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Metabotropic Glutamate 2/3 Receptors and Epigenetic Modifications in Psychotic Disorders: A Review

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Abstract: Schizophrenia and Bipolar Disorder are chronic psychiatric disorders, both considered as "major psychosis"; they are thought to share some pathogenetic factors involving a dysfunctional *gene x environment* interaction. Alterations in the glutamatergic transmission have been suggested to be involved in the pathogenesis of psychosis. Our group developed an epigenetic model of schizophrenia originated by Prenatal Restraint Stress (PRS) paradigm in mice. PRS mice developed some behavioral alterations observed in schizophrenic patients and classic animal models of schizophrenia, *i.e.* deficits in social interaction, locomotor activity and prepulse inhibition. They also showed specific changes in promoter DNA methylation activity of genes related to schizophrenia such as reelin, BDNF and GAD67, and altered expression and function of mGlu2/3 receptors in the frontal cortex. Interestingly, behavioral and molecular alterations were reversed by treatment with mGlu2/3 agonists. Based on these findings, we speculate that pharmacological modulation of these receptors could have a great impact on early phase treatment of psychosis together with the possibility to modulate specific epigenetic key protein involved in the development of psychosis.

In this review, we will discuss in more details the specific features of the PRS mice as a suitable epigenetic model for major psychosis. We will then focus on key proteins of chromatin remodeling machinery as potential target for new pharmacological treatment through the activation of metabotropic glutamate receptors.

Keywords: DNA methylation, epigenetics, mGlu receptors, prenatal stress, psychosis.

INTRODUCTION

Schizophrenia and Bipolar Disorder are severe and chronic psychiatric disorders, both considered as "major psychosis" that are thought to share several pathogenetic factors involving a dysfunctional "gene x environment" interaction. Epigenetic events in telencephalic GABAergic and glutamatergic neurons contribute to the pathogenesis of psychotic symptoms in patients affected by schizophrenia [1, 2]. Atypical antipsychotics and valproic acid, drugs commonly used to treat psychosis, have been demonstrated to exert a potential effect on epigenetic mechanisms and to modulate chromatin structure remodeling by inducing DNA demethylation and histone modifications [3, 4]. The term "epigenetic" refers to stable alterations in chromatin structure and genes expression that occur without modifying the underlying DNA sequence [5]. These alterations strongly depend on gene-environment interactions, can be transmitted through generations of cell divisions and may induce phenotypic modifications in the organisms [6]. Epigenetic modifications of the genome may occur *via* different pathways: (i) cytosine DNA methylation at specific clusters of dinucleotyde sequence (CpG) by a family of enzymes called DNA methyltransferases (DNMTs); (ii) covalent modifications of histones at their amino (N)-terminal tails, the most important of which being acetylation and methylation; (iii) RNA interference [7-10] (Fig. **1**). In recent years, an increasing interest arose among the scientific community on this topic: epigenetic mechanisms have been identified in the pathogenesis of many diseases, including psychiatric diseases [11-16], and the development of drugs with specific ability to modulate the epigenetic machinery is at the moment a very intriguing and promising therapeutic strategy.

MODELING THE MOLECULAR EPIGENETIC PROFILE OF PSYCHOSIS IN PRENATALLY STRESSED MICE

Evidence from neuroimaging, neuropathology, and epidemiological studies has led to the conclusion that schizophrenia (SZ) and bipolar (BP) disorders are likely to be neurodevelopmental disorders that originate before birth [17-19]. Moreover, an association between prenatal stress and risk for developing SZ and associated psychiatric disorders including anxiety and affective disorders has been established [20-22].

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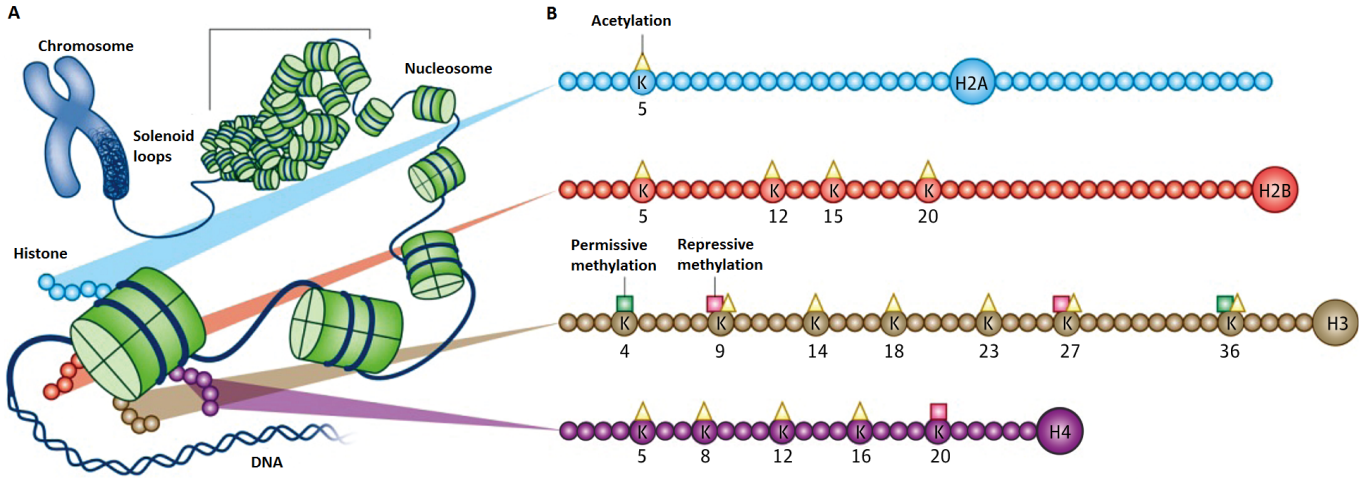

Fig. (1). A schematic organizations of chromatin structure (a). DNA is classically wrapped around histone proteins forming the basic units known as nucleosomes. These complexes consist in two copies of H2A, H2B, H3, and H4 proteins. H2B, H3, and H4 have their N-terminal tails protruding from the nucleosome. H2A has also the C-terminal protruding from the nucleosome. The tails undergo to posttranslational modifications such as acethylation and methylation as shown in (b) in addition to DNA epigenetic modifications such as methylation at CpG islands and demethylation (not shown). This image was originally published in Sun HS, Kennedy PJ, Nestler EJ. Epigenetics of the depressed brain: role of histone acetylation and methylation. *Neuropsychopharmacology Rev*., **2013**, *38*, 124137. The author retains the copyright.

Adult offspring of mice exposed to repeated episodes of restraint stress during pregnancy, here defined as prenatally restraint stressed mice or *PRS mice*, exhibit a SZ-like behavioral phenotype characterized by hyperactivity, stereotyped and compulsive behaviors, deficits in social interaction, pre-pulse inhibition (PPI), fear conditioning, and object recognition, and hypersensitivity to N-methyl D-aspartate (NMDA) receptor blockers. This behavioral phenotype recapitulates positive and negative symptoms, as well as cognitive dysfunction displayed by patients affected by SZ or BP disorder [23]. PRS mice also show a deficit in cortical GABAergic innervation [23-25], which is expected to cause abnormal synchronization of the firing rate of pyramidal neurons, a putative electrophysiological substrate of cognitive dysfunction in psychotic patients [1, 26, 27] and neurodevelopmental animal models of SZ [28].

From a molecular standpoint, PRS mice show a disrupted signature of chromatin remodeling at genes typically expressed in GABAergic and glutamatergic neurons, *i.e.*, genes encoding for glutamate decarboxylase-67 (*GAD1*), reelin (*RELN*) and brain derived neurotrophic factor (*BDNF*) respectively [23, 29, 30]. These molecular changes in PRS mice are similar to those observed in the brain of SZ and BP patients, [2, 31-35] strongly suggesting that aberrant epigenetic GABAergic/glutamatergic mechanisms may underlie psychotic symptoms.

The epigenetic endophenotypes common to the Frontal Cortex (FC) and hippocampus of PRS mice and the brain of patients affected by SZ and BP disorder are the following: (i) increased mRNA and protein levels of the DNA methylating enzymes, DNA methyltransferase 1 (DNMT1) and ten-eleven metylcytosine dioxygenase -1 (TET1); (ii) enhanced DNMT1 binding to *GAD1*, *RELN*, and *BDNF-IX* promoters; (iii) increased levels of 5-methylcytosine (5MC) and

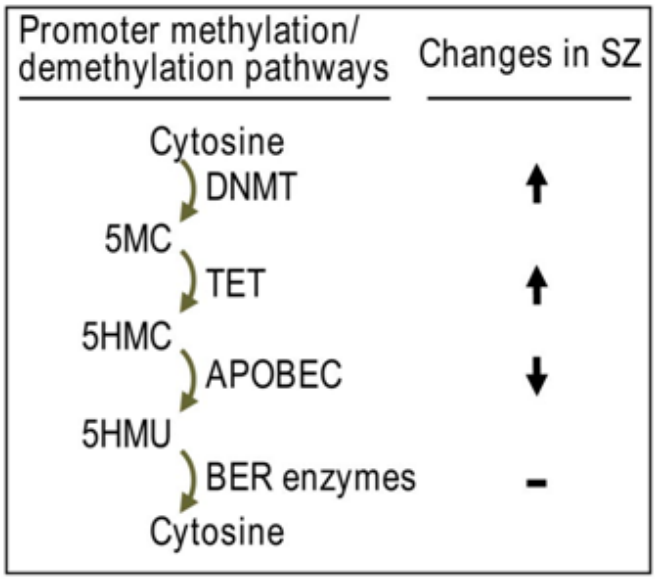

Fig. (2). A schematic enzyme-based reaction cascade of DNA methylation and de-methylation at cytosine residues involving DNA methylatransferase (DNMT), Ten-eleven translocation (TET) enzymes, which hydroxylate 5-methyl cytosine (5MC) to form 5-hydroxymethylcytosine (5HMC), and cytidine deaminases (Apobec), that, through a growth arrest and DNA damage (GADD45) protein-coordinated process (not shown), convert 5HMC to 5-hydroxymethyuracil (5HMU), which can be excised by thymine glycosylases, leading to the non-methylated state. Base Excision Repair [BER] enzymes remove methyl groups from cytosines. This image was originally published in Guidotti A, Dong E, Tueting P, Grayson DR. Modeling the molecular epigenetic profile of psychosis in prenatally stressed mice. *Prog. Mol. Biol. Trans. Sci.,* **2014**, *128*, 89-101. The author retains the copyright.

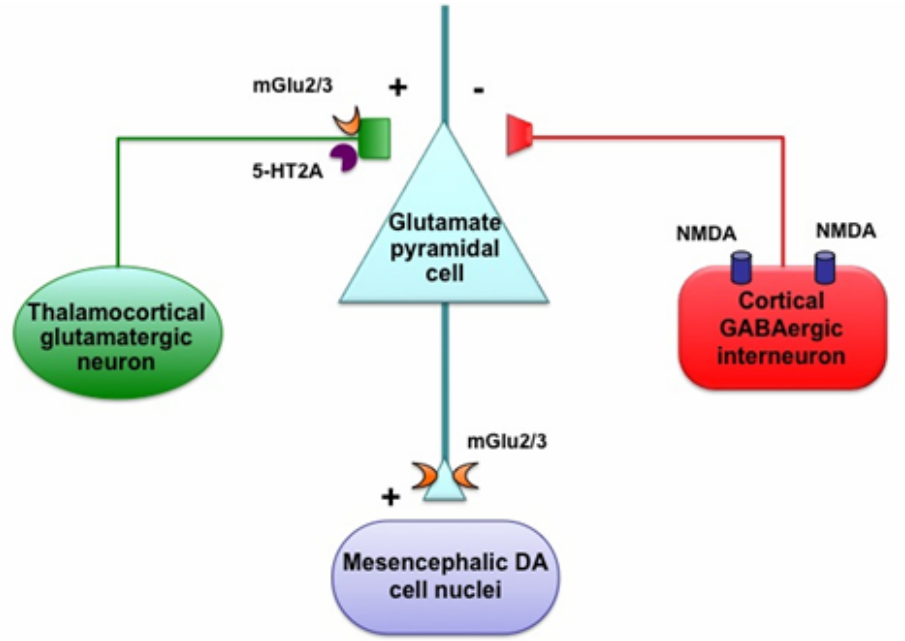

Fig. (3). A schematic cartoon showing the interactions between GABAergic and glutamatergic neurotransmission at cortical level. Unbalanced GABA/glutamate neurotransmission is proposed as the main pathogenetic mechanism underlying psychosis. Hypofunction of GABA interneurons *via* NMDA receptors leads to disinhibition of cortical glutamatergic pyramidal cells causing an excessive activation and glutamate release. The same fibers project to subcortical areas causing an excessive firing and dopamine release. The cartoon shows also different types of receptors, such as mGlu2/3 receptors at presynaptic level and 5-HT2a as potential target for pharmacological interventions to restore the normal balance between GABA and glutamate. DA = dopaminergic.

5-hydroxymethylcytosine (5HMC) at *GAD1*, *RELN*, and *BDNF* promoters; (iv) increased binding of methyl CpG binding protein (MeCP2) to *GAD1* and *RELN* promoters; and (v) a reduced expression of *GAD1*, *RELN* and several *BDNF*-splice variants [2, 23, 30, 31, 36-38] (Fig. **2**).

Different studies [23, 39, 40] have shown that the SZ-like behavioral alterations present in neurodevelopmental rodent models of SZ are corrected by systemic administration of valproate and clozapine. In the present review, we address the question of whether PRS mice may provide a behavioural and molecular epigenetic animal model of SZ valuable for the study of the antipsychotic-like activity of types-2/3 metabotropic glutamate (mGlu2/3) receptor agonists and their interaction with typical or atypical antipsychotic drugs.

METABOTROPIC GLUTAMATE 2/3 RECEPTOR AGONISTS AND SCHIZOPHRENIA

Metabotropic glutamate receptors are GTP-binding-protein (G-protein) coupled receptors that modulate both excitatory and inhibitory synaptic transmission in the CNS, and are considered as candidate drug targets for neurological and psychiatric disorders [41].

In the last decade, a role for mGlu2/3 receptors in schizophrenia has been suggested by preclinical and clinical studies. Pharmacological activation of mGlu2/3 receptors decreases glutamate release in the CNS, thereby reducing synaptic firing. This effect is shared with atypical antipsychotic drugs such as clozapine, which suppress serotonin-induced post-synaptic excitation in different brain regions [42] (Fig. **3**). Both orthosteric mGlu2/3 receptor agonists and selective mGlu2 receptor enhancers reverse the effect of NMDA receptor antagonists on working memory, sensorimotor gating, locomotor activity, and cortical glutamate efflux in rodents [43-47]. In addition, drugs that activate mGlu2/3 receptors restrain the electrophysiological and behavioral effects of agents acting on 5-HT_{2A} serotonin receptors, *i.e.* hallucinogens [48-52]. In patients affected by schizophrenia, systemic treatment with pomeglumetad methionyl, an oral prodrug of the mGlu2/3 receptor agonist, LY404039, displayed significant antipsychotic activity in a Phase-2 clinical trial [53], but not in subsequent trials [54, 55]. Perhaps a reason for these contrasting findings was the recruitment of patients that had been treated with atypical antipsychotic drugs, which are known to epigenetically down-regulate mGlu2 receptors in mice [56, 57]. Thus, mGlu2/3 agonists might still be promising for the treatment of targeted subpopulations of patients affected by SZ.

PHARMACOLOGICAL ACTIVATION OF mGlu2/3 RECEPTORS CORRECTS THE PATHOLOGICAL PHENOTYPE OF PRS MICE

As outlined above, PRS mice exhibit a SZ-like phenotype characterized by alterations in in social interaction, prepulse inhibition, and locomotor activity, and, by an epigenetic down-regulation of reelin, BDNF, and GAD67 proteins in the frontal cortex and hippocampus [23].

Interestingly, PRS mice also showed significant reductions in mGlu2 and mGlu3 receptor mRNA and protein levels in the frontal cortex, which was manifest at birth and, at least for mGlu2 receptors, persisted in adult life. This

reduction was associated with an increased binding of DNMT1 to CpG-rich regions of the *GRM2 and GRM3* gene promoters and an increased binding of MeCP2 to the *GRM2* receptor promoter [2].

More recently, we have found a significant increase of TET1 (the enzyme that catalyzes the transformation of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC) in the frontal cortex of PRS mice [30].

All epigenetic and behavioral changes of PRS mice were reversed by treatment with the mGlu2/3 receptor agonist, LY379268 [29]. A reduced expression of *DNMT1* and *DNMT3a* was also observed in response to LY354740 (another potent mGlu2/3 receptor agonist) (Authors' unpublished observation).

In addition LY379268 increased both mRNA and protein levels of growth arrest and DNA damage 45-β (Gadd45-β), a protein involved in the regulation of DNA demethylation, in mice frontal cortex and hippocampus. The binding of Gadd45-β to specific promoter regions of *RELN, BDNF*, and *GAD1* genes was also increased after treatment with LY379268 [58].

The effects of mGlu2/3 receptor agonists in PRS mice were similar to those produced by the atypical antipsychotic clozapine and by valproate, a drug that has multiple mechanisms of action including the inhibition of HDACs [59]. In contrast, the classical antipsychotic, haloperidol, had no effect on PRS mice.

All together, these findings suggest that treatment with mGlu2/3 receptor agonists or mGlu2 receptor PAMs might correct epigenetic alterations typical of patients affected by SZ.

5HT$_{2A}$-mGlu2/3 RECEPTOR INTERACTIONS

A large body of evidence suggests that 5-HT$_{2A}$ and mGlu2 receptors tightly interact in regulating the response of cortical pyramidal neurons to thalamic inputs in the prefrontal cortex, and that this interaction is relevant to the pathophysiology of schizophrenia. Activation of 5-HT$_{2A}$ receptors enhances glutamate release in the apical dendritic region of cortical layer V pyramidal cells, and this effect is blocked by pharmacological activation of presynaptic mGlu2/3 receptors [60]. Gonzales-Maeso and his associates have shown that mGlu2 physically associates with 5-HT$_{2A}$ receptors *via* specific amino acid residues located at the C-terminal end of the 4th transmembrane (TM) domain, and that activation of mGlu2 receptors restrains specific signaling pathways activated by hallucinogens acting at 5-HT$_{2A}$ receptors [61-63]. The finding that expression of mGlu2 receptors is reduced, and expression of 5-HT$_{2A}$ receptors is increased, in post-mortem cortical tissue of patients affected by SZ [61] suggests that an imbalance between mGlu2 and 5-HT$_{2A}$ receptors contributes to the pathophysiology of SZ and can be targeted by therapeutic intervention. This imbalance was also shown in prenatally stressed mice, and in the adult progeny of dams exposed to viral infection during pregnancy. In both models, early life stress caused a down-regulation of mGlu2 receptors and an up-regulation of 5-HT$_{2A}$ receptors in the cerebral cortex in the adult life [64, 65]. These changes in receptor expression might not be interrelated because it is the blockade or genetic deletion of 5-HT$_{2A}$ receptors that epigenetically down-regulate mGlu2 receptors [56, 57]. 5-HT$_{2A}$ receptor knockout mice showed a reduced expression of mGlu2 receptors in the frontal cortex, which was associated with a reduced H3 and H4 histone acetylation and an increased H3-K27 tri-methylation at the *GRM2* gene promoter, two epigenetic changes that cause gene repression [56]. Similar changes were found in normal mice chronically treated with atypical antipsychotic drugs inhibiting 5-HT$_{2A}$ receptors [56].

CONCLUSIONS

All these findings suggest that mGlu2 and 5-HT$_{2A}$ receptors are specifically targeted by a pathological epigenetic programming that is triggered by adverse events occurring early in life and that ultimately results in a long-lasting dysfunction of thalamic-cortical transmission, which is a key event in the pathophysiology of SZ. Pharmacological activation of mGlu2 receptors seems to have a favorable impact on epigenetic changes associated with SZ, but the effect of mGlu2/3 receptor agonists or mGlu2 receptor PAMs might be limited by the down-regulation of mGlu2 receptors, a process associated with the disorders and even amplified by the use of atypical antipsychotics. Thus, mGlu2 receptors should be targeted in drug-naïve patients, preferentially at the first episode of psychosis, or at least in the early phase of the disease. One can easily predict that "epigenetic drugs" acting at the *GRM2* gene promoter to enhance the expression of mGlu2 receptors may have a permissive effect with mGlu2 receptor agonists/PAMs in the treatment of SZ. Accordingly, pharmacological inhibition of HDACs has been shown to reverse the epigenetic down-regulation of mGlu2 receptors caused by atypical antipsychotics [56]. L-acetylcarnitine enhances mGlu2 receptor expression *via* a mechanism mediated by acetylation of histones or transcription factors [66-68]. L-Acetylcarnitine is currently marketed for the treatment of neuropathic pain and shows an excellent profile of safety and tolerability. It will be interesting to examine whether L-acetylcarnitine is able to reverse the epigenetic programming triggered by early life stress by enhancing the expression of mGlu2 receptors. If so, a combination of mGlu2/3 receptor agonists (or mGlu2 receptor PAMs) and L-acetylcarnitine might be proposed as a valuable strategy in the treatment of SZ.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

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