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Mini Review

Alterations in dopamine and glutamate neurotransmission in tetrahydrobiopterin deficient *spr-/-* mice: relevance to schizophrenia

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Tetrahydrobiopterin (BH_4) is a pivotal cofactor for enzymes responsible for the synthesis and release of monoamine neurotransmitters including dopamine and serotonin as well as the release of glutamate. Deficiencies in BH_4 levels and reduced activities of BH_4-associated enzymes have been recently reported in patients with schizophrenia. Accordingly, it is possible that abnormalities in the biochemical cascades regulated by BH_4 may alter DA, 5-HT and Glu neurotransmission, and consequently contribute to the pathophysiology of different neuropsychiatric diseases including schizophrenia. The development of a novel strain of mutant mice that is deficient in BH_4 by knocking out the expression of a functional sepiapterin reductase gene (*spr* -/-) has added new insights into the potential role of BH_4 in the pathophysiology and improved treatment of schizophrenia. [BMB reports 2010; 43(9): 593-598]

INTRODUCTION

Schizophrenia is one of the most common neuropsychiatric diseases affecting 1% of the general population. This rate is fairly uniform throughout the world, even though the environmental and socioeconomical factors vary among different countries (1-4). The symptoms of schizophrenia start to develop in late adolescence or early adulthood. These include thought disorder, perceptual disturbances, visual and auditory hallucinations and delusions. These symptoms are designated as "positive" symptoms to differentiate them from "negative" symptoms, which include schizophrenia patients, neglect of hygiene, social isolation and withdrawal from interaction with other people (2-4). Genetic, neurochemical and environmental factors have been proposed to contribute to the development of the disease. Injuries in the normal development of human brain including maldevelopment of the anatomical organization and connectivity of cortical afferents innervating the limbic regions may contribute to neurobiological substrates for schizophrenia (5). Disturbances in the concentrations and subsequent alterations in the neurotransmission of different neurotransmitters, including dopamine (DA), serotonin (5HT) and glutamate (Glu), in different cortical and limbic and extrapyramidal pathways have been also proposed to underlie the pathophysiology of schizophrenia (6-8).

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DOPAMINE/GLUTAMATE HYPOTHESIS OF SCHIZOPHRENIA

The involvement of DA in the pathophysiology of schizophrenia is supported by the findings that antipsychotic drugs block dopmaine D_2 receptors in a direct correlation with their antipsychotic efficacy (9), by the ability of amphetamine and other psychostimulants to produce psychotic symptoms in subjects with no prior history of psychosis (10), and by the exacerbation of psychotic symptoms in schizophrenic patients if challenged with a low dose of amphetamine or DA agonists that would not produce these symptoms in healthy subjects (11).

Postmortem studies have shown a significant increase in striatal D_2 receptor binding in brain tissues from schizophrenia patients (12, 13). Positron emission tomography (PET) studies which examined striatal D_2 receptor levels in antipsychotic-naive schizophrenia patients reported inconsistent results, as one study reported a significant elevation of striatal D_2 receptors (14) while two other studies failed to detect significant changes in striatal D_2 receptors in drug-naive patients diagnosed with schizophrenia (15, 16). Repeated treatment of laboratory animals with various *first*- and *second*-generation antipsychotic drugs altered concentrations of different DA receptor subtypes in cortical, limbic and extrapyramidal brain regions of mature and developing rats (17-20).

Deficiencies in Glu neurotransmission have been linked to the pathophysiology of schizophrenia (8, 21). Ionotropic Glu receptors, particularly N-methyl-D-aspartate (NMDA) receptor subtype, have been implicated as a critical site of action of psychotomimetic agents including phencyclidine and ketamine. These agents can produce symptoms that mimic the core symptoms of schizophrenia, including positive and negative symptoms as well as impairment of cognitive functions in humans (22-24). Agonists at the modulatory glycine binding site of the NMDA receptor complex were reported to improve negative symptoms of schizophrenia (8, 21) though these findings require further validation. Laboratory studies found that genetically modified mice that lack the expression of functional NMDA receptors exhibited abnormal behaviors typically observed in animal models of schizophrenia (25).

Abnormal expression of ionotropic Glu receptor subtypes (NMDA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) and kainate) has been reported in postmortem forebrain tissue from schizophrenia patients compared to healthy controls, though the direction of these changes remains inconsistent (8, 26). Preclinical studies indicate that these receptors are altered by treatment with dissimilar antipsychotic drugs. Different studies report increases, decreases, or no change in levels of these receptors after long-term treatment with various antipsychotic agents (27-31). Moreover, contradictory and often opposite findings have been reported in the expression of subunits composing different Glu receptors after repeated administration of dissimilar antipsychotic agents (32-34). However, there is a general agreement that antipsychotic drugs mediate their actions, at least in part, via ionotropic Glu receptors.

TETRAHYDROBIOPTERIN (BH_4) BIOSYNTHESIS

Enzymatic activities of GTP cyclohydrolase I (GTPCH I), 6-pyruvoyl-tetrahydrobiopterin synthase (PTPS), and sepiapterin reductase (SR) all participate in the biosynthesis of BH_4 (Fig. 1) (35, 36). BH_4 is also regenerated by the serial actions of pterin-4α-carbinolamine dehydroxylase (PCD) and dihydropteridine reductase (DHPR) (Fig. 1) (35). Furthermore, the biosynthesis of BH_4 can partially occur by the combined activity of aldose and carbonyl reductases (AR, CR) when enzymatic activity of SR is absent (37). Gene mutations in GTPCH, PTPS, (biosynthesis pathway), PCD or DHPR (regeneration pathway) can lead to BH_4 deficiency, which in turn contributes to the pathophysiology of different diseases (36). BH_4 deficiency can be detected through phenylketonuria (PKU) screening tests (36).

ROLES OF TETRAHYDROBIOPTERIN IN NEUROTRANSMISSION

BH_4 plays a pivotal role in the hydroxylation of aromatic amino acids including phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) (35, 36, 38-40) and the production of nitric oxide (NO) by NO synthase (NOS) (35, 41, 42). In addition, BH_4 is essentially required for the enzymatic activities of three aromatic amino acid hydroxylases (AAAH) including phenylalanine hydroxylase (PAH), tyrosine hydroxylase (TH), and tryptophan hydroxylase (TPH) (38-40).

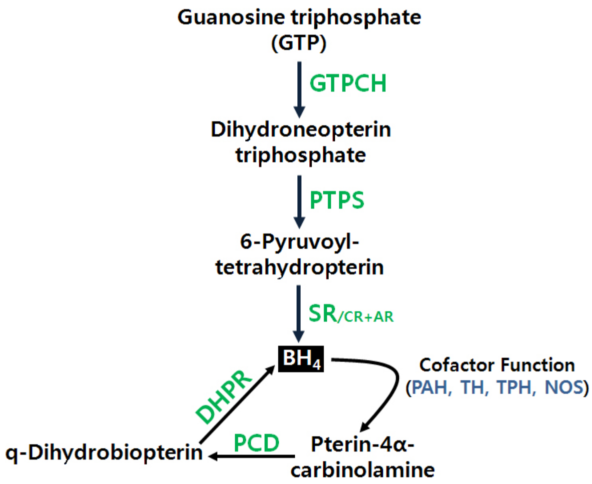

Fig. 1. Biosynthesis and Regeneration of BH_4 (35-37). GTPCH, GTP cyclohydrolase I; PTPS, 6-pyruvoyl-tetrahydrobiopterin synthase; SR, sepiapterin reductase; PCD, pterin-4α-carbinolamine dehydratase; DHPR, dihydropteridine reductase; CR, carbonyl reductase; AR, aldose reductase; PAH, phenylalanine hydroxylase; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; NOS, nitric oxide synthase.

In DA synthesis, PAH converts phenylalanine to tyrosine (38). Consequently, TH, a rate-limiting enzyme in DA and noradrenaline synthesis, converts tyrosine to *L*- 3,4-dihydroxyphenylalanine (L-DOPA), a precursor for DA. L-DOPA is finally converted to DA by DOPA decarboxylase (39). Normal levels of BH_4 appear to be important to localize and stabilize TH enzyme in DArgic neurons (43, 44) and to prevent the nitration of TH by reactive oxygen species (ROS) (45). In 5HT synthesis, TPH converts tryptophan to 5-hydroxytryptophan, which in turn is converted to 5HT by the enzyme aromatic L-amino acid decarboxylase (Fig. 2).

BH_4 is also a critical cofactor for the synthesis of nitric oxide (NO) catalyzed by three types of nitric oxide synthase (NOS) which are endothelial, inducible, and neuronal NOS (35, 41, 42). Optimal levels of BH_4 are required to synthesis NO since lower levels of BH_4 can trigger NOS to produce ROS rather than NO (46, 47). NO has been suggested to play the role of a neurotransmitter rather than a neuromodulator (48), and in this capacity, NO has been shown to regulate DA, noradrenaline and 5HT neurotranmission (49-51) (Fig. 2).

Additional *in vivo* microdialysis studies have suggested that BH_4 regulates the release of different neurotransmitters. BH_4 perfusion stimulated the release of DA, 5HT, and Glu in neuronal cells (52). BH_4-induced release of DA was detected in rat striatum *in vivo* (53) as well as in striatal slices *in vitro* (54). Exogenous BH_4 also elevated levels of 5HT in rat hippocampal slices (55) (Fig. 2).

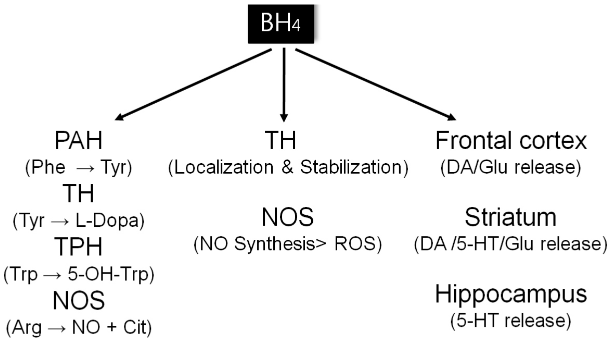

Fig. 2. Roles of Tetrahydrobiopterin in Neurotransmission (35, 36, 43-45, 52-55). PAH, phenylalanine-4-hydroxylase; TH, tyrosine-3-hydroxylase; TPH, tryptophan-5-hydroxylase; NOS, nitric oxide synthase; Phe, phenylalanine; Tyr, tyrosine; Trp, Tryptophan; Arg, arginine; NO, nitric oxide; Cit, citrulline; DA, dopamine; 5-HT, serotonin; Glu, glutamate; L-Dopa, L-3,4-hydroxyphenylalanine; ROS, reactive oxygen species.

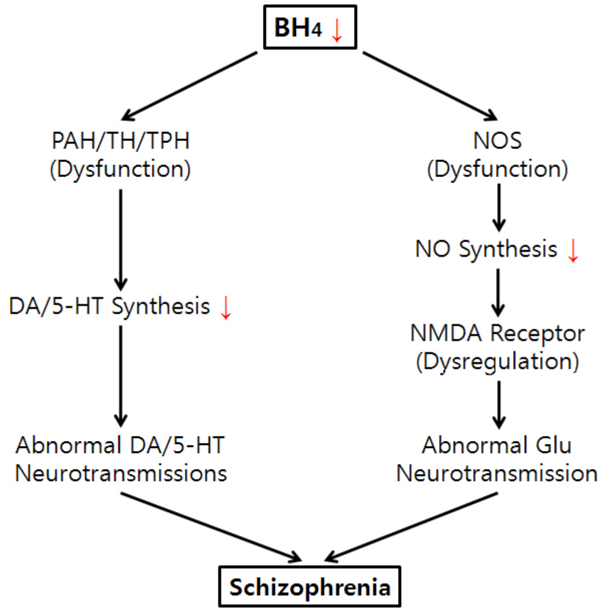

Fig. 3. Hypothesis of BH_4 Deficiency in Schizophrenia. PAH, phenylalanine-4-hydroxylase; TH, tyrosine-3-hydroxylase; TPH, tryptophan-5-hydroxylase; NOS, nitric oxide synthase; NO, nitric oxide; DA, dopamine; 5-HT, serotonin; NMDA, N-methyl-D-aspartate; Glu, glutamate.

TETRAHYDROBIOPTERIN IN SCHIZOPHRENIA

Analytical studies have considered the measurement of total biopterin levels in urine, plasma, or cerebrospinal fluid (CSF) of diseased patients and healthy control as a functional index of accurately evaluating BH_4 levels. Total biopterin represents BH_4, dihydrobiopterin (BH_2), and biopterin, and according to Fiege and colleagues, about 80% of total biopterin in urine, plasma or CSF is derived from BH_4 (56).

Earlier studies that investigated the role of BH_4 in schizophrenia failed to detect significant differences in total biopterin level in urine and CSF of schizophrenia patients versus healthy volunteers (57, 58). Another study reported that total biopterin levels were only low in plasma and but not in urine of patients diagnosed with schizophrenia (59). In contrast, more recent studies found profound reductions in plasma total biopterin levels in schizophrenia and schizoaffective patients (by 34% and 25%, respectively) compared to normal controls (60, 61). Meta-analysis studies for the International Database of BH_4 Deficiencies found significant correlation between biopterin levels in plasma and CSF but failed to establish such a correlation between biopterin levels in urine and CSF of schizophrenia patients (60).

Alterations in functional activities of BH_4 and related enzymes may represent the missing link that connects the reported abnormalities in DA, 5HT and Glu neurotransmission with the pathophysiology of schizophrenia. Several studies reported found lower levels of DA and 5HT metabolites in CSF of schizophrenia patients (62-65) and it highly possible that deficiencies in BH_4 functions might have contributed to the observed lower levels of metabolites of both neurotransmitters in schizophrenia patients. In addition, abnormalities in BH_4-linked NO signaling cascades might lead to disturbances in the closely associated NMDA receptor- mediated Glurgic neurotransmission. Moreover, lower levels of *Nurr1*, which regulates the expression of BH_4-synthetic enzyme GTPCH (66, 67), have been reported in prefrontal cortex of schizophrenia brain tissue (68). These findings support our hypothesis that deficits in BH_4 levels and subsequent alterations in BH_4-associated enzyme activities may contribute to the pathophysiology of schizophrenia via dysregulation of several neurotransmitter systems that closely associate with the disease (Fig. 3).

DEVELOPMENT AND BEHAVIOR OF *SPR-/-* MICE

Yang and colleagues developed a novel strain of genetically modified mice with deficient sepiapterin reductase functional activity (*spr-/-*) (44). These mice produced about 40% and 1% of BH_4 in brain and liver compared to their wild-type littermates, respectively. High-performance liquid chromatography (HPLC) studies showed that *spr-/-* mice exhibit significant changes in different neurotransmitter levels. 5HT and norepinephrine were barely detected in the caudate putamen and cerebellum of mutant mice (44). In addition, a profound reduction (over 90%) in levels of DA and its major metabolite levels 3,4-dihydroxyphenylacetic acid (DOPAC) were detected in caudate putamen and cortex of *spr-/-* mice compared to wild-type littermates (44).

In preliminary behavioral experiments, we found that mutant *spr-/-* mice (4-6 week old) displayed increased (3-4 folds) and sustained locomotor activity compared to wild-type controls. Interestingly, such hyperactivity was recorded in the presence of very low levels of DA. The hyperactivity was not altered by the administration of the psychostimulant amphetamine or the non-competitive NMDA receptor antagonist phencyclidine (Choi *et al.* in preparation). Lack of amphetamine effect suggests that the observed hyperactivity may result from low DA transporter levels or functions (69). The observed hyperactivity was very similar to that reported in genetic mice lacking the expression of DA transporters, which were also insensitive to the stimulatory actions of amphetamine (70). In addition, low NMDA receptor-mediated glutamatergic neurotransmission appears to be involved in locomotor hyperactivity of *spr-/-* mice since phencyclidine failed to attenuate the observed locomotor hyperactivity. The same result was also observed from the reduced NMDA receptor type 1 mice ($Nr1^{neo}$-/-), another potential animal model that mimics the symptoms of schizophrenia (25).

CONCLUSION

Evidence suggests that BH_4 plays a role in the pathophysiology of schizophrenia. The development of mutant *spr-/-* mice provided new insights into the contribution of BH_4 to the DA/Glu hypothesis of schizophrenia. In addition, mutant *spr-/-* mice would be valuable in screening the activity of novel antipsychotic drugs developed for improved treatment of schizophrenia and other idiopathic psychotic disorders.

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