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Dysregulation of methionine metabolism in multiple sclerosis

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ABSTRACT

We report a significant reduction in plasma methionine concentrations in relapse remitting multiple sclerosis (MS) patients compared to controls. *In vivo* studies demonstrate that changes in peripheral methionine levels in mice can regulate histone H3 methylation and expression of DNA methyltransferase 3A (DNMT3A) centrally, in the cerebral cortex. Therefore, we propose that decreases in circulating methionine represent one of the earliest manifestations of dysregulated methionine metabolism in MS with potential impacts on both histone H3 and DNA methylation in the central nervous system.

1. Introduction

Multiple sclerosis (MS) is an inflammatory neurodegenerative disease that results in progressive neurological disability (Noseworthy et al., 2000; Bjartmar et al., 2000). In early relapse remitting stages of disease (RRMS), inflammatory demyelination results in less efficient conduction of nerve impulses and neurological impairment. These impairments generally resolve over a period of weeks due to redistribution of sodium channels and remyelination (England et al., 1990). Over time most patients progress to secondary progressive stages of disease (SPMS) where accumulation of damage and loss of axons and neurons leads to permanent disability (Vukusic and Confavreux, 2003). It has been established that cortical pathology including extensive gray matter lesions and cortical atrophy contribute to disease and disability (Bö et al., 2006; Fisher et al., 2008). Mounting evidence points to involvement of mitochondrial deficits and metabolic disturbances in MS cortical pathology (Dutta et al., 2006; Pandit et al., 2009; Clements et al., 2008; Broadwater et al., 2011; Witte et al., 2014) and changes in B_{12}- dependent methionine metabolism have been found to be linked to mitochondrial abnormalities in MS (Singhal et al., 2015). Methionine is an essential amino acid that not only participates in protein synthesis but is also an important intermediate metabolite involved in methyl group transfer to histones and DNA. Methylation of these targets is known to regulate the expression of thousands of genes by epigenetic mechanisms. A schematic showing the reactions involved in methionine metabolism is shown in Fig. 1A. In a previous report, levels of methionine metabolites were measured by liquid chromatography tandem-mass spectrometry (LC-MS/MS) and reduced concentrations of cystathionine and the methyl donors S-adenosylmethionine (SAM) and betaine were identified in postmortem MS cortical tissue when compared to controls (Singhal et al., 2015). As a result, we suggest that methionine metabolism is dysregulated in MS cortical tissue, however, it is not presently clear whether levels of circulating methionine metabolites are also altered in MS. In the current study we have measured methionine metabolite levels in plasma from early RRMS and compared them to those obtained from control individuals. Further, we treated mice with methionine to determine whether alterations in circulating methionine levels could impact the levels of the methyl donor SAM in the brain and subsequently modify downstream methylation reactions in the brain including histone methyltransferase H3 (H3K4me3) and DNA methyltransferase 3A (DNMT3A).

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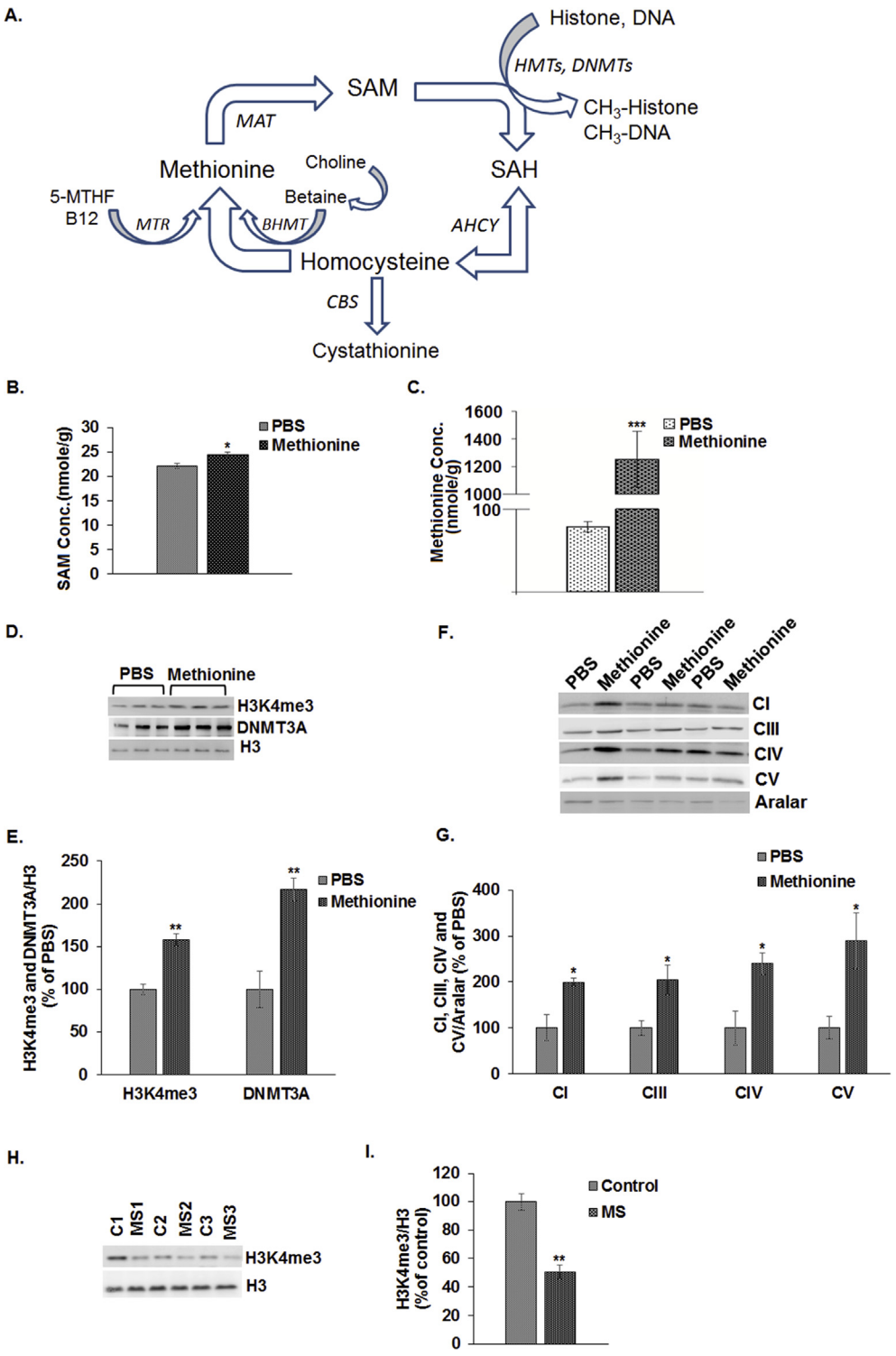

Fig. 1. Analysis of methionine metabolism metabolites and their effects on methylation in methionine treated mice and postmortem human brain tissue. **(A)** Schematic diagram depicting methionine metabolism. Homocysteine is remethylated to methionine with the transfer of methyl groups from either 5-MTHF or betaine by the enzymes MTR or BHMT respectively. Methionine is converted to SAM by MAT then SAM donates methyl groups to histone/DNA for methylation by HMT/DNMT enzymes. Once SAM donates a methyl group it is converted to SAH. Then SAH is converted to homocysteine by the enzyme AHCY. BHMT, betaine homocysteine S-methyltransferase; MTR, methionine synthase; 5-MTHF, 5-methyl tetrahydrofolate; MAT, methionine adenosyl transferase; HMTs, histone methyltransferases; DNMTs, DNA methyltransferases; AHCY, S-adenosylhomocysteine hydrolase. **(B and C)** SAM and methionine levels in brain were analyzed by LC-MS/MS in methionine treated and control mice. Both methionine and SAM were increased in methionine treated mice as compared to control **(D)** Representative Western blots for H3K4me3, DNMT3A and histone H3 from brain nuclear fractions isolated from control mice and mice treated with methionine. **(E)** Densitometry was performed for Western blots from three separate experiments. H3K4me3 and DNMT3A are increased in the brains of methionine treated mice compared with controls. **(F)** Representative Western blots for NDUFS4 (CI), UQCRC2 (CIII), COX5B (CIV), ATP5F1 (CV) and aralar from brain mitochondrial fractions isolated from control mice and mice treated with methionine. **(G)** Densitometry was performed for Western blots from three separate experiments. Protein subunits of CI, CIII, CIV, and CV are increased in the brains of methionine treated mice compared with controls. **(H)** Representative Western blots for H3K4me3 from nuclear fractions of three control and three MS cortical tissue samples. **(I)** Densitometry was performed from three separate experiments. Levels of H3K4me3 are normalized to histone H3. Data are expressed as a percentage of control with the highest control set at 100%. Error bars indicate SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.