New Insights into the Regulation of Methyl Group and Homocysteine Metabolism

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Abstract

Hepatic folate, methyl group, and homocysteine metabolism are interrelated pathways that when disrupted are associated with numerous pathologies. Maintenance of normal methyl group and homocysteine homeostasis is dependent on the balance between: S-adenosylmethionine (SAM)-dependent transmethylation, which utilizes methyl groups and produces homocysteine; remethylation of homocysteine back to methionine by folate-dependent and -independent mechanisms; and homocysteine catabolism via the transsulfuration pathway. Recent studies have demonstrated that hormonal imbalance is a factor in the control of key proteins that regulate these pathways. A diabetic state is characterized by increased expression of specific methyltransferases that utilize SAM-derived methyl groups and produce homocysteine. Although the supply of methyl groups from the folate-dependent 1-carbon pool appears to be diminished under diabetic conditions, the increased production of homocysteine is compensated for by stimulation of folate-independent remethylation and catabolism by transsulfuration, resulting in hypohomocysteinemia. Similar changes have been observed with glucocorticoid administration and in a growth hormone-deficient model, which can be prevented by insulin and growth hormone treatment, respectively. Taken together, these reports clearly indicate that hormonal regulation is a major factor in the metabolic control of folate, methyl groups, and homocysteine, thereby providing a potential link between the pathologies associated with these pathways and hormonal imbalance.

Introduction

Folate, methyl group, and homocysteine metabolism are important metabolic pathways required for optimal health. A number of nutritional, hormonal, and genetic factors may result in metabolic disruption of these interrelated pathways that is associated with various pathological conditions, including cardiovascular disease, cancer development, neurodegenerative diseases, and birth defects (1–4). Thus, a clear understanding of how these factors, hormones in particular, impact the metabolism of folate, methyl groups, and homocysteine is essential for optimizing health and minimizing adverse consequences.

Metabolic interrelations between folate, methyl groups, and homocysteine

Folate, methyl group, and homocysteine metabolism are inextricably linked processes (Fig. 1). S-adenosylmethionine (SAM) is the universal methyl group donor for a variety of methyltransferases, resulting in the methylation of substrates such as nucleic acids, lipids, and proteins (5). The transmethylation pathway is present in most mammalian tissues and SAM is generated via the activation of methionine by methionine adenosyltransferase (MAT) (6). All SAM-dependent methyltransferase reactions result in the production of S-adenosylhomocysteine (SAH), which can subsequently be converted to homocysteine by SAH hydrolase. Homocysteine may be remethylated back to methionine by either folate-dependent or folate-independent mechanisms. For folate-dependent remethylation, the B12-dependent enzyme methionine synthase (MS) utilizes a methyl group from 5-methyltetrahydrofolate (5-CH3-THF). Betaine-homocysteine methyltransferase (BHMT) catalyzes the folate-independent remethylation of homocysteine using betaine, a methyl group donor derived from choline oxidation. Alternatively, homocysteine can be catabolized through the transsulfuration pathway to cysteine, beginning with the irreversible conversion to cystathionine by cystathionine β-synthase (CBS). Cysteine can be further metabolized into other important biological compounds such as glutathione. Whereas SAM-dependent transmethylation occurs in nearly all tissues, the transsulfuration pathway and the remethylation of homocysteine by BHMT are tissue specific, existing primarily in the liver and kidney (7,8).

SAM-dependent transmethylation

Although there are many methyltransferases that utilize SAM as a substrate, 3 methyltransferases that play a major role in the generation of homocysteine and regulation of methyl group metabolism include guanidinoacetate methyltransferase (GAMT), phosphatidylethanolamine N-methyltransferase (PEMT), and glycine N-methyltransferase (GNMT). GAMT and PEMT are considered to be the largest consumers of methyl groups derived from SAM and are important in the production of creatine and phosphatidylcholine (PC), respectively. Historically, GAMT has been proposed to consume up to 70% of methyl groups derived from SAM. However, a recent review by Stead et al. (9) suggests that PEMT may be the primary consumer of methyl groups and have the greatest impact on homocysteine levels.

1 Abbreviations used: BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; 5-CH3-THF, 5-methyltetrahydrofolate; CTR, CTP; phosphocholine cytidylyltransferase-γ; GAMT, guanidinoacetate methyltransferase; GNMT, glycine N-methyltransferase; MAT, methionine adenosyltransferase; MS, methionine synthase; MHFR, 5,10-methylenetetrahydrofolate reductase; PC, phosphatidylcholine; PEMT, phosphatidylethanolamine N-methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; STZ, streptozotocin; ZDF, Zucker diabetic fatty.
is also tissue-specific, as it has been reported to exist in liver, methyl groups when the supply is excessive. GNMT expression when methyl group availability is compromised and disposes of dependent transmethylation reactions of biological importance system ensures that methyl groups are conserved for SAM-dependent transmethylation reactions (15). However, methyltransferases are inhibited by SAH, the product of all regulation of GNMT and methylation capacity of the cell. Most As a proposed regulator of methyl group supply and GNMT.

PEMT. PEMT catalyzes a series of reactions by which 3 methyl groups derived from SAM are donated to phosphatidylethanolamine, thereby producing PC and 3 molecules of homocysteine. PC is important for the synthesis of membranes, bile, and lipoproteins, as well as cell signaling. It is estimated that one-third of PC production is via the SAM-dependent PEMT pathway, whereas the remainder is synthesized via the CDP-choline pathway.

Recently, PEMT has been proposed as a regulator of homocysteine homoeostasis due to its prominent role in homocysteine production. The effect of hepatic PEMT regulation on plasma homocysteine has been investigated using a liver-specific CTP:phosphocholine cytidylyltransferase-α (CTα) knockout mouse (10). Because CTα-deficient mice lack the capability to synthesize PC via the CDP-choline pathway, methylation of phosphatidylethanolamine by PEMT was increased 100%. This increase in flux via PEMT resulted in higher plasma homocysteine concentrations despite elevated BHMT activity. Increased PEMT activity was also implicated as a primary determinant of homocysteine levels in a cell culture model in which PEMT activity was found to significantly correlate with plasma homocysteine concentrations (11). However, other studies have found PEMT activity was not associated with concomitant changes in homocysteine concentrations. PEMT expression was elevated by streptozotocin (STZ)-induced diabetes and in response to glucocorticoid administration (12–14). This upregulation of PEMT would be expected to have a profound impact on plasma homocysteine levels; however, it appears that homocysteine catabolism was enhanced in the early stages of type 1 diabetes, leading to hypohomocysteinemia.

GNMT. As a proposed regulator of methyl group supply and utilization, GNMT is the most abundant hepatic methylenetetrahydrofolate reductase and functions to regulate the SAM:SAH ratio (i.e. methylation capacity). Allosteric mechanisms play a key role in the regulation of GNMT and methylation capacity of the cell. Most methylenetetrahydrofolate reductases are inhibited by SAH, the product of all the SAM-dependent transmethylation reactions (15). However, GNMT is less sensitive to inhibition by SAH and is allosterically inhibited by 5-CH3-THF (16). Taken together, this regulatory system ensures that methyl groups are conserved for SAM-dependent transmethylation reactions of biological importance when methyl group availability is compromised and disposés of methyl groups when the supply is excessive. GNMT expression is also tissue-specific, as it has been reported to exist in liver, kidney, pancreas, and intestine (17).

Because of the importance of GNMT, it is vital to identify and understand the hormonal factors that may regulate its expression and/or function. Similar to PEMT, the activity and/or expression of GNMT has been reported to be upregulated in the STZ-induced (type I) diabetic rat, the Zucker (type II) diabetic fatty (ZDF) rat, and in response to administration of glucocorticoids (18–20). Moreover, treatment of diabetic rats with insulin has been shown to prevent upregulation of GNMT (K. M. Nieman and K. L. Schalinske, unpublished results). Growth hormone has also been reported to have an impact on GNMT expression (21). The Ames dwarf mouse is characterized by an increased lifespan and a lack of differentiation of portions of the pituitary, including the somatotropic cells, which produce growth hormone. GNMT activity and mRNA abundance were also increased in the Ames dwarf mouse (22,23); however, treatment of 3- or 12-mo-old Ames dwarf mice for 7 d with growth hormone significantly lowered GNMT activity. In rats, it is well known that thyroid status alters the activity of 5,10-methylenetetrahydrofolate reductase (MTHFR), which would be expected to result in an alteration of GNMT activity via allosteric regulation by 5-CH3-THF (24). In support of this, recent studies have found that triiodothyronine reversed retinoic acid-meditated elevations in GNMT activity but was without effect on abundance, suggesting post-translational regulation of GNMT in the hyperthyroid state (25).

Recent studies using knockout mouse models have revealed additional observations on both GNMT function and methyl group homeostasis. Using a newly developed gnmt-deficient mouse, Luka et al. (26) reported that the hepatic expression of GNMT was abolished, resulting in an ~100-fold increase in the SAM:SAH ratio in the homozygous mouse; however, hepatic methionine, SAM, and SAH concentrations were similar between gnmt−/− and wild-type mice. This supports the earlier observations of Wagner et al. (16) that GNMT does function in part to regulate transmethylation potential. The unique role GNMT may play in regulation of methyl group balance is further emphasized by the report that GNMT activity and expression is elevated in the brain of PEMT knockout mice, whereas the activity of all other methyltransferases examined were downregulated (27). GNMT was expressed at very low levels in the brain in wild-type mice; however, because PEMT is a major consumer of methyl groups, it was proposed that GNMT was upregulated in pemt−/− mice to dispose of the methyl groups that are not being consumed by PEMT.

Interestingly, GNMT expression is lost during hepatocarcinoma development, suggesting that it may be a biomarker of carcinogenesis and that abnormal regulation of methylation potential contributes to the pathogenesis of cancer (28). Further supporting this hypothesis, overexpression of GNMT has been associated with the downregulation of oncogenes (29).

SAM:SAH ratio. The ratio of SAM:SAH serves as an index of transmethylation potential. Transmethylation reactions have been shown to be compromised because of a decreased SAM:SAH ratio, particularly when SAH levels are elevated (30,31). It might be expected that the SAM:SAH ratio could be altered due to changes in activity of SAM-dependent methyltransferases, particularly GNMT and PEMT. Diabetics with nephropathy have a decreased lymphocytic SAM:SAH ratio and decreased transmethylation flux (32,33). Furthermore, in the brain of the PEMT knockout mouse, increased availability of SAM appears to drive hypermethylation of DNA and proteins (34). Transmethylation by PEMT and GNMT was increased in CTα knockout mice and ZDF rats, respectively (10,19). Accordingly, there appears to be
an increase in SAM production for these SAM-dependent transmethylations, as evidenced by increased MAT activity in both of these species. Thus, the relative production of SAM and SAH remains in balance and the SAM:SAH ratio is maintained.

A radiolabeled tracer study indicated that hepatic transmethylation flux was upregulated in the Ames dwarf mouse despite a decrease in the SAM:SAH ratio. This alteration in transmethylation flux was accompanied by increased MAT and GNMT activity and increased expression of MAT, GNMT, and SAH hydrolase mRNA levels (22, 23). Moreover, essential methyllylation processes, specifically DNA methylation, were not compromised. This suggests that regulation of methyltransferases by SAM and SAH concentrations can be overwhelmed by hormonal and other regulatory factors.

Remethylation

**BHMT.** Folate-independent homocysteine remethylation by BHMT is also proposed to have an impact on methyl group and homocysteine metabolism. BHMT utilizes a methyl group donated by betaine to remethylate homocysteine to methionine, which can then be reactivated to SAM. BHMT appears to play a regulatory role in homocysteine homeostasis. Hepatic BHMT activity and/or mRNA expression has been shown to be elevated in diabetic rats and by treatment of rat hepatoma cells with glucocorticoids (18, 19, 25). As previously discussed, multiple transmethylases (i.e., GNMT, PEMT) are upregulated under diabetic conditions, suggesting increased production of homocysteine. However, elevated BHMT function contributes to increased remethylation of homocysteine, resulting in the lowering of plasma homocysteine levels (35). Induction of BHMT by diabetes was prevented by administration of insulin, with concurrent partial restoration of the SAM:SAH ratio (35). This reversal by insulin appears to be due to decreased abundance and de novo transcription of the BHMT mRNA. BHMT function was also elevated in the Ames dwarf mouse, which, similar to the diabetic rat, exhibits upregulated transmethylation and low homocysteine levels (23). In CTα knockout mice, BHMT activity was increased although it was not sufficient to normalize plasma homocysteine levels (10).

Despite failure to return plasma homocysteine levels to normal in some models, the possibility of BHMT as a key modulator of plasma homocysteine levels is supported by the recent experiments of Collinson et al. (36). Mice receiving 6 injections of S-(δ-carboxybutyl)-DL-homocysteine, a specific inhibitor of BHMT, exhibited a 7-fold elevation in plasma homocysteine concentrations. The authors note that decreased CBS activity contributed to the increase, but most interestingly, BHMT protein expression was significantly elevated, which might be expected with the concurrent decrease in SAM, thus relieving any potential inhibition of BHMT transcription by SAM. Additional experiments demonstrated that a single injection of S-(δ-carboxybutyl)-DL-homocysteine given to mice had no effect on the activity of other enzymes involved in homocysteine metabolism, whereas BHMT activity decreased 90% and homocysteine levels rose significantly.

**MS and MTHFR.** The impact of hormonal balance on folate-dependent remethylation is somewhat unclear. Results from Wijekoon et al. (19) using ZDF rats at 11 wk of age showed no changes in either hepatic MS or MTHFR activity. Interestingly, there was a transient increase in MTHFR activity and decrease in MS activity during the prediabetic, insulin-resistant stage at 5 wk of age. In contrast, it has been reported that hepatic MS activity was decreased in STZ-induced diabetes (18). There is some evidence that these effects may also be tissue specific, based on a report by Jacobs et al. (37) in which the hepatic activities of MS and MTHFR were unchanged in the STZ-diabetic rat, whereas both renal MS and MTHFR activities were decreased. Insulin administration restored renal MS activity but failed to restore renal MTHFR activity. Taken together, the data suggest that remethylation by MS is not a primary determinant in maintenance of homocysteine homeostasis under diabetic conditions. However, a diabetic state appears to be characterized by a diminished ability to provide methyl groups from the folate-dependent 1-carbon pool.

Transsulfuration

**CBS and γ-cystathionase.** Transsulfuration results in the irreversible catabolism of homocysteine. The ability of CBS to regulate serum homocysteine levels was investigated by Wang et al. (38) using a transgenic mouse model where the human CBS gene was controlled by the metallothionein promoter. When given water supplemented with zinc, the CBS transgene was expressed, resulting in increased CBS activity in the liver and kidney and decreased serum homocysteine. Moreover, this induction of CBS was effective in lowering homocysteine levels even in mice fed a high methionine-low folate diet. This suggests that the upregulation of CBS and/or γ-cystathionase is an important factor in the determination of plasma homocysteine concentrations.

CBS and γ-cystathionase activities were elevated in the Ames dwarf mouse liver (22). The effect of increased activities of these enzymes has been further confirmed in the Ames dwarf mouse, which exhibits increased flux through the transsulfuration pathway in the liver, kidney, and brain, as well as lower plasma homocysteine concentrations (23). Hepatic CBS and γ-cystathionase activities were also increased in both STZ-diabetic and ZDF rats (18, 19). Treating the STZ-diabetic rat with insulin prevented alterations in both activity levels and plasma homocysteine (37). Hypohomocysteinemia in type 1 diabetic patients is thought to be explained, at least in part, by enhanced transsulfuration (39). However, plasma homocysteine levels in both type 1 and 2 diabetics increased with the severity of renal dysfunction (32). This hyperhomocysteinemia has recently been attributed to suppressed homocysteine clearance, as indicated by kinetic studies in type 2 diabetics with nephropathy (33). Thus, differences in homocysteine disposal between diabetics with and without renal dysfunction might provide a mechanistic explanation for the transition from hypo- to hyperhomocysteinemia in diabetes.

In summary, folate, methyl group, and homocysteine metabolism have a wide range of functions, including the synthesis and metabolism of numerous biological compounds, epigenetic regulation of gene expression, and maintenance of redox status. Due to the association of homeostatic perturbation of these metabolic systems with numerous pathologies, a greater understanding of factors, such as hormones, involved in the regulation of these pathways is important for the subsequent development of strategies for preventing or minimizing metabolic abnormalities. Although the impact of individual enzymes has been evaluated using specific inhibitors and knockout models, future research using in vivo flux studies (41) and mathematical modeling (42) may help to further clarify the relative contribution of each pathway toward methyl group and homocysteine homeostasis, thereby identifying the most promising targets for intervention. Moreover, the impact of marginal nutritional status (i.e., vitamin deficiencies) and the increased methyl group needs for specific populations, as well as the role of genetic polymorphisms, also need to be adequately addressed.

Methyl group and homocysteine metabolism 313
Literature Cited


