Hepatic Phosphatidylethanolamine N-Methyltransferase, Unexpected Roles in Animal Biochemistry and Physiology*

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In 1961, when Bremer and Greenberg (1) characterized the methylation reactions that convert phosphatidylethanolamine (PE) to phosphatidylcholine (PC), it was unlikely that they would have predicted the physiological impact of this biosynthetic conversion. Similarly, when Ridgway and Vance (2) succeeded in purification of the hepatic enzyme PE N-methyltransferase (PEMT), we considered this enzyme to be important only for making PC in the liver. Subsequent research has now clearly shown that PEMT has critical roles that are additional to the important role of supplying PC for the liver. This review will summarize research on PEMT and its impact on animal biochemistry and physiology.

Biochemical Characterization of PEMT

PEMT is localized to the endoplasmic reticulum (ER) and the mitochondrial associated membranes (a subfraction of the ER) of the liver (3, 4). PEMT spans the membrane with four transmembrane sequences (5). PEMT catalyzes all three methylation reactions in the conversion of PE to PC (Fig. 1). The methyl donor is S-adenosylmethionine (AdoMet). The binding site on PEMT for AdoMet has been localized to the cytosolic surface of the ER (6). Residues critical for binding of PE to PEMT have not been identified.

PEMT and Its Evolutionary Significance

PEMT is essentially a hepatocyte-specific enzyme (7). Also present in the liver is the CDP-choline pathway for PC biosynthesis (Fig. 2) first described by Kennedy and Weiss (8, 9). This pathway is essential because cells that lack the rate-limiting enzyme CTP:phosphocholine cytidylyltransferase (CT) do not survive (10) and undergo apoptosis (11). Similarly, mice with an inactive gene (Pcyt1a) that encodes CTα die early during embryogenesis (12). Another gene (Pcyt1b) encodes the isoform CTβ (13). Deletion of the part of this gene that encodes CTβ2 is not embryonically lethal but does result in gonadal dysfunction (14). CTα accounts for ~85% of the CT activity in the liver (15). Because the liver has ample PC biosynthetic capacity via the CDP-choline pathway, why did PEMT survive in evolution? In the 1990s, the obvious and unambiguous approach to answer this question was to construct mice that lacked PEMT.

The PEMT gene is localized to chromosome 11 and spans 25 kb with seven exons and six introns (16). A targeting vector was constructed and introduced into mice, yielding homozygous PEMT gene-disrupted mice (17). All PEMT activity was eliminated in the Pemt−/− mice, yet the levels of hepatic PC and PE were minimally affected. Pemt−/− mice displayed no abnormal phenotype, normal hepatocyte morphology, normal plasma lipid levels, and no differences in bile composition. Thus, it seemed that PEMT was dispensable. This result was not unexpected because the CDP-choline pathway remained in the livers of Pemt−/− mice. To attenuate PC biosynthesis via the CDP-choline pathway, we fed the mice a choline-deficient (CD) diet. After 3 days, the CD-Pemt−/− mice exhibited end-stage liver failure (18). The concentration of PC in the liver decreased by 50% compared with that in CD-Pemt+/+ mice, and the concentrations of plasma triacylglycerols (TG) and cholesterol were decreased by ~90%. These data suggest that PEMT has survived in evolution to provide PC and/or choline (derived from PC via catabolism) at times when choline is insufficient in the diet (19).

PEMT, Steatosis, and Steatohepatitis

The CD-Pemt−/− mice also provided a unique model for the induction of steatohepatitis. Previously, a model in which both choline and methionine were removed from the diet had been used to study development of steatosis and steatohepatitis (20). The limitation of choline and methionine deficiency is that methionine has many critical roles other than in PC biosynthesis, such as protein biosynthesis and the provision of carbons for the >50 additional transmethylation reactions (21). The elimination of PEMT would directly affect only the conversion of PE to PC.

The dramatic decrease in hepatic PC in CD-Pemt−/− mice (19) caused us to consider how this might occur. The liver secretes lipoproteins and bile that utilize large amounts of PC. The biliary PC that is secreted from the liver is ~23 mg/day for a 20-g mouse (22). The total amount of PC in the liver would be ~20 mg. Thus, each day the mouse liver secretes into the bile the equivalent of its entire pool of PC. When PC replenishment is compromised, as in the CD-Pemt−/− mice, the rapid decrease in hepatic PC is not unexpected. PC biosynthesis is also
required for normal very low density lipoprotein (VLDL) secretion (23). In an attempt to estimate the relative importance of PEMT-derived PC to VLDL secretion, we traced the fate of [3H-methyl]methionine after injection into the tail vein of a mouse. After 5 h, 5–7% of the radioactivity was recovered in plasma PC compared with 2–6.5% recovered in bile PC (24). Thus, utilization of PEMT-derived PC for lipoprotein secretion is highly significant.

In 1993, Borst and co-workers (25) reported the disruption of the mouse gene encoding a flippase (MDR2 [multiple drug-resistant protein 2], ABCB4) responsible for transfer of PC into bile. Thus, we bred the Pemt−/− mice with the Mdr2−/− mice and obtained a strain that lacked both functional genes. When compared with choline-supplemented mice, some of the mice developed steatosis and lived for at least 90 days (26). Surprisingly, by 21 days after the CD diet was initiated, the PC concentration in the livers of CD-fed mice was not due simply to this decrease in PC, as PC was decreased in both mouse models, one of which survived (Pemt−/−/Mdr2−/−), whereas the other (Pemt−/−) did not.

One difference that became apparent was that the levels of hepatic PE did not decrease in the Pemt−/− mice fed the CD diet, whereas the levels of PE did decrease in the livers of Pemt−/−/Mdr2−/− mice (27).

FIGURE 1. Pathway for conversion of PE to PC and AdoHcy catalyzed by PEMT. Pmme, phosphatidylmonomethylethanolamine; PDME, phosphatidylidi- methylethanolamine.

FIGURE 2. The CDP-choline (Kennedy) pathway for PC biosynthesis. Choline kinase (CK) is encoded by two genes that produce the α- and β-isomers. CT is also encoded by two genes that yield the α- and β-isomers. DG, diacylglycerol.

PC is a cylindrically shaped molecule, whereas PE usually has an inverted cone shape (28). We reasoned that if the amount of PC, but not PE, decreased in the livers of Pemt−/− mice, some of the PE might replace the PC on the cell surface (27). This could disrupt the packing of the lipids in the membrane that would result in increased permeability. To test this hypothesis, we used a biotinylated peptide that specifically binds PE (29).

Hepatocytes from Pemt−/− mice fed the CD diet for 2 days were incubated with the biotinylated PE-specific binding peptide Ro98-019 at 4 °C for 30 min and subsequently with fluorescence-conjugated streptavidin for 30 min. This figure was adapted from Ref. 28 with permission. CS, choline-supplemented.

The CDP-choline (Kennedy) pathway for PC biosynthesis. Choline kinase (CK) is encoded by two genes that produce the α- and β-isomers. CT is also encoded by two genes that yield the α- and β-isomers. DG, diacylglycerol.

Hepatocytes from Pemt−/− and Pemt−/−/Mdr2−/− mice were incubated with the biotinylated PE-specific binding peptide Ro98-019 at 4 °C for 30 min and subsequently with fluorescence-conjugated streptavidin for 30 min. This figure was adapted from Ref. 28 with permission. CS, choline-supplemented.

FIGURE 3. PE exposure on the cell surface. Hepatocytes from Pemt−/− and Pemt−/−/Mdr2−/− mice were incubated with the biotinylated PE-specific binding peptide Ro98-019 at 4 °C for 30 min and subsequently with fluorescence-conjugated streptavidin for 30 min. This figure was adapted from Ref. 28 with permission. CS, choline-supplemented.
the RNA interference vector was injected into the tail veins of CD-Pemt−/− mice, steatohepatitis was attenuated; the PC/PE ratio increased; and there was a 75% reduction in plasma alamine aminotransferase.

The results provide strong support for the hypothesis that CD-Pemt−/− mice develop steatohepatitis because of increased permeability of the cell membrane that occurs due to a decreased ratio of PC to PE. Studies on the development of nonalcoholic steatohepatitis in humans have shown that patients with this disease have a significant reduction (p = 0.001) in the PC/PE ratio in hepatic biopsies (27).

A correlation between PEMT and hepatic steatosis has also been observed in a strain of mice with impaired biosynthesis of PC via both pathways (30). This strain, RCS8, showed decreased hyperglycemia and hyperinsulinemia when treated with the diabetic drug rosiglitazone. Treatment of RCS8 mice with rosiglitazone further inhibited PEMT as well as enzymes in the choline pathway with enhanced hepatosteatosis. Rosiglitazone effects on PEMT have since been shown to be dependent on peroxisome proliferator-activated receptor-γ action (31).

The regulation of PEMT appears to be complicated because induction of type I diabetes by streptozotocin increases PEMT expression (32). Treatment of diabetic rats with insulin prevents the increase in PEMT activity. Thus, there might be a direct relationship between PEMT activity and insulin action. To test this hypothesis, we evaluated the susceptibility of Pemt−/− mice to diet-induced obesity. Whereas Pemt+/+ mice showed weight gain, plasma hyperlipidemia, and insulin resistance, Pemt−/− mice were strikingly protected against these changes.5

**PEMT and Lipoprotein Homeostasis**

PC biosynthesis is required for normal secretion of VLDL into the medium of primary rat hepatocytes (23) as demonstrated by removal of both choline and methionine from the culture medium. Choline cannot be replaced by dimethylthanolamine, monomethylethanolamine, or ethanolamine (33). However, if only choline, but not methionine, is removed from the culture medium, VLDL secretion is not diminished (34). The specific requirement for choline (in the absence of methionine) in mice is further supported by the inability of dimethylethanolamine (35) or isopropanolamine (36) to substitute for choline in the survival of Pemt−/− mice.

With the generation of Pemt−/− mice, the role of PEMT in the secretion of VLDL was examined. Secretion of TG was decreased by 50% in cultured hepatocytes derived from male Pemt−/− compared with Pemt+/+ mice (37). In agreement, there was a 70% inhibition of secretion of apoB100, but not apoB48. Complementary experiments in which a cDNA for PEMT was transfected into McArdle rat hepatoma cells (which lack PEMT) showed a stimulation of TG secretion (37). Studies in mice showed that male, but not female, Pemt−/− mice had lower levels of TG, apoB100, and apoB48 in plasma (38). Measurement of secretion of apoB100 in the livers of male Pemt−/− mice showed a decrease compared with Pemt+/+ mice. The reason for this sexual dimorphism is not evident (24).

Because the PEMT pathway is necessary, at least in male mice, for normal VLDL secretion, we wondered if the CT pathway for PC biosynthesis is also required. We generated mice that specifically lacked CTα in liver hepatocytes (15). Compared with wild-type mice, these mice had lower levels of serum TG, PC, and apoB100 and exhibited a decreased secretion of VLDL into plasma independent of gender. Interestingly, there was an almost 2-fold increase in the activity of PEMT that did not compensate for the loss of CTα activity (15). Thus, both pathways for PC biosynthesis are independently required for VLDL secretion.

Because the levels of VLDL were decreased in the plasma of PEMT mice, we were curious if Pemt−/− mice might be protected against atherosclerosis. Because mice do not readily develop atherosclerosis, we bred the Pemt−/− mice with atherosclerosis-prone mice that lacked the low density lipoprotein receptor and fed the mice a high fat/high cholesterol diet for 16 weeks. There was a striking 85% decrease in atherosclerosis in the Pemt−/−/low density lipoprotein receptor knock-out mice compared with the Pemt+/+/low density lipoprotein receptor knock-out mice.6

The major lipoprotein in mice is high density lipoprotein (HDL). The levels of cholesterol and PC of HDL were also decreased in both genders of Pemt−/− mice (38). Similarly, the levels of HDL-associated lipids were decreased by ~50% in the liver-specific CTα knock-out mice (15). One possible mechanism for why PEMT deficiency results in a decrease in HDL lipids is that efflux of lipids to plasma apoA-I was impaired. However, this was not altered in hepatocytes from Pemt−/− mice.6 Rather, there was increased expression of a receptor for HDL, scavenger receptor class B, type I, and this increased the removal of HDL lipids from the medium of hepatocytes.6

HDL appears to have an important role in hepatic PC homeostasis. When Pemt−/− mice are fed a CD diet, male mice show signs of liver damage within 24 h, whereas female mice do not (39). By 2 days of choline deficiency, liver damage is evident in both male and female Pemt−/− mice. A potential explanation for the gender difference is that in the female mice, there is an increase in plasma HDL during the first day of choline deficiency, and some of the HDL-associated PC is delivered to the liver (39). HDL is implicated in “reverse cholesterol transport,” in which cholesterol from extrahepatic tissues is delivered to the liver, where it can be converted into bile acids and excreted. From the data available to date, it seems that HDL also delivers PC to the liver and participates in hepatic PC homeostasis.

**PEMT and Homocysteine**

The other product of the PEMT reactions is S-adenosylhomocysteine (AdoHcy) (Fig. 1). AdoHcy is hydrolyzed to adenosine and homocysteine (Hcy) by the action of AdoHcy hydrolase. Hyperhomocysteinemia is an independent risk factor for the development of cardiovascular diseases such as atherosclerosis and stroke (40) and myocardial infarction (41). Elevated plasma Hcy has also been correlated with increased risk of Alzheimer disease (42), dementia (43), and bone fractures (44).

5 Y. Zhao and D. E. Vance, unpublished data.

6 J. Robichaud and D. E. Vance, unpublished data.
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It is clear that biological methylation and Hcy metabolism are closely related. At present, there are ~50–100 known mammalian methyltransferases (21). This number might be an underestimate because a bioinformatics analysis has shown that ~0.6–1.6% of open reading frames in microbial, plant, and animal genomes might encode methyltransferases (21). Because PEMT accounts for the formation of 30% PC made in the liver and 3 AdoHcy molecules are synthesized for every PC molecule generated, we hypothesized that PEMT might contribute significantly to plasma Hcy. Remarkably, deletion of the PEMT gene in mice resulted in a 1.7-fold increase in the liver-specific PEMT reaction (48, 58). Hence, to satisfy PC production, the liver would need to supply one-third of this PC (1.65 mmol) is probably generated by the PEMT reaction (48, 58). Hence, to satisfy PC production, the PEMT reaction might consume 5 mmol of AdoMet/day, which is 2-fold higher than the original estimate for PEMT-dependent AdoMet consumption. Clearly, PEMT is a major player in AdoMet consumption and Hcy production in addition to its role in PC biosynthesis and function.

PEMT and Gene Expression

The PEMT gene was first characterized in 1996 (16). Recently, the first report on alteration of the Pemt gene was published. Zeisel and co-workers (59) demonstrated an induction of PEMT by incubation of primary mouse or human hepatocytes with 17β-estradiol for 24 h. There was an increase in mRNA, protein, and PEMT activity in a dose-dependent manner. An estrogen response element was found. These results provide the first indication as to why premenopausal women are more protected against choline deficiency than are men or postmenopausal women.

A polymorphism in the human PEMT gene is associated with nonalcoholic fatty liver disease (60). Controls and patients with liver disease were evaluated for a Val-to-Met substitution at residue 175 of the PEMT human protein. Met/Met at position 175 occurred in 68% of the patients with liver disease and in 41% of control subjects. Dong et al. (61) found a similar association of the V175M polymorphism and liver disease. In apparent contrast, no association was found between the V175M polymorphism and steatosis in the Dallas Heart Study (62). The patients examined in the Dallas Heart Study included multiple races (63), whereas in the study by Song et al. (60), most subjects were Caucasian. When only Caucasian subjects were compared, there was a similar relationship between the V175M polymorphism and liver disease (63). Because not all of the subjects had steatosis, the polymorphism does not necessarily lead to fatty liver. Rather, the polymorphism may predispose people to fatty liver when TG synthesis is enhanced (63).

When the wild-type versus V175M PEMT was expressed in McArdle hepatoma cells, there was ~40% less activity (60). It was not possible to measure PEMT activity in the patients. Whether or not the level of PEMT protein determines the rate of the PEMT reaction has not been clearly determined in rodents or humans.

Conclusion

We now know that PEMT has important roles that go beyond simply providing PC in the liver. PEMT has a role in maintaining a correct ratio of PC to PE in the liver particularly when the supply of dietary choline is restricted. Decreased PEMT activity can lead to steatosis and steatohepatitis under certain conditions. PEMT has a special role in VLDL secretion that cannot be substituted by the choline pathway. PEMT has an important function in regulating the supply of plasma Hcy. The possible role of PEMT in insulin sensitivity and atherosclerosis is intriguing.

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