Properties of a partially purified phosphodimethylethanolamine methyltransferase from rat brain cytosol

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The presence of cytosolic *S*-adenosylmethionine-dependent *N*-methyltransferase(s) activity(ies) capable of converting phosphoethanolamine into phosphocholine has been recently demonstrated in the rat brain. At least two enzymes are involved in the methylation of phosphoethanolamine to phosphocholine and these are separable by ammonium sulphate fractionation. The enzyme catalysing the last step of this methylation process is present in the 50–80 % ammonium sulphate fraction. A 220-fold purified enzyme has been obtained with sequentially employed Q-Sepharose fast flow and octyl-Sepharose CL4B column chromatography. The maximum enzyme activity was at pH 9.5. The K_m values for *S*-adenosylmethionine, the methyl donor, and phosphodimethylethanolamine, the methyl acceptor, were 125 μ M and 750 μ M respectively. This phosphodimethylethanolamine *N*-methyltransferase was found to be calcium-dependent, with a 4-fold increase in activity at 0.5 mM- CaCl_2 . *S*-Adenosylhomocysteine at 0.5 mM caused 100 % inhibition of the activity. The effects of various structural analogues on the phosphodimethylethanolamine *N*-methyltransferase activity were also investigated and these results suggest that the enzyme is specific to the substrate. These results provide evidence for the existence of the pathway for the methylation of phosphoethanolamine to phosphocholine in rat brain cytosol.

INTRODUCTION

Choline (Cho) is the polar head group characterizing the Cho-containing phospholipids, which are components of all mammalian membranes. In brain tissue Cho also serves as a precursor for acetylcholine (AcCho) synthesis by the cholinergic neurons. The major source of Cho for the brain is its uptake from the blood circulation [1–5]. However, the brain is also capable of producing free Cho through the catabolism of membrane phosphatidylcholine (PtdCho) [6–8]. PtdCho is synthesized primarily by a pathway involving phosphorylated and cytidine nucleotide intermediates [9], but can also be synthesized by the sequential methylation of phosphatidylethanolamine (PtdEtn) [10–12], catalysed by PtdEtn *N*-methyltransferase (N-MT; EC 2.1.1.17) [13,14] and by the base-exchange reaction [15–17].

These pathways contribute to the maintenance of the PtdCho content of the brain at a relatively constant level, and it has been shown that a co-ordinate relationship may exist between them. Indeed, it has been reported that the inhibition by 3-deazaadenosine (3-DZA) of the methylation of PtdEtn to PtdCho led to an increase in PtdCho biosynthesis by the *de novo* pathway in hepatocytes [18,19]. A 2-fold increase in the PtdEtn N-MT activity of a rat hepatoma cell line was observed when the cells were grown in the absence of Cho compared with the activity in cells grown in the presence of Cho [20]. This observation would suggest that when the uptake of Cho is impaired, or unavailable, thus restricting PtdCho synthesis by the *de novo* pathway, cells compensate for this decrease by increasing the PtdEtn N-MT pathway. However, in brain tissue only 5 % of total PtdCho synthesis can be provided by the methylation of PtdEtn [21].

Another mechanism of PtdCho production might be through the formation of phosphocholine (PCho) by the sequential methylation of phosphoethanolamine (PEtn). We have pre-

viously reported the existence of such a pathway both in primary neuronal cultures and in the intact rat brain [22]. These studies suggested that PEtn was a preferred substrate for methylation(s) compared with either free Etn or PtdEtn. The potential contribution of this methylation pathway in providing Cho and/or Cho-containing compounds may play a part in the maintenance of the Cho steady-state concentration, and therefore in the regulation of PtdCho biosynthesis. Moreover, in investigations utilizing pharmacological agents, it was observed that the methyltransferase(s) responsible for the conversion of PEtn into PCho was (were) different from that (those) for the conversion of PtdEtn to PtdCho [23]. We have demonstrated the existence of a cytosolic enzyme system present in rat brain which converts PEtn into PCho by sequential methylation [24]. These preliminary observations prompted us to further investigate this novel methylation pathway and to attempt to purify the PEtn N-MT. We now describe the partial purification of phosphodimethylethanolamine (PMe_2Etn) N-MT from rat brain cytosol; this enzyme catalyses the final step in the methylation pathway beginning with PEtn and yielding PCho.

MATERIALS AND METHODS

Materials

$\text{S-}[^3\text{H}]\text{Adenosylmethionine}$ ($^3\text{HAdomet}$) was purchased from Amersham Corp. Unlabelled Adomet and EDTA were from Boehringer Mannheim Canada. Dowex 50W $\times$ 8 (H^+ form), 3-(cyclohexylamino)-1-propanesulphonic acid (CAPS), 3-(cyclohexylamino)-2-hydroxy-1-propanesulphonic acid (CAPSO), Bicine, Hepes, phenylmethanesulphonyl fluoride (PMSF), PEtn, PCho, noradrenaline, adrenaline, catechol, 2-hydroxyethylhydrazine, β -phenylethylamine, nicotinamide, choline, *S*-adenosylhomocysteine (AdoHcy), L-homocysteine thiolactone, poly-

Abbreviations used: Adomet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; AcCho, acetylcholine; CAPS, 3-(cyclohexylamino)-1-propanesulphonic acid; Cho, choline; 3-DZA, 3-deazaadenosine; Etn, ethanolamine; N-MT, *N*-methyltransferase; PEG, poly(ethylene glycol); PMSF, phenylmethanesulphonyl fluoride; PEtn, phosphoethanolamine; PCho, phosphocholine; PMe_2Etn , phosphodimethylethanolamine; PMeEtn , phosphomonomethylethanolamine; PtdEtn, phosphatidylethanolamine; PtdCho, phosphatidylcholine.

(ethylene glycol) (PEG), sucrose and BSA, were obtained from Sigma, St. Louis, MO, U.S.A. Ammonium sulphate was purchased from ICN Biomedicals Inc., Costa Mesa, OH, U.S.A. Q-Sepharose fast flow and octyl-Sepharose gels were supplied by Pharmacia-LKB Biotechnology. Scintiverse II was supplied locally by Fisher Scientific. 2-Aminoethylphosphonic acid, diethylamine hydrochloride, 2-aminoethanethiol hydrochloride, 2-dimethylaminoethanethiol hydrochloride, ethanolamine, monomethylethanolamine, dimethylethanolamine, *N*-methylphenethylamine and *NN*-dimethylphenethylamine were purchased from Aldrich, Milwaukee, WI, U.S.A. 2-Amino-3-phosphonopropionic acid was purchased from Research Biochemicals. *N*-Phenylethanolamine was from Fluka, and 3-DZA was from Southern Research Institute, Birmingham, AL, U.S.A. The Coomassie Blue protein assay reagent was purchased from Pierce Chemical Co.

Phosphomonomethylethanolamine (PMeEtn) and PMe₂Etn were chemically synthesized according to the method of Weisburger & Schneider [25] with a slight modification. Briefly, an equimolar mixture of H₃PO₄ and monomethylethanolamine (or dimethylethanolamine) was heated overnight at 170 °C. The sample was allowed to cool, diluted 5-fold with water, the pH adjusted to 6 and the sample loaded on to a Dowex 50W × 8 H⁺ column. Under these conditions the unreacted free base was retained on the column and the phosphobase was recovered in the water effluent. This effluent was evaporated to dryness and dissolved in water. Samples of the solution containing these products were heated at 110 °C in 6 M-HCl for 18 h and separate aliquots of the hydrolysate were used to quantify the phosphorus content by the procedure of Bartlett [26] and to quantify the amino alcohol with choline oxidase employing the individual amino alcohol as standard [27,28]. The molar ratios of phosphorus and amino alcohol for both PMeEtn and PMe₂Etn were approximately 1.0. T.l.c. analysis of the products was performed using butanol/methanol/HCl/water (10:10:1:1, by vol.) as solvent, and the individual spots were located by exposure to iodine vapour. The *R_f* values were 0.67 for PEtn, 0.57 for PMeEtn, 0.42 for PMe₂Etn and 0.28 for PCho. The pH of the phosphobase solution to be employed as substrate was adjusted to 9.5 before use. All other materials were of reagent grade.

Purification of PMe₂Etn N-MT

Preparation of the cytosolic fraction. Male Sprague-Dawley rats (75–90 g) were killed and their brain tissue was homogenized in 0.32 M-sucrose containing 10 mM-CAPS and 100 μM-PMSF at pH 9.5 (10:1, v/wt). The homogenate was centrifuged at 20000 *g* for 20 min and the resulting supernatant was centrifuged at 105000 *g* for 60 min. The supernatant (S3) was removed and dialysed overnight against the homogenizing buffer.

Ammonium sulphate precipitation. The dialysed S3 fraction was precipitated stepwise with increasing concentrations of ammonium sulphate of 0–30%, 30–40%, 40–50% and 50–80%. The individual precipitates were suspended in 10 mM-CAPS buffer containing 100 μM-PMSF, pH 9.5, and dialysed overnight against the same buffer.

Q-Sepharose fast flow chromatography. The 50–80% ammonium sulphate precipitate containing the bulk of the PMe₂Etn N-MT activity was applied to a Q-Sepharose fast flow anion-exchange column (10 ml bed volume) previously equilibrated with 10 mM-CAPS containing 100 μM-PMSF, pH 9.5. The column was first washed with 50 ml of the equilibrating buffer and then eluted with 200 ml of a linear gradient of NaCl from 0 to 0.6 M at a flow rate of 20 ml/h and individual 2 ml fractions were collected. The fractions that possessed the PMe₂Etn N-MT activity were pooled, dialysed against 10 mM-CAPS containing 100 μM-PMSF, pH 9.5, and concentrated using PEG.

Octyl-Sepharose CL4B chromatography. The pooled sample from the Q-Sepharose column was loaded on to an octyl-Sepharose CL4B hydrophobic column of 2 ml bed volume previously equilibrated with 10 mM-CAPS containing 100 μM-PMSF and 0.6 M-NaCl, pH 9.5. The column was washed with 10 ml of the starting buffer and eluted with 40 ml of a linear reverse gradient of NaCl from 0.6 to 0 M, followed by 50% ethylene glycol in the buffer at a flow rate of 20 ml/h; 1 ml fractions were collected. The fractions containing the PMe₂Etn N-MT activity were pooled, dialysed against 10 mM-CAPS containing 100 μM-PMSF, pH 9.5, and concentrated with PEG.

Determination of PMe₂Etn N-MT activity

To 24 μl of an incubation solution containing 1 mM-PM₂Etn, 0.5 mM-CaCl₂, 66.67 mM-CAPS buffer, pH 9.5, and 250 μM-[³H]Adomet (specific radioactivity 12500 d.p.m./nmol) was added 36 μl of the enzyme preparation. Controls were prepared with heat-inactivated enzyme samples. After 60 min of incubation at 37 °C, the reaction was stopped by the addition of 20 μl of cold 1 M-HClO₄. The samples remained on ice for 10 min and were then centrifuged at 5000 rev./min for 10 min. To the resulting supernatant was added 20 μl of 1.2 M cold KOH, and after 10 min on ice the sample was centrifuged again at 5000 rev./min for 10 min. An aliquot of the supernatant was diluted 5-fold with water and applied to a Dowex 50W × 8 H⁺ form with a 0.5 ml bed volume previously washed with 1 M-HCl and then washed with water until neutral. After the application of the sample, the column was washed with 1 bed vol. of water and with another 10 ml of water. An aliquot of the water effluent was added to 10 ml of Scintiverse II and the radioactivity was determined.

The incubation conditions and the assays for PEtn N-MT and PMeEtn N-MT were similar to that described for PMe₂Etn N-MT.

Other methods

The protein concentrations were determined by the method of Lowry *et al.* [29], except for the column fractions where the Coomassie Blue protein assay procedure was used [30]. The *K_m* and *V_{max}* values were determined according to the Lineweaver-Burk procedure. The noradrenaline N-MT assay was performed according to a previously described method [31].

RESULTS

Ammonium sulphate precipitation

As shown in Table 1, there are at least two enzymes involved in the methylation of PEtn to PCho, and these are separable by ammonium sulphate fractionation. The methyltransferase(s) responsible for the conversion of PEtn to PMeEtn, and of PMeEtn to PMe₂Etn, is (are) recovered in the 0–40% ammonium sulphate precipitate. The enzyme responsible for converting PMe₂Etn to PCho is recovered principally in the 50–80% ammonium sulphate precipitate, with only 15% found in the 0–40% ammonium sulphate fractions.

Q-Sepharose fast flow column

The protein elution profile and the enzyme activities of the Q-Sepharose column are shown in Fig. 1. The peak of PMe₂Etn N-MT activity appears at approx. 0.3 M-NaCl. The contents of tubes 50–65 containing the activity were pooled, dialysed overnight, concentrated and found to have a specific activity of about 0.25 nmol/min per mg of protein.

Octyl-Sepharose CL4B column

The pooled sample from the Q-Sepharose column was loaded

Table 1. Distribution of phosphobase N-MT activities in various ammonium sulphate fractionations

The cytosol prepared from rat brain homogenate was subjected to ammonium sulphate fractionation and assayed with *PEtn*, *PMeEtn* or *PMe₂Etn* as substrate as described in the Materials and methods section. Values are the average of four separate experiments. Each assay was performed in duplicate. WH, whole homogenate; S3, cytosol.

Fraction	Protein (mg)	Specific activity (pmol/mg per min)			Total activity (pmol/min)			Recovery (%)		
		<i>PEtn</i>	<i>PMeEtn</i>	<i>PMe₂Etn</i>	<i>PEtn</i>	<i>PMeEtn</i>	<i>PMe₂Etn</i>	<i>PEtn</i>	<i>PMeEtn</i>	<i>PMe₂Etn</i>
WH	1527	5.0	4.8	4.7	7772.4	7360.1	7299.0	100	100	100
S3	560	11.5	12.7	12.9	6473.6	7117.6	7274.4	83.2	96.7	99.6
0–30 %-(NH ₄) ₂ SO ₄	124	14.2	38.4	4.0	1767.0	4769.0	498.4	22.7	64.7	6.8
30–40 %-(NH ₄) ₂ SO ₄	96	42.9	22.9	5.2	4124.1	2205.1	500.1	53.0	29.9	6.8
40–50 %-(NH ₄) ₂ SO ₄	25	0	0	0	0	0	0	0	0	0
50–80 %-(NH ₄) ₂ SO ₄	125	0	0	37.8	0	0	4726.2	0	0	64.7

Fig. 1. Q-Sepharose fast flow column chromatography of *PMe₂Etn* N-MT activity

The 50–80%–ammonium sulphate fraction was applied to a Q-Sepharose fast flow column as described in the Materials and methods section. ○, Protein concentration was determined by the Coomassie Blue method; ●, enzyme activity was measured in the presence of 1 mM-*PMe₂Etn*; —, NaCl gradient.

on to an octyl-Sepharose column, and the elution profile is shown in Fig. 2. There is a single peak of *PMe₂Etn* N-MT activity which is eluted with 50% ethylene glycol in the buffer. The contents of tubes 95–105 were pooled, dialysed overnight and concentrated; the specific activity was determined to be 1 nmol/min per mg of protein.

Purification of *PM₂Etn* N-MT

Purification of the *PMe₂Etn* N-MT from rat brain homogenate is summarized in Table 2. A 220-fold purified enzyme with a specific activity of 1 nmol/min per mg of protein was obtained from the octyl-Sepharose column.

General properties

pH curve. *PMe₂Etn* N-MT activity was measured as a function of pH and it was found that the optimum was at pH 9.5. At neutral pH the activity was almost undetectable, and it was decreased by 80% at pH 11 (results not shown).

Effect of bivalent cations. For these studies the enzyme sample had been dialysed against a solution containing 10 mM-CAPS, 5 mM-EDTA and 100 μM-PMSF, pH 9.5, overnight, and then dialysed against the same solution without EDTA overnight prior to investigating the effect of cations. The *PMe₂Etn* N-MT activity was increased 4-fold by the presence of 0.5 mM- CaCl_2 in the incubation mixture, compared with the control incubated in the absence of calcium. In the presence of 0.5 mM- MgCl_2 there

Fig. 2. Octyl-Sepharose CL4B chromatography of *PMe₂Etn* N-MT

The pooled fractions from the Q-Sepharose fast flow column were applied to an octyl-Sepharose CL4B column as described in the Materials and methods section. ○, Protein concentration was determined by the Coomassie Blue method; ●, enzyme activity was measured in the presence of 1 mM-*PMe₂Etn*; —, NaCl reverse gradient.

Table 2. Purification of *PMe₂Etn* N-MT from rat brain cytosol

Details are provided in the text.

Fraction	Protein (mg)	Specific activity (pmol/min per mg)	Total activity (pmol/min)	Purification (fold)
WH	1527	4.7	7299.0	1.0
S3	560	12.9	7274.4	2.7
50–80 %-(NH ₄) ₂ SO ₄	125	37.8	4726.2	7.9
Q-Sepharose pool	8	218.4	1747.5	45.6
Octyl-Sepharose pool	1.3	1051.9	1420.0	220.0

was a 1.5-fold stimulation of activity. In contrast, ZnCl_2 at the same concentration caused a slight inhibition and MnCl_2 dramatically decreased the activity by 90% (results not shown).

Affinity for Adomet and *PMe₂Etn*. When the concentration of *PMe₂Etn* was held constant and the concentration of Adomet was varied, the K_m for Adomet, the methyl donor, was found to be 125 μM and the V_{max} was 1.33 nmol/min per mg of protein (Fig. 3). In a similar manner, the K_m for *PMe₂Etn*, the methyl

Fig. 3. Affinity of PMe_2Etn N-MT for Adomet

PMe_2Etn N-MT was assayed as described in the Materials and methods section in the presence of various Adomet concentrations. The data are expressed as a double-reciprocal plot ($1/v$ versus $1/[S]$). The inset displays the velocity as a function of Adomet concentration.

acceptor, was found to be $750 \mu\text{M}$, and the V_{max} was $1.25 \text{ nmol/min per mg of protein}$ (Fig. 4).

Product identification. A single spot was found on autoradiogram prepared by t.l.c. of the Dowex 50 H^+ effluent; this corresponded to the PCho standard.

Effect of various analogues. The effects of a variety of structurally related compounds upon the PMe_2Etn N-MT activity were examined. The results showed that the phosphobases PEtn , PMeEtn and PCho , the free bases Etn , MeEtn , Me_2Etn and Cho , the phospholipids PtdEtn , PtdMeEtn and PtdMe_2Etn , and the analogues 2-amino-3-phosphopropionic acid, 2-aminoethylphosphonic acid, 2-aminoethanethiol, 2-dimethylaminoethanethiol, diethylamine and 2-hydroxyethylhydrazine did not affect the PMe_2Etn N-MT activity. The other compounds examined are either the substrates for or the products of other previously described mammalian cytosolic methyltransferases, and included noradrenaline, adrenaline, phenylethanolamine, phenylethylamine, N -methylphenethylamine, NN -dimethylphenethylamine, catechol, nicotinamide and histamine. These compounds had no effect on PMe_2Etn N-MT activity when present in the incubation medium at 4 mM , except for noradrenaline and adrenaline which caused a slight decrease in activity (results not shown). However, this inhibitory effect was not dose-dependent and, moreover, the purified PMe_2Etn N-MT preparation failed to catalyse the methylation of noradrenaline to adrenaline (results not shown).

Effects of AdoHcy and 3-DZA. The inhibitory effect of 3-DZA on PtdEtn N-MT activity is due to its conversion into a deaza-analogue of AdoHcy which becomes a strong inhibitor of AdoHcy hydrolase. 3-DZA had only a slight effect on the PMe_2Etn N-MT activity that did not appear to be concentration-dependent. However, when $50 \mu\text{M}$ -AdoHcy was added to the incubation, a 75 % decrease in the PMe_2Etn N-MT activity was observed, and a concentration of 0.5 M caused complete inhibition (Table 3).

Distribution of PMe_2Etn N-MT activity in various rat tissues. PMe_2Etn N-MT activity in the cytosolic portions of the brain, liver, lung, spleen, kidney, heart, muscle and adrenals was determined (Table 4). The lowest activity was found in lung tissue and the highest in brain and adrenals. An investigation of

Fig. 4. Affinity of PMe_2Etn N-MT for PMe_2Etn

PMe_2Etn N-MT activity was assayed in the presence of various concentrations of PMe_2Etn . The data are expressed as a double-reciprocal plot ($1/v$ versus $1/[S]$). The inset displays the velocity as a function of PMe_2Etn concentration.

Table 3. Effect of 3-DZA and AdoHcy on the PMe_2Etn N-MT activity

PMe_2Etn N-MT activity was assayed as described in the text in the presence of various concentrations of the inhibitors. Values represent the averages $\pm$ S.D. of two separate experiments, each carried out in triplicate.

Concentration (mM)	3-DZA		AdoHcy	
	Activity (pmol/min per mg)	Inhibition (% of control)	Activity (pmol/min per mg)	Inhibition (% of control)
0.05	888.6 ± 46.3	16.7	258.1 ± 5.2	75.8
0.5	965.7 ± 8.4	9.5	0	100.0
5	767.7 ± 70.5	28.0	0	100.0

Table 4. Tissue distribution of PMe_2Etn N-MT in the rat

The assays of PMe_2Etn N-MT activity were carried out as described in the text. Values represent the averages $\pm$ S.D. of two separate experiments. Each experiment was performed in duplicate.

Tissue	PMe_2Etn N-MT activity (pmol/g per mg)
Adrenals	877.8 ± 175.2
Brain	813.3 ± 61.6
Heart	423.3 ± 40.8
Kidney	237.9 ± 33.3
Liver	163.4 ± 14.8
Lung	25.1 ± 24.8
Muscle (gastromemia)	707.4 ± 91.9
Spleen	354.1 ± 102.6

the cytosolic PMe_2Etn N-MT activity in various regions of the rat brain revealed that the activity in the hippocampus was 2.5-fold and that of the hypothalamus was 1.5-fold greater than in the whole rat brain. The frontal cortex, the caudate putamen, the cerebellum and the medial septum had the same range of activity as in the cytosol from the whole brain (results not shown).

DISCUSSION

The pathways have been described for PtdCho synthesis in the rat brain which are also present in other tissues. The main metabolic route in mammalian tissues is the *de novo* pathway [9]. The two other minor pathways are the methylation of PtdEtn [13,14] and the base-exchange reactions [15–17]. However, studies demonstrated that in neuronal cells, as well as in rat brain *in vivo*, PEtn could be converted into PCho by a stepwise methylation process [22] which is catalysed by a cytosolic N-MT [24].

In the present report we describe the partial purification and properties of a PM_e_2 Etn N-MT from rat brain cytosol which catalyses the final step in the progressive PEtn methylation pathway leading to the synthesis of PCho. This is the first experimental evidence documenting the existence of such a conversion in mammalian tissue. Although its existence had been suggested [32], the pathway had only been described to be present in plants. It was shown that the Adomet-dependent N-methylation of PEtn is the sole pathway for PtdCho synthesis in *Lemna* (duckweed), but in the carrot both phosphobase and phospholipid methylations occurred [33].

We investigated the distribution of PM_e_2 Etn N-MT in different rat tissues (Table 4). In the liver, which is the tissue containing the greatest activity of PtdEtn N-MT, accounting for up to 20 % of total hepatic PtdCho synthesis, the activity of the cytosolic PM_e_2 Etn N-MT is only 20 % of that present in the brain cytosol. There seems to be a regional variation of this PEtn N-MT activity in brain tissue, with enrichments in the hippocampus and the hypothalamus. A major concern was whether or not the PM_e_2 Etn N-MT was similar to any previously described cytosolic methyltransferase activity. A variety of potential substrates for these previously described methyltransferases were examined and these did not affect, nor were they substrates for, the PM_e_2 Etn N-MT activity. These observations suggest that this enzyme is specific for PM_e_2 Etn as the methyl acceptor. Another unusual property of PM_e_2 Etn N-MT is its calcium-dependency. The cytosolic methyltransferases that have been described were not found to be metal-dependent with the exception of the catechol O-methyltransferase, which is inhibited by calcium [34]. The alkaline pH optimum of 9.5 for the PM_e_2 Etn N-MT is similar to that of the PtdEtn N-MT but different from those of other cytosolic methyltransferases.

These studies demonstrate the existence of cytosolic enzymes capable of carrying out the progressive methylation of PEtn to PCho in the rat brain. They also provide direct evidence that the methylation of Etn-containing compounds into Cho-containing compounds is not restricted to the phospholipid level. However, the biological significance of such a pathway is unexplored.

Numerous studies focused at the phospholipid level reported the effects of hormones on PtdEtn N-MT activity. Conflicting results were obtained and, in general, no correlation was shown between effects of hormones and PtdEtn N-MT activity when studied *in vitro*, or in intact cells [35–38]. Several explanations were offered for these differences, and one of the contributing factors was the controversy about the nature of the PtdEtn N-MT [39,40]. Pajares and co-workers claimed that the methylation of PtdEtn into PtdCho was catalysed by a methyltransferase with a molecular mass of 50 kDa, and several *in vitro* studies have been carried out using this enzymic preparation [41–43]. However, Ridgway & Vance have provided evidence that the 50 kDa protein is not PtdEtn N-MT, since they successfully purified the enzyme to apparent homogeneity [44]. Therefore most of the hormonal studies on PtdEtn N-MT should be re-evaluated. Based on the fact that the levels of PEtn N-MT and PtdEtn N-MT appear to have reciprocal relationships, it will be interesting to investigate the possible involvement of P Etn N-MT in these

hormonal regulation and signal transduction phenomena. The role of this enzyme in the regulation of AcCho and PtdCho syntheses must also be considered.

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