Original Research

Tetrahydrobiopterin May Be Transported into the Central Nervous System by the Folate Receptor α

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The aim of this study was to determine if tetrahydrobiopterin (BH$_4$), a cofactor that is essential for several critical neurometabolic pathways, is transported across the blood-brain barrier (BBB) using the same transport mechanism as folate. In this study we examined 30 children with ASD (mean age 7.5 years, standard deviation 3.2 years; 23% female). We studied autoantibodies that interfere with the binding of folate to the folate receptor α (FRα) – a receptor that is critically involved in the transport of folate across the BBB. The relationships between cerebrospinal fluid (CSF) BH$_4$ concentrations with FRα autoantibody titers as well as with the interaction between CSF 5-methyltetrahydrofolate (5MTHF) concentration and FRα autoantibody titers were studied. CSF BH$_4$ concentration was found to be lower in individuals with higher FRα blocking, but not binding, autoantibody serum titers, suggesting that interference with FRα dependent BBB transport interferes with BH$_4$ transport across the BBB. This effect was not explained by lower CSF 5MTHF concentrations, thereby reducing the possibility that low CSF BH$_4$ concentrations were secondary to low central folate. In addition, CSF BH$_4$ concentration was inversely correlated with the interaction between CSF 5MTHF and FRα blocking autoantibody titers suggesting that BH$_4$ competes with folate for FRα dependent transport across the BBB. These data suggest that FRα dependent transport mechanisms may be involved in the transportation of BH$_4$ across the BBB.

INTRODUCTION

Tetrahydrobiopterin (BH$_4$) is an essential cofactor for several critical metabolic pathways, including those responsible for monoamine neurotransmitter production, phenylalanine metabolism and nitric oxide production. BH$_4$ is synthesized *de novo* from guanosine-5'-triphosphate or recycled from 7,8-dihydrobiopterin through a salvage pathway.[1] A deficiency in BH$_4$ synthesis or recycling can result in neurological disorders including phenylketonuria type IV and dopamine-responsive dystonia.[2] BH$_4$ deficiency is also associated with neurodevelopmental disorders such as cerebral folate deficiency[3] and autism spectrum disorder (ASD).[4,5] Low cerebrospinal fluid (CSF) BH$_4$ concentrations have been reported in children with ASD[6] and several clinical trials have shown that children with ASD have a favorable response to BH$_4$ supplementation.[5] The reason for the low CSF BH$_4$ concentrations in children with ASD is not known, but it has been suggested that BH$_4$ overuse and poor recycling could contribute to a deficiency or insufficiency.[4]

BH$_4$ supplementation has been shown to improve cognition and behavior in children with ASD[5] and has the potential to improve cognitive outcomes in BH$_4$-responsive phenylketonuria[7] but the mechanism by which BH$_4$ cross the

blood-brain barrier (BBB) remains to be defined. Studies have shown that subcutaneous,[8,9] intraperitoneal[10] and intravenous[11] administration of BH$_4$ increases CSF BH$_4$ concentrations in mice, rats and monkeys, respectively, and that oral BH$_4$ administration in mice increases tyrosine hydroxylase activity, a BH$_4$-dependent enzyme, in the central nervous system.[12] In humans, CSF BH$_4$ concentrations were increased in a boy with hyperphenylalaninemia after 6 oral administrations of 10mg/kg BH$_4$ given every 12 hours[13] and after 3 months of oral treatment with 3mg/kg/day of BH$_4$ divided into two daily doses in children with ASD.[14] Although these studies provide evidence that BH$_4$ crosses the BBB in both humans and animals, the mechanisms involved in transport of BH$_4$ across the BBB and into the central nervous system remain unknown.[5]

Since both folate and BH$_4$ are pterin derivatives, we postulate that BH$_4$ may cross the BBB using mechanisms involved in folate transport. One of the major mechanisms of folate transport across the BBB involves the folate receptor α (FRα). The FRα is located on both sides of the epithelial surface of the choroid plexus and is essential for the transportation of folate derivatives across the BBB through adenosine triphosphate dependent receptor-mediated endocytosis resulting in a 2-4 fold higher concentration of folate in the CSF as compared to the blood.[15] The FRα has gained attention recently since two FRα autoantibodies, one

called the binding autoantibody and one called the blocking autoantibody, have been found to bind to the FRα on the apical side of the choroid plexus.[16,17] Although both of these autoantibodies are believed to impair FRα receptor function, it is the blocking FRα autoantibody that has been associated with a neurodevelopmental disorder known as cerebral folate deficiency[16,18] and has been shown to be inversely correlated with CSF concentrations of 5MTHF.[19] Both autoantibodies have been associated with ASD.[19]

In order to determine if the FRα might be involved in BH_4 transportation across the BBB, in this study the relationship between CSF BH_4 concentrations and FRα autoantibodies titers is examined with the assumption that FRα autoantibodies also block BH_4 from binding to the FRα just as they block folate from binding to the FRα. Low CSF folate concentrations could metabolically contribute to lower CSF BH_4 concentrations since folate is needed to produce guanosine-5'-triphosphate, the BH_4 precursor, and is involved in BH_4 recycling.[20] To rule-out the possibility that low CSF folate is responsible for low CSF BH_4 concentrations, in this study it is demonstrated that there is no relationship between CSF folate and BH_4 concentrations. In addition, in this study it is demonstrate that the interaction between CSF folate and the FRα autoantibody titers is inversely correlated with CSF BH_4 concentration, suggesting that folate competes with BH_4 for the FRα. Lastly, in this study it is demonstrated that supplementation with folinic acid in a child with cerebral folate deficiency secondary to a mitochondrial disorder results in a decrease of CSF BH_4 concentration as CSF 5MTHF increases, suggesting a competition between folate and BH_4 to bind to the FRα and cross the BBB.

METHODS

Parents of patients seen in a medically-based ASD clinic were asked to consent to allow information from the medical record, including neurological and metabolic testing, to be anonymously abstracted into a database under an institutional review board approved protocol. Approximately 98% of parents approached signed the consent. Patients met the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition – Text Revision[21] criteria for ASD and had previously been diagnosed by a developmental pediatrician, pediatric neurologist or clinical psychologist with ASD.

As part of the workup for medical-conditions associated with ASD, FRα autoantibody titer testing was offered. Approximately 1ml of serum was collected and sent to the laboratory of Dr. Edward Quadros, Ph.D. at the State University of New York, Downstate (Brooklyn, NY). The assay for both the blocking and binding FRα autoantibody titers has been described previously.[16,17] The FRα autoantibody titers can be categorized as negative, low, medium or high (blocking autoantibody: negative = not detected, low < 0.5, medium = 0.5-1.0, high > 1.0, expressed as pmoles of folic acid blocked per ml of serum from binding to FRα; binding autoantibody: negative = not detected, low < 2.0, medium = 2-10, high > 10, expressed as pmoles of IgG antibody per ml of serum).

Parents of children who were FRα autoantibody positive were offered a diagnostic lumbar puncture (LP) and 30 parents requested the diagnostic LP. CSF was obtained through an LP under general sedation and fluoroscopy guidance. CSF was collected with standardized reagent tubes, frozen at -80° C and sent out for analysis of 5-methyltetrahydrofolate (5MTHF) and BH_4 (Medical Neurogenetics, Atlanta, GA). In all cases included in this report, CSF demonstrated a normal number of white and red blood cells, protein, glucose, and amino acids. We have not included serum values of folate or BH_4 in this report since serum BH_4 concentrations cannot be readily measured and since serum laboratory measures of folate were inconsistently reported across clinical laboratories with many laboratories reporting an exact number only if serum folate was below the normal range.

In addition to examining the relationship between CSF BH_4 and FRα autoantibodies titers as well as the relationship between CSF BH_4 and CSF 5MTHF, the relationship between CSF BH_4 and the interaction between FRα autoantibody titers and CSF 5MTHF concentration [Interaction = FRα autoantibodies titers x CSF 5MTHF concentration] was also examined. This was done to determine if BH_4 competes with 5MTHF for the FRα in the context of FRα autoantibodies. In the context of FRα autoantibodies, greater competition with folate for FRα would be expected to be an additional mechanism that would inhibit BH_4 transport across the BBB. This assumes that the CSF 5MTHF concentration reflects the magnitude of folate transport across the BBB and that the FRα has a lower affinity for BH_4 as compared to folate.

Pearson r correlations were used to determine the relationship between CSF BH_4 and FRα autoantibodies titers as well as the CSF 5MTHF concentration and the interaction between these latter two values.

Lastly, we present the longitudinal change in CSF 5MTHF and BH_4 in a case of a child with cerebral folate deficiency due to mitochondrial disease.

RESULTS

Participants had a mean age of 7 years 6 months (standard deviation 3 years 2 months; range 2 years 2 months to 12 years 11 months) and 7 of the 30 (23%) were female. Higher blocking (r = -0.44, p = 0.05), but not binding (r = -0.01), FRα autoantibody titers were significantly related to lower CSF BH_4 concentrations (**Figure 1A**). CSF 5MTHF concentrations were not correlated with CSF BH_4 concentrations (r = 0.04) suggesting that low BH_4 concentrations were not depressed secondary to decreased folate availability. CSF BH_4 concentration was significantly (r = -0.50, p < 0.005) related to the interaction between CSF 5MTHF concentration and serum blocking FRα autoantibody titers (**Figure 1B**).

Lastly, if folate does compete with BH_4 for FRα, then high dose folate supplementation should result in a relatively lower CSF BH_4 concentration. In order to illustrate this

phenomenon, we present CSF 5MTHF and BH$_4$ concentrations over a 16 month period for a 3 year old boy with cerebral folate deficiency secondary to a mitochondrial disorder who was supplemented with high dose folinic acid

(~2mg/kg). With oral high-dose folate supplementation, CSF 5MTHF increased (**Figure 2A**) while CSF BH$_4$ decreased (**Figure 2B**), with the latter effect becoming more significant over time.
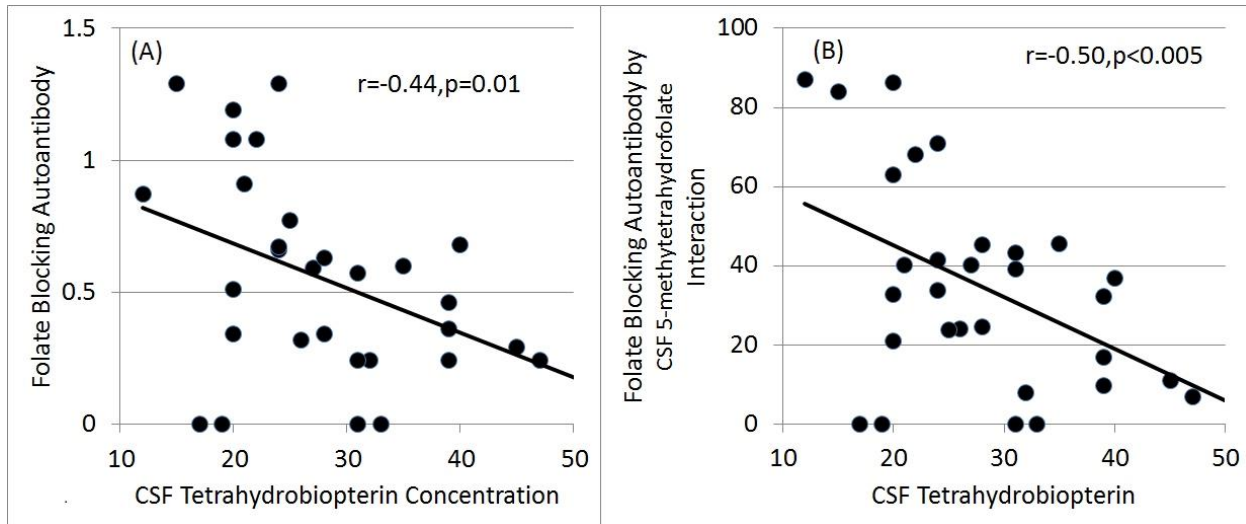
Figure 1. Relationship between cerebrospinal fluid (CSF) tetrahydrobiopterin (BH$_4$), CSF 5-methyltetrahydrofolate and the folate receptor α autoantibody. **(A)** CSF BH$_4$ concentration is inversely correlated with the blocking folate receptor α autoantibody titer. **(B)** The interaction between CSF 5-methyltetrahydrofolate and the blocking folate receptor α autoantibody titer is also inversely proportional to the CSF BH$_4$ concentration.
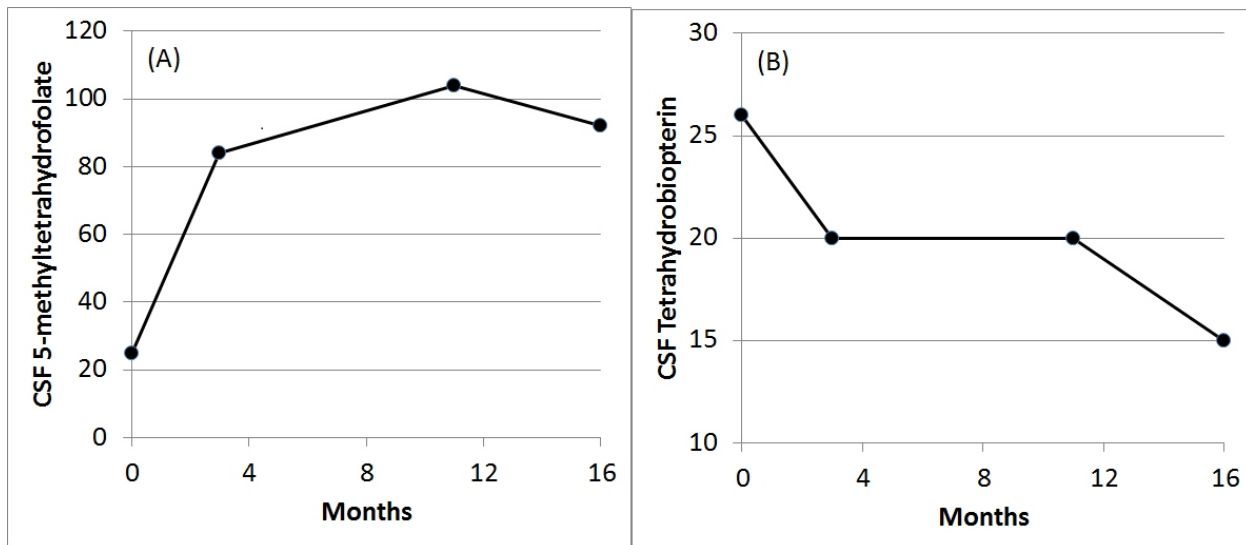
Figure 2. Supplementation with high levels of folate in a child with cerebral folate deficiency result in a decreased in cerebrospinal fluid (CSF) tetrahydrobiopterin (BH$_4$) suggesting that folate is competing with BH$_4$ for the folate receptor α. The decrease of both 5-methyltetrahydrofolate and BH$_4$ between 11 and 16 months most likely reflects the fact that BH$_4$ concentrations are now dependent on 5-methyltetrahydrofolate for *de novo* production in the central nervous system due to reduced transportation across the blood-brain barrier.

CONCLUSIONS

In this study we sought to clarify mechanisms of BH$_4$ transport across the BBB into the central nervous system. We hypothesized that BH$_4$ may use some of the same mechanisms as folate since both folate and BH$_4$ are pterin derivatives. To support this hypothesis we established that

there was a relationship between serum FRα autoantibodies titers and CSF BH$_4$ concentration. Although this relationship could be due to a blockage of binding of serum BH$_4$ with the FRα on the apical side of the choroid plexus, it is also possible that low CSF BH$_4$ concentrations could be due to an

insufficient level of central folate. To rule out this latter possibility we demonstrated that the CSF BH_4 concentration had no relationship to the CSF 5MTHF concentration. Lastly, we hypothesized that if both BH_4 and folate use the same mechanism to cross the BBB then they should compete for the available FRα resulting in relatively lower CSF BH_4 concentrations when the serum FRα autoantibodies are elevated (because there is less available FRα) especially in the context of relatively higher CSF 5MTHF concentrations since relatively higher CSF 5MTHF concentration would indicate that more folate was being transported across the BBB leaving fewer FRα receptors to transport BH_4. Our data support the notion that the higher serum FRα autoantibodies and higher folate flux across the BBB combine to inhibit BH_4 transportation across the BBB. We feel that this data supports the notion that BH_4 may use some of the same mechanisms as folate to cross the BBB.

To further support the notion that serum folate competes with BH_4 for transport across the BBB we demonstrate that supplementation with high doses of a reduced form of folate, folinic acid, in the context of cerebral folate deficiency results in a decrease in CSF BH_4 concentration presumably due to competition between folate and BH_4 for the FRα. It may be speculated that, like reduced folate, BH_4 at pharmacological doses may also be transported across the BBB via the reduced folate carrier, an alternative mechanism for folate transport that has lower affinity for folate as compared to FRα. This would also decrease central BH_4 in the context of cerebral folate deficiency as folate would also be competing for the reduced folate carrier in order to cross the BBB in such a context.

Further research is needed to define the mechanisms for the transport of BH_4 across the BBB in neurodevelopmental disorders. Polymorphisms in genes associated with enzymes involved in the production and recycling of BH_4 may also significantly contribute to CSF BH_4 concentrations. Most likely, the use of animal models and/or *in vitro* binding studies in which these factors can be systematically manipulated would be optimal for answering these questions. The exact contribution of systemic and central BH_4 production will no doubt need to be better elucidated in order to better understand to what extent transportation of BH_4 contributes to the availability of BH_4 in the central nervous system.

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