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Short Communication

Methylenetetrahydrofolate reductase deficiency alters levels of glutamate and γ-aminobutyric acid in brain tissue

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme key regulator in folate metabolism. Deficiencies in MTHFR result in increased levels of homocysteine, which leads to reduced levels of *S*-adenosylmethionine (SAM). In the brain, SAM donates methyl groups to catechol-O-methyltransferase (COMT), which is involved in neurotransmitter analysis. Using the MTHFR-deficient mouse model the purpose of this study was to investigate levels of monoamine neurotransmitters and amino acid levels in brain tissue. MTHFR deficiency affected levels of both glutamate and γ-aminobutyric acid in within the cerebellum and hippocampus. *Mthfr*$^{-/-}$ mice had reduced levels of glutamate in the amygdala and γ-aminobutyric acid in the thalamus. The excitatory mechanisms of homocysteine through activation of the N-methyl-D-aspartate receptor in brain tissue might alter levels of glutamate and γ-aminobutyric acid.

1. Introduction

Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism since it generates the main circulating form of folate, 5-methyltetrahydrofolate (5-methylTHF), which can serve as a methyl donor for the remethylation of homocysteine to methionine and subsequently the generation of *S*-adenosylmethionine (SAM) (Fig. 1). Through methylation SAM is involved in lipid metabolism and also donates a methyl group to catechol-O-methyltransferase (COMT), which catalyzes synthesis of neurotransmitters in the brain [1,2]. Increased levels of homocysteine, as a result of folate deficiency, have been linked to low levels of monoamine neurotransmitter levels in patients with depression [3].

Many polymorphisms of MTHFR have been described in the human population and the most common is a polymorphism at base pair 677, a conversion from C to T which results in a missense mutation causing an alanine to valine conversion [4]. Homozygosity has been reported in 5–20% of North American and European populations [5]. These individuals have reduced enzyme activity (heterozygotes 65% and homozygotes 30% compared to controls [6]) and slightly elevated levels of plasma homocysteine, which is highly dependent on folate status [5,7]. Individuals with the TT genotype have increased risk of developing cognitive impairment [8], late onset Alzheimer's disease [9], as well as depression and schizophrenia [10]. Another condition involved in MTHFR deficiency is an inborn error of metabolism, which results in a severe deficiency in the enzyme and leads to homocystinuria. Patients with a severe MTHFR deficiency have significantly elevated levels of homocysteine and reduced levels of methionine and present with neurological and vascular complications [11–13].

In order to investigate the effects of MTHFR deficiency on neurological function in vivo, a knockout mouse model for MTHFR was developed, *Mthfr*$^{+/-}$ and *Mthfr*$^{-/-}$ mice have elevated levels of plasma homocysteine [14]. *Mthfr*$^{+/-}$ mice model the polymorphism at base pair 677, as they have elevated levels of plasma homocysteine when compared to wildtype mice but are phenotypically normal. Whereas *Mthfr*$^{-/-}$ mice model the severe form of MTHFR deficiency, these mice have significantly elevated levels of homocysteine and have motor and cognitive impairments [15]. In brain tissue, both *Mthfr*$^{+/-}$ and *Mthfr*$^{-/-}$ mice have significantly reduced levels of DNA methylation and *S*-adenosylhomocysteine [14] and *Mthfr*$^{-/-}$ mice have reduced levels of 5-methylTHF [14,16] and SAM [14]. Additionally, behavioral studies have reported impairments in cognitive and motor function

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-methylTHF, 5-methyltetrahydrofolate; COMT, catechol-O-methyltransferase; HPLC, high performance liquid chromatography; MTHFR, methylenetetrahydrofolate reductase; SAM, *S*-adenosylmethionine; GABA, γ-aminobutyric acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin.

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[15,17]. The purpose of this study was to investigate whether levels of monoamine neurotransmitters and amino acids are altered in different brain regions of MTHFR-deficient mice.

2. Materials and methods

All experiments were approved by the Landesamt für Gesundtheit und Soziales Berlin and performed in accordance with the German Animal Welfare Act. At 3-months of age blood and brain tissue from C57BL/6 male mice was collected and processed for post-mortem neurochemical analyses using high performance liquid chromatography (HPLC) as described previously [18]. The levels of dopamine, serotonin, and their metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) in the amygdala, caudate putamen, cerebellum, dorsal raphe, hippocampus, medial prefrontal cortex, nucleus accumbens, and thalamus, amygdalae were measured by HPLC with electrochemical detection. Glutamate and γ-aminobutyric acid (GABA) were determined by HPLC with fluorescence detection. We worked with male mice in this study to avoid sex differences that have been previously described in MTHFR mice [19] and neurotransmitter analysis [20].

Blood samples taken from the same mice were centrifuged at 7000 ×*g* for 7 min at 4 °C to obtain the plasma. HPLC was used to measure plasma homocysteine concentrations by a diagnostic laboratory at Charite University Medicine (Labor 28, Berlin).

One-way analysis of variance (ANOVA) was used to compare genotype groups for each measurement followed by Tukey's post-hoc test if applicable. Significance level was set at $p \leq 0.05$.

3. Results

Mthfr$^{+/-}$ and *Mthfr*$^{-/-}$ had significantly higher plasma homocysteine levels when compared to *Mthfr*$^{+/+}$ mice (Table 1, $p < 0.05$).

There were few changes in monoamine neurotransmitter levels and respective metabolites. Serotonin levels were increased in *Mthfr*$^{-/-}$ in the amygdala when compared to *Mthfr*$^{+/+}$ mice (Table 1, $p < 0.05$). Dopamine levels within the cerebellum were different between genotype groups (Table 1, one-way ANOVA, $F_{(2, 23)} = 8.45$, $p < 0.01$). 5-HIAA was increased in the cerebellum of *Mthfr*$^{+/-}$ (Table 1, $p < 0.05$) and there was a genotype effect in the hippocampus (Table 1, one-way ANOVA, $F_{(2, 21)} = 3.35$, $p < 0.05$).

Glutamate levels were different between genotype groups within the amygdala (Table 1, one-way ANOVA, $F_{(2, 21)} = 3.45$, $p < 0.05$). *Mthfr*$^{-/-}$ mice had reduced levels of glutamate in the cerebellum (Table 1, $p < 0.05$) compared to *Mthfr*$^{+/-}$ mice, and hippocampus (Table 1, $p < 0.05$) compared to *Mthfr*$^{+/+}$ mice. Cerebellar GABA levels were increased in *Mthfr*$^{+/-}$ and *Mthfr*$^{-/-}$ when compared to *Mthfr*$^{+/+}$ mice (Table 1, $p < 0.05$). While, GABA levels were reduced in hippocampus (Table 1, $p < 0.05$) and thalamus (Table 1, $p < 0.05$) of *Mthfr*$^{-/-}$ mice compared to *Mthfr*$^{+/+}$.

4. Discussion

Increased levels of plasma homocysteine have been linked to impaired monoamine synthesis as a result of reduced levels of SAM [1]. In the present study using a hyperhomocysteinemic MTHFR-deficient mouse model we investigated levels of the monoamine neurotransmitters, dopamine, 5-HT, and their respective metabolites, as well as the amino acids, glutamate and GABA in different brain areas. We confirmed that *Mthfr*$^{+/-}$ (~14 μM) and *Mthfr*$^{-/-}$ (~70 μM) have significantly elevated levels of plasma homocysteine as previously reported in earlier work [14,15,21]. We identified that *Mthfr*$^{-/-}$ have increased levels of serotonin in the amygdala, whereas *Mthfr*$^{+/-}$ have increased levels of cerebellar dopamine levels. Levels of 5-HIAA are increased in *Mthfr*$^{+/-}$ mice in the cerebellum and reduced in the hippocampus of *Mthfr*$^{+/-}$ and *Mthfr*$^{-/-}$ mice. Additionally, hippocampal levels of HVA

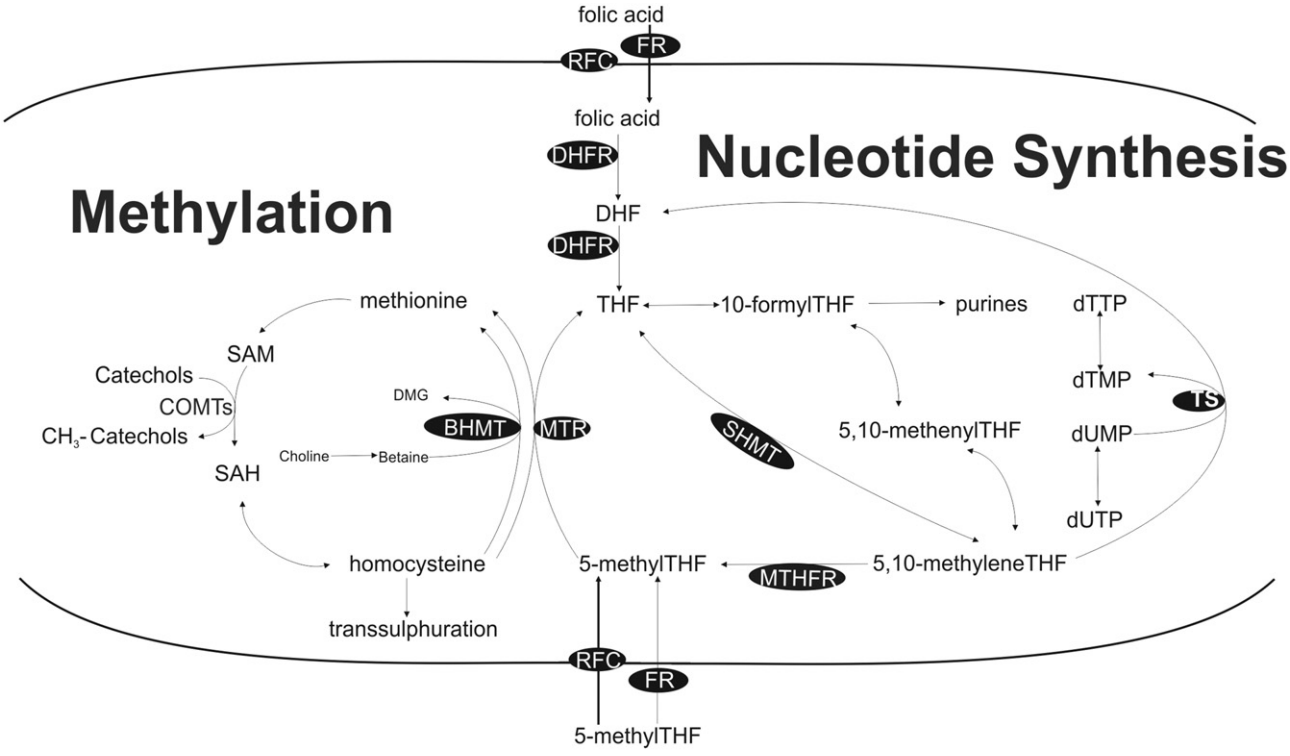

Fig. 1. One-carbon metabolism and related biological roles (methylation and nucleotide synthesis). Enzymes indicated in black circles. Abbreviations: BHMT — betaine homocysteine methyltransferase; CHDH — choline dehydrogenase; COMT — catechol-O-methyltransferase; DHFR — dihydrofolate reductase; dTMP — deoxythymidine monophosphate; dTTP — deoxythymidine triphosphate; dUMP — deoxyuridine monophosphate; dUTP — deoxyuridine triphosphate; FR — folate receptor; MTHFR — methylenetetrahydrofolate reductase; MTR — methionine synthase; MTRR — methionine synthase reductase; RFC — reduced folate carrier; SAM — *S*-adenosylmethionine; SAH *S*-adenosylhomocysteine; and TS — thymidylate synthase.