vineland

Adaptive Behavior scales, the PL-ADOS, or the CARS. NMDA receptor antagonists are very helpful for hyperexcitability, stimming behavior, sometimes aggressive behavior in autism (Muehlmann and Devine, 2008). Another clinical observation suggest that ketamine anesthesia, a specific blocker for NMDA receptors, has beneficial effects in calming and focused attention of children with autism (Shah et al., 2009). There are reports which demonstrate blockage of the phenomenon of behavioral sensitization to amphetamine-induced stereotypy in mice in response to NMDA antagonists (Karler et al., 1990). Memantine is a moderate affinity antagonist of the NMDA glutamate receptor, this drug was hypothesized to potentially modulate learning, block excessive glutamate effects that can include neuroinflammatory activity, and influence neuroglial activity in autism and PDD-NOS (Chez et al., 2007). Chronic maintenance therapy with the drug showed significant improvements in open-label use for language function, social behavior, and self-stimulatory behaviors, although self-stimulatory behaviors comparatively improved to a lesser degree. Several studies indicate that exaggerated signaling through GRM5 can account for multiple cognitive and syndromic features of fragile X syndrome, the most common inherited form of mental retardation and autism. In humans, fragile X syndrome is associated with an increased incidence of autistic behavior, anxiety disorders, epilepsy, sensory processing disorders, and delays in speech and language function. 2-Methyl-6-phenylethynyl-pyridine (MPEP), an antagonist of the mGluR5 metabotropic glutamate receptor, blocks aberrant phenotypes in the Fmr1 mouse model of fragile X (Dolen and Bear, 2008). An inbred mouse strain BTBR showed behavioral phenotypes similar to all three of the diagnostic symptoms of autism, including well-replicated deficits in reciprocal social interactions and social approach, unusual patterns of ultrasonic vocalization and high levels of repetitive self-grooming. Treatment with mGLUR5 antagonist, MPEP significantly reduced repetitive self-grooming in BTBR mouse model (Silverman et al., 2010). The above findings suggest that antagonists of mGluR5 receptors may have selective therapeutic efficacy in treating different behavioral abnormalities in autism.

11. Concluding remarks

The above review establishes the intricate relationship between autism, a neurodevelopmental disorder and glutamate, a major excitatory neurotransmitter. The various regulatory proteins of the glutamatergic system, which includes glutamate receptors (like GluR6, GluR8, NMDA, AMPA1 and 2 glutamate receptors) as well as glutamate transporters (like glial glutamate transporters, GLT1, GLAST, SLC1A1 and EAAT-1,2) are highly implicated in the pathophysiology of the disorder. But, the exact mechanism leading to the manifestation of the behavioral phenotype in ASD is not well understood. An understanding of how glutamate receptor and transporter biology is affected in autism may lead to future development of strategies for regulating its expression and/or function to achieve better treatment prospects for the disorder. Hence, further research in this direction will help to design specific molecules targeting these signaling proteins and possibly siRNA to modulate the expression dynamics of these selective glutamate receptors, transporters for their therapeutic application in autism.

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