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Prospects of Using Platelets as Peripheral Marker to Study the Role of GABA in Autism

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Abstract

Literature indicated that platelets could be used as a model for neuronal receptors such as γ-amino butyric acid (GABA) and serotonin. Research work exhibited the presence of low levels of GABA and high levels of serotonin concentration in the platelets of autistic children as compare to their healthy counter parts. There are also other evidences pointing out to the significant role of GABA in autism such as association of g-band frequency with the cortical concentration of GABA and gabapentin (GABA analogue) specifically inhibits the cytosolic branched chain amino transferase (BCATc); an enzyme responsible to modulate glutamate availability for the synthesis of GABA.

Keywords

Autism, GABA, Serotonin, Platelets, Neurotransmitters

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1. Introduction

It was previously suggested that platelets could be used as a model for neuronal receptors such as amino butyric acid (GABA) and serotonin [1] [2]. Our work in progress and literature [3], revealed the presence of low levels of GABA and high levels of serotonin concentration in the platelets of autistic children as compare to their age matched healthy counter parts. There are also other evidences pointing out to the significant role of GABA in autism such as association of g-band frequency with the cortical concentration of GABA [4] and gabapentin (GABA analogue) specifically inhibit cytosolic branched chain amino transferase (BCATc); an enzyme responsible to modulate glutamate availability for the synthesis of GABA [5].

2. GABA and Other Neurotransmitters

There are many neurochemicals such as GABA, Glutamate, serotonin, dopamine, and acetylcholine present before the neuronal differentiation does occur in the fetal brain and play modulatory role in neuronal differentia-

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tion, proliferation and migration (**Table 1**). The developmental abnormalities in autism may be related to the expression of numerous genes that normally silenced during the post natal development [6]. Many genes remain switched on which stunned axodendrtic development but still GABA seems to be the most distinguished candidate for autism. At the early stage of development neuronal GABA receptors function as excitatory due to the high Cl^- concentration inside the cell, and resultant efflux of Cl^-. Dysregulation of monoamines neurotransmitters such as serotonin can modify neural activity widely across the forebrain, and thereby affect the progressive refinement and emergent efficiencies of all forebrain-processing systems. GABA and its relationship with other neurotransmitters and neuromodulators may involve in triggering autism and autistic behaviors. GABA related changes in neurotransmitters, branched chain amino acids, cell adhesion molecules and neurotropic factors effect on the developing fetus and newborn.

3. Neurotransmitters Interaction with GABA and Development of Autism

During the early stage of development GABAergic excitation cooperates with N-methyl-D-aspartate receptors (NMDARs) to drive spontaneous synchronous activity (SSA) by removal of Mg^+ blockade of NMDA and influx of Ca^{++} [7]. SSA is fundamentally important for developing neuronal network and suppressed GABAergic inhibition involves in pathophysiology of autism through this pathway. Similarly, reduced availability of glutamic acid decarboxylase (GAD), enzyme responsible for the synthesis of GABA can lead to delayed myelination and synaptic maturation, learning and memory processes. While decrease numbers of GABA interneuron per units of cortical minicolumns and low levels of GABA concentration at the synapse shown to be involved in autism. GABAergic neurons are sensitive to glutamate analog (NMDA) resulting in the loss of inhibitory control which in turn damage the large pyramidal and multipolar neurons and may contribute to the pathology of autism [8]. Significant loss of Purkinje cells and pyramidal neurons in the frontal cortex, and in limbic system were observed in autism. GABAergic dysfunction may either result in direct alterations in GABA systems or in neuromodulation of GABAergic neurons via several neuromodulators that are reported to be involved in such changes, potentially with synergistic effects (**Table 2**). Acetylcholine is one of them cholinergic dysfunction may have an indirect contribution in the development of autistic symptoms via its influence on GABAergic neurons, a correlate of prior GABAergic dysfunction, or work as a direct contributor through its influence on synaptic development [9]. The $\alpha 7$ nicotinic acetylcholine receptor which has been reported to be found on the surface of GABA inhibitory neurons promote, GABA release and can restore diminished inhibitory tone. While $\alpha 4\ \beta 2$ nicotinic acetylcholine receptor which has regulatory effect on GABAergic neurons have shown to be decreased in the cerebral neocortex and in the cerebellum of autistic patients. The serotonergic system is involved in the regulation

Table 1. Modulatory action of Serotonin receptors on GABAergic receptors neurotransmission in various brain regions.

Table Serotonin modulatory effect on GABA				
Serotonin/receptors	GABA/ receptors	Mechanism involved	Location in brain regions	References
5HT	$GABA_B$	5-HT inhibits$GABA_B$ mediated IPSCs acting both pre and post synaptically	CA3 pyramidal neurons	[17]
5HT	$GABA_B$	5-HT and $GABA_B$ receptors increase and decrease Ttype Ca^{2+}	Interneurons from stratum lacunosum-moleculare	[18]
$5\text{-}HT_3$	GABA	Stimulates GABA release	Basolateral amygdala (from interneurons)	[19] [20]
$5\text{-}HT_2$ and $5\text{-}HT_4$	$GABA_A$	Modulate post synaptically $GABA_A$ mediated effect	Pyramidal neurons from prefrontal cortex	[21]
$5\text{-}HT_2$	$GABA_A$	Promotes Phosphorylation of $GABA_A$ receptors by activating on protein kinase C (PKC) which reduces $GABA_A$ mediated Cl^- currents.	Pyramidal neurons from prefrontal cortex	[21]
$5\text{-}HT_4$	$GABA_A$	Modulates $GABA_A$ mediated current depending on protein kinase A (PKA)activation level	Pyramidal neurons from prefrontal cortex	[21]
5HT	GABA	↑GABA release, strengthen local GABAergic inhibition and modulate thalamic processing of sensory signals	Dendrites of thalamic interneurons	[22]
$5\text{-}HT_2$	$GABA_A$	Enhances $GABA_A$ induced Cl^- current acting through a protein kinase dependent pathway	Spinal dorsal horn,	[23]-[25]

Table 2. Represent the role of neurotransmitters in the development of Autism and other Neurological disorders.

Pathological maturation of neurotransmitter systems in the developmental of Autism			
Brain regions/ migration ↔	Synaptic integration ↔	Network activity/plasticity ↔	Behavioral clinical phenotype
Glutamate Cortical region	Blockade of GABAergic activities	↑Cortical excitatory inputs	Autism
Cortical region	↑(5HT2$_A$) receptor activity on GABAergic interneurons	↓Glutamate signaling	Developmental disorder such as Autism
Pyramidal and multipolar neurons	GABAergic neurons are sensitive to glutamate analog (NMDA)	Damage the large pyramidal and multipolar neurons	Autism
GABA Cortex	Postnatal ↓ in cortical GABAergic neurons	↑Excitation and ↑Noise in Cortex	Autism
Acetylcholine Cerebral neocortex	↓α4 β2 nicotinic acetylcholine receptor on GABAergic neurons	↓GABAergic activity	Autism
Cerebellum	↓α4, α2 nicotinic acetylcholine receptor	↓GABAergic activity	Reported in autistic patients
Hippocampus	Prenatal stress	↑Level of acetylcholine	Developmental disorders including Autism
Cerebral cortex	↓α4 β2 acetylcholine receptor	↓Interneuron GABAergic neurotransmission	Autism
Serotonin Cortex	Destruction of 5HT afferents by using Pchlorophenylalanine at a critical period(E12 to E17)	Abnormal distribution of GABAergic interneurons	Developmental disorders
Prefrontal cortex	5HT2$_A$ receptor agonists	Reduced GABA$_A$ currents by activation of protein kinase (PKC) which decreases GABA$_A$ mediated Cl$^-$ currents	Autism
Prefrontal cortex pyramidal neurons	5HT$_4$ receptor	±GABA$_A$ mediated current depending on Protein Kinase (PKA) levels	Autistic Spectrum Disorder
Dopamine cerebral cortex	Pysiological changes in dopamine D1 and D2 receptors	Cause alteration in GABA neuron migration at the embryonic stage	Autism
Telencephalic regions	DAergic innervation	significant GABA dysfunction	Neuronal disorders including schizophrenia

of emotional processes and cognitive behaviors. There are several 5HT receptors; most of them belongs to G protein family, while 5-HT$_3$ receptor is a ligand-gated ion channel receptor and expressed on GABAergic neurons in neocortex and suggested to be involved in controlling excitation and inhibition of cortical columns. Activation of 5HT$_3$ induces a transient enhancement of inhibitory postsynaptic currents (IPSCs) in neocortex and hippocampus [10]. 5-HT$_{2A}$ receptor agonists can reduce GABAA currents by activating protein kinase C (PKC) in most of prefrontal cortex pyramidal neurons and reduce GABAA mediated Cl$^-$ currents. The overlapping between expression of 5HT$_{2A}$ and GABA$_A$ receptors suggested that they may be co localized at some synapses of pyramidal neurons in the prefrontal cortex (**Figure 1**, [28]).

Similarly, 5HT$_4$R are also located on pyramidal neurons of prefrontal cortex and has dual effect on GABA$_A$ mediated currents, *i.e.* can either enhance or depress depending on protein kinase A (PKA) levels. Dopamine (DA), a catecholamine synthesized from tyrosine by tyrosine hydroxylase is present in mesolimbic, nigrostrial, and mesocortical systems and are involved in controlling variety of functions such as cognition, motor function and reward mechanism. Ventral tegmental area (VTA) a group of neurons that are found on the floor of midbrain can mediate activation of mesofrontal DA system which effect on various neurotransmitters including 5HT, NE, acetylcholine, GABA and opioid peptides [11]. Any alteration in dopamine D1 and D2 receptors can cause modification in GABA neuronal migration to the cerebral cortex at the embryonic stage. Hence dopamine disparity during development can have an impact on GABA neurons expansion in multiple brain regions [12].

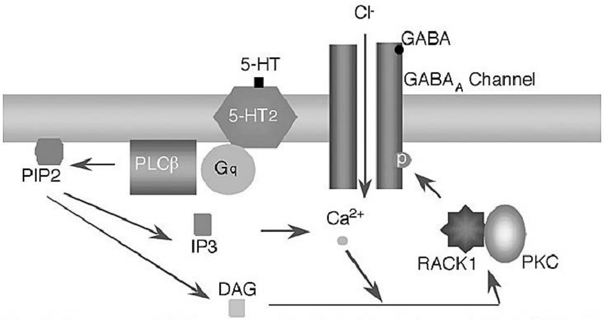

Figure 1. Diagram represent GABA$_A$ receptor regulation by signal transduction cascade through 5-HT$_2$ in prefrontal cortex. 5-HT$_2$R stimulates Phospholipase C results in the release of IP3 and DAG. Whereas PKC and RACK1 leads to phosphorylation of GABA$_A$R and hence reducing GABA currents.

Prenatal intake of cocaine or DA receptor agonists can disrupt tangential migration of GABAergic neurons because GABAergic neurons in forebrain regions receive dopaminergic innervation when migrate to cortex during embryonic period. It has been reported that significant GABA dysfunction in multiple telencephalic regions is associated with multiple neuronal disorders including autism. Similarly, brain drive neurotropic factor (BDNF) attenuates inhibitory transmission and decrease the efficacy of inhibitory transmission by acute postsynaptic down regulation of Cl^- transport. Similarly, cell adhesion molecules neurexins and neuroligins are trans-synaptic cell adhesion pair and are involved in synaptic functions. The interaction between neurexins and neuroligins are thought to trigger postsynaptic differentiation [13] and the balance between inhibitory GABA and excitatory glutamate inputs [14]. Other studies on mice carrying neuroligin3 (Nlgn3) gene mutation shows behavioral phenotypes related to ASD suggesting that the R451C mutation switches Nlgn3 synaptic specificity from glutamergic to GABAergic [15]. The branched chain amino acid (BCAA) is the combination of three essential amino acid leucine, isoleucine and valine and metabolism of BCAA is different from metabolic pathway of other amino acids. Mutation of Branched Chain alpha-Keto acid Dehydrogenase Kinase (BCKDK) gene which inactivates BCKD-kinase complex prevents the breakdown of BCAA. This BCKD-kinase mutation was reported in consanguineous families with autism, and total loss of kinase activity was present in homozygous participants [16]. Imbalanced excitation or inhibition of neurochemicals may be responsible for cytotoxicity in the developing brain and resultant behavioral deficits. GABA seems to be the most influential neurotransmitter during fetal development and any change in GABAergic migration and neurotransmission by monoamine neurotransmitters such as serotonin can alter GABAergic neuronal activity, migration and distribution. Suppressed GABAergic activity during critical period of development might result in the developmental disorders like autism and a peripheral marker such as platelet is essential for timely diagnosis of ASD and treatment effects.

GABAergic activities suggested to be crucial in pathophysiology of depressive behaviors and decreased GABA activity which would probably be a feature of a subset of mood disorder patients, possibly representing a genetic susceptibility to develop unipolar or bipolar disorder. However, neurotransmission of GABA appears to be involved in the mechanism of action of antidepressant and mood stabilizers. GABAergic pathways that appear to modulate monoaminergic and serotonergic systems, it is speculate that low basal GABA level can cause reduced levels of monoaminergic and serotonergic transmission and deficit in GABAergic neurotransmission in mood disorders would be complementary to the well-established alteration in monoaminergic and serotonergic systems which would suggest that an alteration in balance neurotransmission of these neurotransmitters (GABA, Serotonin) in depressive behaviors.

Depression can occur with autism however, clinical studies support that it is most common psychiatric illness seen in autism. In some cases depression in autism could occur by chance, or it could result from combination of genetic or environmental factors or both. The diagnostic criteria for people with depression in autism represent wide range of symptoms such as social withdrawal and appetite and sleep disturbance, and these are also core symptoms of depression. Depression can be reliably diagnosed in high functioning persons using same criteria as for the general population. Impairments in verbal and nonverbal skills can mask the symptoms of depression whereas, symptoms associated with autism such as obsession and self-injury may be increased during an episode of depression in autistic individuals [26]-[28].

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