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CONTRIBUTION OF SUBSTRATES FOR PHOSPHOLIPID N METHYLATION BY THE BASE EXCHANGE ENZYME IN RAT BRAIN MICROSOMES

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Summary: Monomethylethanolamine and dimethylethanolamine stimulatd the SAM phospholipid N-methylation activity by a Ca^{+2} dependent reaction. Presumably these bases are converted into their corresponding membranous phospholipid which become substrates for a phospholipid methyl transferase also present in these membranes.

Modification of the polar moieties of phospholipids present in biological membranes has been postulated as an early occurrence in membrane events. A major hypothesis focuses on the phosphatidylinositol - phosphatidic acid effect catalyzed by a phosphatidylinositol specific phospholipase C that may be related to cation transport (1). Another suggestion is that the stepwise transfer of three methyl groups to phosphatidylethanolamine producing phosphatidylcholine is a mechanism for transmitting biological signals across cellular membranes (2). Alterations of the polar portion of membrane phospholipids has been suggested to modulate their receptor function for a variety of pharmacological agents (3).

The base exchange enzymes catalyze the incorporation of L-serine, choline and ethanolamine into their corresponding phospholipid presumably at the expense of a pre-existing phospholipid molecule (4). This communication provides preliminary evidence for the ability of the base exchange reaction to provide substrates for the phospholipid N-methylation enzymes.

Abbreviations: [^{3}H]SAM is S adenosyl [^{3}H-CH_3] methionine; PMME is phosphatidylmonomethylethanolamine; PDME is phosphatidyldimethylethanolamine; MME is monomethylethanolamine; DME is dimethylethanolamine, PE is phosphatidylethanolamine, TLC is thin layer chromatography.

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Materials and Methods

Adenosyl-L-methionine, S-[C^3H_3], ^3H-SAM, was purchased from New England Nuclear, Mass., and its specific activity adjusted to about 68,896 cpm/nmole with the non-radioactive compound. 2-Dimethyl [1,2-^{14}C] aminoethanol with a specific activity of 1.3 mCi/nmol after repurification was obtained from I.C.N., California. [1,2-^{14}C] ethanolamine with a specific activity of 2.5 mCi/nmole was from N.E.N., Boston, Mass. PMME and PDME were obtained from Gibco, Ohio and MME and DME were from Aldrich Chemical, Wisconsin. Silica gel 60 thin layer chromatography plates were purchased from Brinkman, N.Y. All other reagents and standards were from the appropriate commercial suppliers.

The assay procedure I for the base exchange activity of rat brain microsomes and recipies for incubation mixtures have been described previously (5). Incubations for the phospholipid N-methylations contained 5 nmoles SAM[^3H], 10 umoles HEPES pH 9.0, $CaCl_2$ 1 umole, $MgCl_2$ 0.5 umoles, approximately 1 mg protein and where indicated 0.1 umole MME, or 0.15 umole DME, or 0.2 umole ethanolamine or 0.2 mg PE, PDME or PMME in a total volume of 0.15 mls. The reaction tubes were incubated for 1 hour and processed as in procedure I (5). Aliquots of the final chloroform phase were removed for radioactivity determination by liquid scintillation spectrometry. The remaining sample was applied to a TLC plate with appropriate phospholipid standards and developed employing a solvent system of n-propanol-proprionic acid-chloroform-water (3:2:2:1) (6). Iodine vapor was employed to visualize standards and the components present in each sample. The plates were sprayed with ENHANCE (N.E.N., Boston) and exposed to Fuji X-ray film to locate the labeled products. The gel was sectioned accordingly and removed from thin plates in order to quantitate the radioactivity distribution.

Results

Stimulation of phospholipid N methylation by MME and DME

The effect of the addition of either MME or DME on the appearance of radioactivity from SAM[^3H] into phospholipids is shown in Fig. 1. This shows (a) that the 4 standards are well separated with this solvent system, (b) that there is a prominent radioactive contamination present in the SAM[^3H] sample which is less polar than PE (lane 2), (c) that small quantities of radioactive product migrating with PMME and PDME are synthesized by the intact enzyme system (lane 4), (d) that the presence of MME or DMME results in a very marked increase of radioactivity present in the corresponding phospholipid possessing one additional methyl group (lanes 5 and 7). These observations are expressed quantitatively in Table I, which also reveals an 8-10 fold stimulation of total counts soluble in organic solvent.

The addition of ethanolamine did not cause any increase in radioactivity incorporated or change in the distribution on the TLC plates.

Incubations were carried out with either labeled DME or ethanolamine in the presence and absence of varying concentrations of non-radioactive SAM.

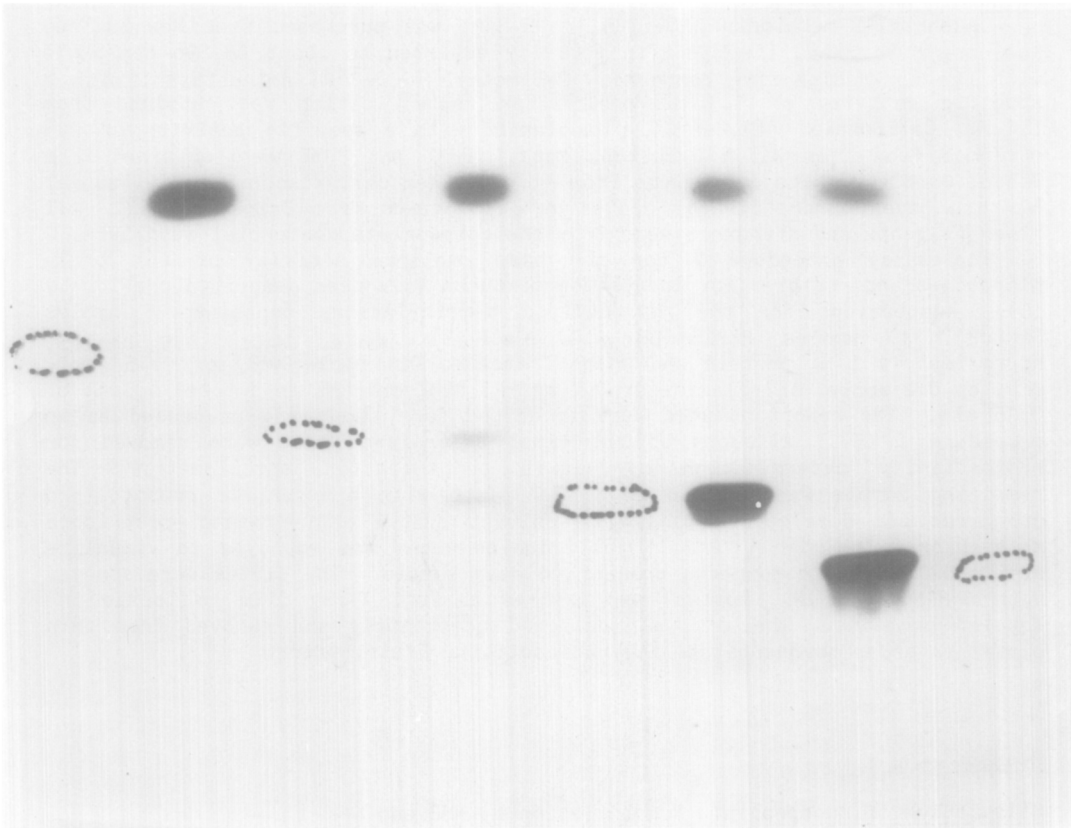

Fig. 1. Effect of DME or MME on the SAM[^{3}H] labeling of phospholipids.
The radioautogram was prepared as described in the text. Lane 1: PE standard, Lane 3: PMME standard, Lane 5: PDME standard, Lane 8: PC standard. Stipled areas represent migration of standards as detected with iodine vapor. Lane 2: boiled enzyme control, Lane 4: no additions to incubation mixtures, Lane 6: MME added to incubations, Lane 7: DME added to incubations.

The lipids were separated by TLC plates, autoradiograms prepared and the plate sectioned and radioactivity associated with various areas quantitated. Under these experimental conditions the products were exclusively the corresponding phospholipids as PE and PDME (data not shown). Presumably if methylation of these products did occur it was undetectable due to the low enzymatic activity and low specific activity of these radioactive precursors.

The MME dependent formation of PMME is a Ca^{+2} requiring reaction as seen in Fig. 2 showing the appearance of an iodine positive product (lane 4) migrating with PMME standards (Lane 6). There is a reduction of the intensity

Table I
Distribution of Radioactivity Recovered from TLC of Creatine Mixtures Containing SAM[^{3}H]

	Boiled Enzyme	Intact Enzyme	+ MME	+ DME
Reagent Contaminant	77%	68%	13%	15%
"PC"	0.5%	1.5%	2.7%	55%
"PDME"	4.2	8.2%	72.6%	3.5
"PMME"	3.6	7.1%	2.4	3.2
Fold increase of [^{3}H] over intact enzyme	--		9.9	7.53

There were 5691 cpms recovered from the TLC scrapings.

of this staining in the absence of Ca^{+2} ion (lanes 5 and 8) which is eliminated completely in the presence of EGTA.

Stimulations by PMME and PDME

The inclusion of either PMME or PDME in the incubation mixtures caused respectively a 3.4 and 3.9 fold stimulation of radioactivity incorporation into the next higher methylated phospholipid. Although there was some increase with addition of ethanolamine plasmalogen, radioautographic examination revealed that this was associated with non-polar material.

Properties of this System

The incorporation of radioactivity from SAM[^{3}H] was maximal at approximately 100 uM with endogenous acceptors while in the presence of 500 uM MME, SAM[^{3}H] saturation occured at 50 uM. The incorporation was linear for 60 minutes in the presence of MME and for only 30 minutes in the presence of DMME.

There are at least 3 separate base exchange enzymes for the incorporation of either choline, serine or ethanolamine (4). It was of interest to learn if these compounds interfered with DME or MME stimulated SAM[^{3}H] incorporation into lipid. These experiments suggest that the DME stimulation is more sensitive to these amino alcohols than the MME stimulation. The serine inhibitions with both substrates is more marked than that caused by choline or ethanolamine (Table II). The MME and DME stimulations of SAM[^{3}H] incorporation into methylated phospholipids is Ca^{+2} dependent but independent of Mg^{+2}.

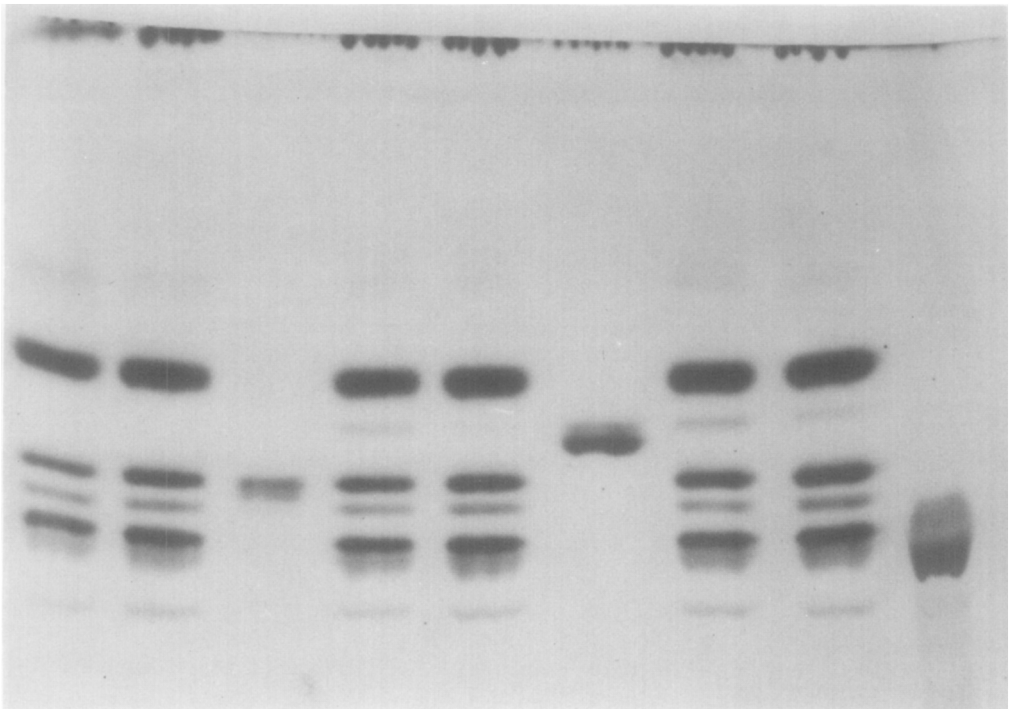

Fig. 2.
Thin layer chromatography of lipids after incubations containing MME. Lane 1: boiled enzyme, Lane 2: intact enzyme, no addition, Lane 3: phosphatidylserine standard, Lane 4: MME added, Lane 5: MME added, Ca^{+2} omitted, Lane 6: PMME standard, Lane 7: MME added, Mg^{+2} omitted, Lane 8: MME added, Ca^{+2} and Mg^{+2} omitted, Lane 9: PC standard. The plates were exposed to I_2 vapor to reveal the location of the spots.

Discussion

This work suggests that the base exchange reactions can be coupled to the phospholipid N methylation reactions. A base exchange enzyme converts MME to PMME and DME into PDME by a Ca^{+2} dependent reaction. In the presence of SAM^{3}H these membrane bound phospholipids are converted to the corresponding phospholipid containing an additional methyl group. Under comparable conditions exogenous PME and PDME were appreciably less stimulatory while ethanolamine and PE were ineffective.

The function of the base exchange enzymes, which are ubiquitous in nature, is obscure except that the biosynthesis of phosphatidylserine in mammalian tissues occurs by this reaction (7). The function of the phospholipid N methylations have attracted much interest since they may be

Table II
Effect of Several Amino Alcohols on the MME and DME Stimulated Incorporation of SAM[^{3}H] into Phospholipids

	%
MME + choline (0.5 umoles)	92
MME + serine (0.5 umoles)	65
MME + ethanolamine (0.5 umoles)	76
DME + choline (0.5 umoles)	68
DME + serine (0.5 umoles)	30

The incubation contained either 0.5 mM MME or DME and, where indicated 2.5 mM choline, or ethanolamine or serine, the activity with endogenous acceptors was 19 pmoles/mg protein, in the presence of MME it was 105 pmoles/mgm protein in the presence of DME, it was 63 pmoles/mgm protein.

The values are expressed as % of that found in presence of either MME or DME alone which usually amounted to around 13,000 cpm. There was no augmentation of label appearance in lipid by choline, serine or ethanolamine alone.

involved in the transmission of messages across biological membranes (2). Neither the base exchange reactions or the phospholipid N methylations cause the de novo synthesis of phospholipid molecules but merely alter the polar portion of nitrogen containing phospholipids.

The significance of the ability of phospholipid molecules produced by the action of the base exchange enzymes to be available as substrates for the phospholipid N methylation reactions is unclear. It has been proposed that 2 separate phospholipid methyl transfers (PMT) exist. PMT I which has low activity methylates PE while PMT II which is more active transfers methyl groups to PMME and PDME (8). Perhaps the base exchange reactions provides a mechanism for bypassing the relatively inactive PMT I and allow the more active PMT II to have access to substrates.

The presence in brain tissue of an SAM[^{3}H] dependent phospholipid N-methylation reaction has been observed by several investigators (8-14)

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