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Lymphocyte and Granulocyte Phosphatidylethanolamine N-Methyltransferase: Properties and Activity in Cystic Fibrosis

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ABSTRACT. Human lymphocyte and granulocyte membranes contain an enzyme, phosphatidylethanolamine N-methyltransferase (PEMT), which catalyzes the transfer of a methyl group from S-adenosylmethionine to the polar head group of phosphatidylethanolamine to form phosphatidylmonomethylethanolamine. This enzyme, in lymphocyte membranes, has K_m for S-adenosylmethionine of 7.01 ± 2.9 (SD) μM, and specific activity 0.57 ± 0.31 pmol/mg protein/15 min, is inhibited by S-adenosylhomocysteine, displays optimal activity at pH 8.0–9.0, and is stimulated by isoproterenol in dose-dependent, propranolol-inhibitable fashion, to a lesser extent by epinephrine, but not by norepinephrine, prostaglandin E_1, concanavalin A, or adenosine 3′:5′ cyclic monophosphate, with or without phosphodiesterase inhibitors. Granulocyte membrane PEMT has K_m for S-adenosylmethionine of 4.4 μM and specific activity 0.54 ± 0.51 pmol/mg protein/15 min, is inhibited by S-adenosylhomocysteine, displays optimal activity at pH 8.0–9.5, and is stimulated by isoproterenol > epinephrine > norepinephrine, but not by prostaglandin E_1, serum-treated zymosan, formyl-methionyl-leucyl-phenylalanine, or adenosine 3′:5′ cyclic monophosphate. Because activation of PEMT reportedly contributes to several processes known to be abnormal in cystic fibrosis, including coupling of the β-adrenergic receptor to adenylate cyclase, activity of PEMT was compared in lymphocyte and granulocyte membrane preparations from cystic fibrosis patients and healthy controls, in which abnormal coupling of β-adrenergic receptor to adenylate cyclase had been demonstrated. For both cell types, the K_m and specific activity of PEMT were comparable in normal and cystic fibrosis samples. Therefore, the hypothesis that reduced PEMT activity accounts for the impaired coupling of β-adrenergic receptor to adenylate cyclase in lymphocytes and granulocytes in cystic fibrosis is rejected. (*Pediatr Res* 20:1290–1296, 1986)

Abbreviations

CF, cystic fibrosis
PEMT, phosphatidylethanolamine N-methyltransferase
SAM, S-adenosylmethionine
PE, phosphatidylethanolamine
PM, phosphatidyl monomethylethanolamine
PC, phosphatidylcholine
PD, phosphatidyl N,N-dimethylethanolamine
GMPPNP, guanyl 5′yl imidodiphosphate
IBMX, isobutylmethylxanthine
fMLP, formyl-methionyl-leucyl-phenylalanine

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Patients with CF may have disordered plasma membrane function. Among the abnormalities reported are altered monovalent ion transport across respiratory and sweat duct epithelium (1, 2), abnormal calcium ATPase in erythrocytes and fibroblasts (3), abnormal coupling of β-adrenergic receptor to adenylate cyclase in lymphocytes and granulocytes (4), and possibly, abnormal secretory processes (5). Disorder of some fundamental membrane process in CF could affect all the other diverse functions. One possible candidate for this basic process is enzymatic phospholipid methylation.

Plasma membranes of many cell types have been reported to contain a two-enzyme system which catalyzes the sequential addition of three methyl groups to phosphatidyl ethanolamine to form phosphatidylcholine. This system has been suggested to be involved in the coupling of β-adrenergic and diazepam receptors to adenylate cyclase, regulation of β-adrenergic receptor number, activation of calcium ATPase, and the secretory process (6–13). The first enzyme, PEMT, catalyzes the transfer of a methyl group from SAM to PE to form PM. Activation of PEMT is associated with facilitated coupling of the β-adrenergic receptor to adenylate cyclase in HeLa cells, C6 astrocytoma cells, and rat reticulocytes (6–9), and with increase in calcium ATPase activity in human erythrocytes (10). The second enzyme in this system, PC-forming enzyme, catalyzes the transfer of two methyl groups to PM to make PC. Activation of PC-forming enzyme is associated with an increase in the apparent number of β-adrenergic receptors in rat reticulocytes or HeLa cells (9, 11). The process of stimulus-secretion coupling, in the mast cells and basophilic leukemia cells at least, appears to depend on activation of the phospholipid methylation system (12, 13): antigen-stimulated histamine release fails to occur in rat basophilic leukemia mutant cell lines which lack either PEMT- or PC-forming enzyme, but these mutants complement each other (13).

Because the phospholipid methylation system (and particularly PEMT) affects several processes which are abnormal in CF, it deserves investigation. However, considerable controversy has arisen over this enzyme system in plasma membranes, because its activity is low, and in some prior reports unambiguous identification of radioactive product was not reported (14). Therefore, before comparison of CF and control samples was attempted, normal PEMT activity was characterized. Lymphocyte and granulocyte membranes were selected for study because, in CF, they contain β-adrenergic receptors which fail to couple appropriately to adenylate cyclase (4). After the initial characterization, PEMT activity in normal and CF preparations was compared in order to test the hypothesis that reduced PEMT

activity accounts for the CF β-adrenergic coupling abnormality in lymphocytes and granulocytes. If this hypothesis is correct, either the K_m for SAM should be increased or the specific activity of this enzyme should be reduced in CF preparations.

METHODS

Subjects. Healthy human subjects who contributed blood for the initial enzyme characterization studies were age 18–40 yr and took no medications. Patients with CF age 20–33 yr with clinical scores (15) 62–90 also donated blood. Each patient with CF was matched with two age-appropriate control subjects. The characteristics of these subjects are shown in Table 1. The study was approved by the Institutional Review Board.

Lymphocyte and granulocyte preparation. Human blood was anticoagulated with acid-citrate-dextrose and centrifuged at 200 × *g* for 20 min to remove platelet-rich plasma. Lymphocytes and granulocytes were separated as previously described (4). Lymphocyte membranes were prepared by homogenization of the cells, suspended in distilled water, in a Brinkman Polytron Homogenizer, setting 8, for 15 s, followed by centrifugation at 40,000 × *g* for 10 min (4). The membranes were washed once in Tris HCl, pH 8.0 (25 mM) and $MgCl_2$ (5 mM) buffer and finally resuspended at protein concentration 1.5–6 mg/ml.

Granulocytes were resuspended in Tris HCl-50 mM, pH 7.4, $MgCl_2$ (10 mM), and lysed by Polytron action (15 s at setting of 8) (4). Membranes were recovered by centrifugation at 40,000 × *g* for 10 min, washed once in Tris HCl, pH 8.0 (25 mM) and $MgCl_2$ (5 mM) buffer and resuspended at protein concentration 2.5–6.5 mg/ml.

Stimulation of cAMP production. Lymphocytes and granulocytes were stimulated to produce cAMP as previously described (4).

Phospholipid methylation assay. The usual enzyme assay contained Tris HCl buffer, pH 8.0, 25 mM, $MgCl_2$ (5 mM), PE (160 μg) and [^{3}H]-SAM (New England Nuclear Corp., Boston, MA) (4 μM, final specific activity 2 Ci/mmol) and 60–250 μg protein in total volume 100 μl. The [^{3}H]-SAM was washed with $CHCl_3$ prior to use. Incubation was at 37° C for 15 min. Reaction was stopped by placing the tubes on ice and adding 2 ml $CHCl_3$:CH_3OH:2N HCl (6:3:1). Following vigorous mixing, the tubes were centrifuged to separate the phases (3 min, 400 × *g*) and the aqueous phase removed and extracted again. The extracts were combined and washed with methanolic KCl. The chloroform was dried with anhydrous Na_2SO_4, evaporated under a stream of nitrogen, and taken up in chloroform-methanol containing carrier phospholipids (GIBCO, Grand Island, NY) and chromatographed on thin layer plates of silica gel using $CHCl_3$:1-propanol:propionic acid:H_2O, 2:3:2:1 as developer (16). The spots were visualized with I_2 vapor, scraped into scintillation vials, extracted with 1 ml methanol, and counted in a cocktail of scintanalyzed Toluene containing Scintiprep (Fisher Chemical Co., Pittsburgh, PA). When plastic-backed plates were used, the spots were cut out with scissors, placed in scintillation vials and eluted with methanol before counting in the cocktail described above.

Table 1. *Subjects*

CF					Control			
No.	Age (yr)	Sex	Score*	Meds†	No.	Age (yr)	Sex	Meds
1	20	F	80	1–3	1a	22	M	None
					1b	23	F	None
2	24	F	90	None	2a	24	M	None
					2b	26	F	None
3	29	M	79	1, 2	3a	29	M	None
					3b	29	F	None
4	33	M	62	1, 2, 4–6	4a	32	F	None
					4b	38	F	None

* Modified Shwachman score (15).

† Medications: 1-pancrease; 2-multivitamins and Aquasol E; 3-tetracycline; 4-trimethoprim/sulfisoxazole; 5-β-adrenergic agents, aerosol and systemic; 6-Colistin by aerosol.

RESULTS

Recovery of product. Commercial [^{14}C]- or [^{3}H]-PC or PE (New England Nuclear Corp.) were used as markers. Two extractions with chloroform resulted in recovery of 78–85% of radioactive marker: further extractions added little. Following evaporation and chromatography, recovery was 65–75%. Thus, overall recovery was 50–60% when plastic-backed chromatography plates are used (lower when glass plates are used). The values reported herein are not corrected for recovery.

Evaluation of the assay procedure. In lymphocyte or granulocyte membranes, only 0.1–0.3% of added radioactivity is incorporated into PM. This amount approaches the level of contamination in commercial radioactive SAM. Washing the [^{3}H]-SAM with $CHCl_3$ or butanol prior to use (done routinely for the assays reported herein) reduced total background radioactivity in the chromatogram by 30–50%. Still, over half the chloroform extractable counts were not found in the PM spot (R_f = 0.45–0.55) but were recovered at or near the solvent front (R_f > 0.9). These counts increase with time when [^{3}H]-SAM is incubated without tissue at 37° C (Fig. 1) and increase at higher pH, consistent with breakdown of [^{3}H]-SAM or a contaminant in alkaline medium. Therefore it is important to demonstrate that the counts recovered with the PM carrier have the properties of bona fide product of enzyme-catalyzed reaction.

No radioactivity accumulates in the PM spot with time in the absence of tissue (Fig. 1). Boiling the lymphocyte or granulocyte membranes for 15 min prior to assay eliminated 80–95% of all the counts in the PM spot. (All values reported for enzyme activity are for intact enzyme minus boiled control.) Radioactivity in the product was four to 20 times that in the same spot for the boiled control or the zero time point (Figs. 2 and 3). Triplicate samples of reaction product chromatographed in three different

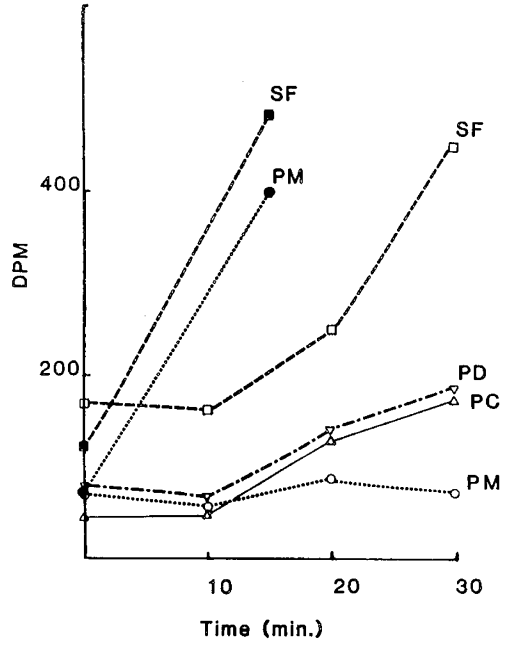

Fig. 1. Accumulation of radioactivity with time in portions of the chromatogram marked by authentic phospholipid carrier. Data are shown from one of five similar experiments. *Open symbols* indicate radioactivity accumulated in the absence of tissue: *closed symbols* show accumulation in the presence of lymphocyte membranes. *SF*, solvent front (includes R_f values >0.9).

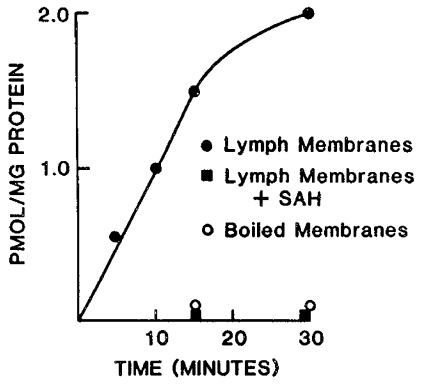

Fig. 2. Production of PM by lymphocyte membranes. *Curve* shows the mean of three experiments. *SAH*, S-adenosylhomocysteine. "Boiled membranes" were boiled 15 min before inclusion in assay.

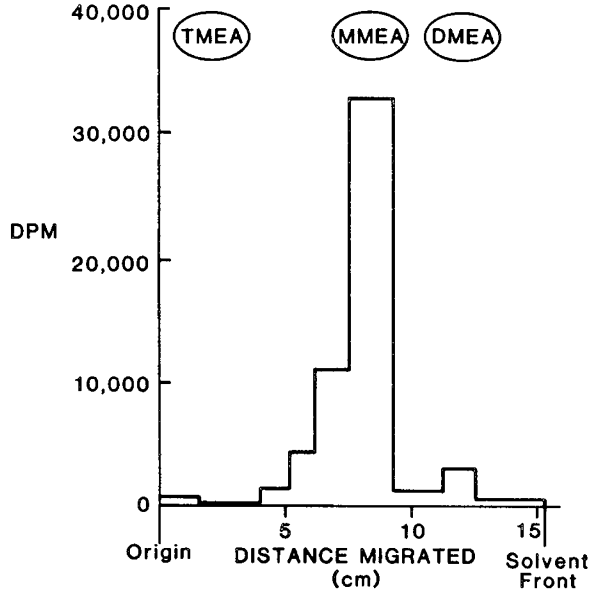

Fig. 3. Distribution of radioactivity in a thin-layer chromatogram of materials derived from hydrolysis (6 N HCl, 3 h, 100° C) of PM separated during routine assay for PEMT. Stationary phase was silica gel G and developer was 1-propanol:NH_4OH, 4:1. *TMEA*, authentic trimethylethanolamine; *DMEA*, authentic dimethylethanolamine; *MMEA*, authentic monomethylethanolamine. Chromatogram illustrates recovery of the majority of counts in PM with MMEA after hydrolysis.

Table 2. *Recovery of PM in different chromatography systems*

	Counts per min in PM spot	
Liquid phase*	Trial 1	Trial 2
I	645	755
II	637	1166
III	752	715

* I = 1-propanol: $CHCl_3$:propionic acid: H_2O, 3:2:2:1. II = 2-propanol: $CHCl_3$: propionic acid: H_2O: 3:2:2:1. III = $CHCl_3$: acetone: methanol: acetic acid: H_2O: 10:4:2:2:1.

solvent systems, in which the relative R_f values for the carrier materials were different, yielded similar counts recovered with carrier PM (Table 2). In a separate experiment, purified PM recovered from the silica gel was hydrolyzed with 6 N HCl for 3 h at 100° C. One volume of methanol was added. The liquid was removed by evaporation and the free base taken up in CH_3OH-H_2O, 1:1 containing carrier ethanolamine, monomethylethanolamine, dimethylethanolamine, and trimethylethanolamine. This material was chromatographed on silica gel using 1-propanol:NH_4OH, 4:1 as developer, spots visualized with I_2 vapor and cut out, extracted with CH_3OH:CH_3COOH:H_2O, 39:1:10, and counted in ACS scintillant (Amersham Co., Arlington Heights, IL). The radioactivity was recovered largely with the monomethylethanolamine spot (Table 3, Fig. 3) (16), indicating that the [^{3}H] label that travelled with PM on the initial chromatogram was located in the polar head group, as expected.

Properties of lymphocyte PEMT activity. Activity was linear with time over 5–20 min but slowed at 45–60 min. Activity was inhibited 85–90% by S-adenosylhomocysteine, a competitor for SAM (Fig. 2). Formation of PM is pH-dependent, optimal at pH 8.0–9.0, and poor at pH 7.0 or pH 10.0. Substrate-velocity curves were performed at concentrations ranging from 1–16 μM SAM: at lower concentrations it is difficult to detect activity reliably, at higher concentrations, significant amounts of PD and PC are formed. At these substrate concentrations there was no increase in radioactivity travelling with PC or PD compared to boiled controls. Double reciprocal plots are linear ($r > 0.977$) (Fig. 4) and give $K_m = 7.01 \pm 2.9$ μM and apparent $V_{max} = 0.57 \pm 0.31$ pmol/15 min/mg protein for enzyme in lymphocyte membranes from 10 healthy unmedicated volunteers 18–40 yr old. Activity is stimulated 5–30% by exogenous PE but does not depend on the addition of exogenous Mg^{++}, although the membranes were not specifically prepared to be magnesium free.

Isoproterenol stimulated PEMT activity in a dose-dependent manner, with maximal stimulation at 10^{-5} M (Fig. 5). Norepinephrine (10^{-4} and 10^{-5} M) had no effect, and epinephrine (10^{-5} M but not lower concentrations) slightly but statistically significantly stimulated PEMT activity. GMPPNP (10^{-4} M) significantly inhibited PEMT. However, GMPPNP did not affect the isoproterenol stimulation of PEMT. Propranolol (10^{-5} M) inhibited the stimulation by isoproterenol or epinephrine (10^{-5} M). IBMX, a phosphodiesterase inhibitor, did not enhance the effect of isoproterenol, and cAMP alone (10^{-6} M) or in combination

Table 3. *Recovery of monomethylethanolamine (MMEA) from hydrolysis of PM*

	DPM	Recovery (% previous step)	Recovery (% initial)
PM spots*	83399	100	100
PM (extracted)	76197	91	91
Total after hydrolysis	58100	76	70
MMEA	52211	90	63

* PM spots from several experiments were pooled to obtain high initial counts.

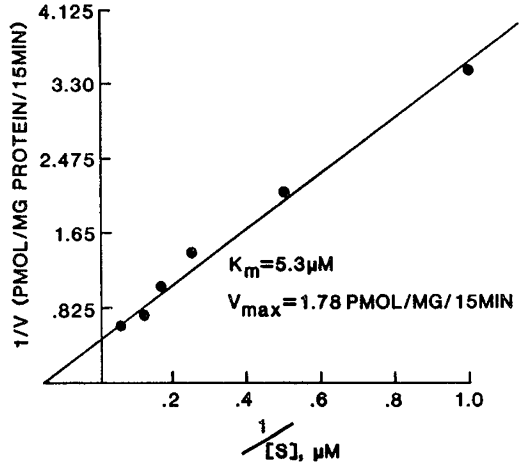

Fig. 4. Representative double reciprocal substrate-velocity plot for PEMT activity in human lymphocyte membranes from a 25-yr-old healthy volunteer.

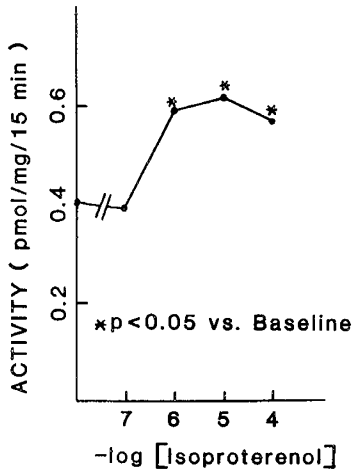

Fig. 5. Response of PEMT to isoproterenol in human lymphocyte membranes. *Curve* shows the mean of five experiments; *bars* are SEM. *Asterisks* indicate significant ($p < 0.05$) differences from baseline values.

with IBMX did not stimulate PEMT. Neither prostaglandin E_1 (10^{-8} to 10^{-6} M) nor concanavalin A (1 μg/ml) stimulated PEMT (Table 4).

Association of activity with lymphocytes. Preparation of lymphocytes on a Ficoll-Hypaque gradient leads to some contamination of the lymphocyte fraction with platelets, even when platelet-rich plasma is removed in an initial centrifugation step, as was done in the present study. However, methods that give platelet-free preparations entail substantial losses of lymphocytes and possibly selection of lymphocyte subpopulations. Platelets contain phospholipid methylating enzymes (17). In order to determine how much contaminating platelets contribute to the activity in lymphocyte preparations, platelets were purified from platelet-rich plasma and homogenized and centrifuged in the same fashion as the lymphocytes. From 150 ml blood, 0.6 mg platelet protein was recovered. PEMT activity was 1.93 pmol/mg protein/15 min (mean of two determinations). Because 98 to 99% of platelets were recovered in the platelet-rich plasma and one to three platelets were counted for each lymphocyte in the final preparations, no more than 1–2% of total blood platelets appear in the lymphocyte preparations. After homogenization and centrifugation, less than 1% of the recovered protein is platelet derived. Therefore, less than 5% of the PEMT activity observed in lymphocyte membranes can be attributed to platelet contamination.

Properties of the granulocyte enzyme activity. Activity was linear with time over 5–30 min. Activity was inhibited >90% by S-adenosylhomocysteine, a competitor for SAM. Formation of PM is pH dependent, optimal at pH 8.0–9.5, and poor at pH 7.0 or pH 10.0. Substrate-velocity curves were performed at concentrations ranging from 1–16 μM SAM: at lower concentrations it is difficult to detect activity reliably, at higher concentrations, significant amounts of PD and PC are formed. At these substrate concentrations, there was no increase in radioactivity traveling with PC or PD compared to boiled controls. Double reciprocal plots are linear ($r > 0.945$) (Fig. 6) and give apparent $K_m = 4.42 \pm 2.5$ μM and apparent $V_{max} = 0.54 \pm 0.51$ pmol/15 min/mg protein for enzyme in granulocyte membranes from 10 healthy unmedicated volunteers 18–40 yr old. Activity is not stimulated by exogenous PE and does not depend on the addition of exogenous Mg^{++}, although the membranes were not specifically prepared to be magnesium free.

Isoproterenol stimulation of enzyme activity was dose dependent and inhibited by propranolol. Maximal stimulation was not achieved even at 10^{-4} M (Fig. 7). GMPPNP (10^{-4} M) stimulated PEMT modestly. However, GMPPNP did not affect the stimulation of PEMT by isoproterenol. The order of potency of β-adrenergic agonists was isoproterenol>>epinephrine>norepinephrine. IBMX did not enhance the effect of isoproterenol, and cAMP alone (10^{-6} M) or in combination with IBMX did not stimulate PEMT. Prostaglandin E_1 (10^{-6} M) inhibited PEMT: activity averaged 78% of control. There was no stimulation of human granulocyte PEMT by fMLP (10^{-9} or 10^{-6} M) or by serum-treated zymosan (Table 5).

Table 4. *Lymphocyte PEMT responses*

Additions (s)	*n*	% Baseline*
Isoproterenol 10^{-5} M	5	155†
Isoproterenol 10^{-5} M	3	(91)‡
+ GMPPNP 10^{-4} M		(162)§
Epinephrine 10^{-5} M	5	117†
Norepinephrine 10^{-5} M	4	104
Isoproterenol 10^{-5} M	4	105
+ Propranolol 10^{-5} M		
Propranolol 10^{-5} M	4	111
Prostaglandin E_1 10^{-6} M	3	105
GMPPNP 10^{-4} M	3	64†
cAMP 10^{-5} M	3	93
cAMP 10^{-5} M + IBMX 10^{-4} M	2	109
IBMX 10^{-4} M	2	72
Concanavalin A 1 μg/ml	4	87

* Baseline values determined in the presence of 4 μM SAM, after 15 min incubation at 37° C.

† Significantly different from baseline, paired *t* test ($p < 0.05$).

‡ Percent of control with isoproterenol alone.

§ Percent of control with GMPPNP alone.

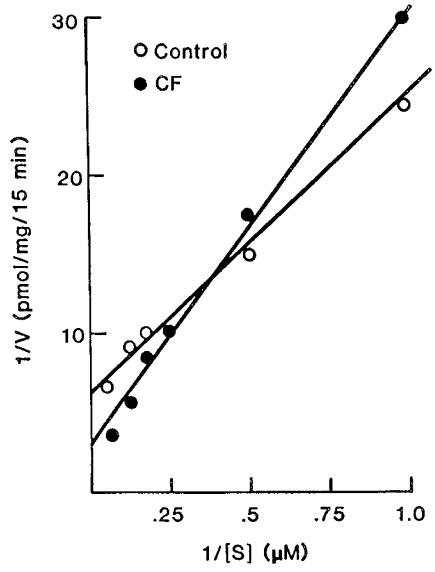

Fig. 6. Representative double reciprocal substrate-velocity plots for PEMT activity in granulocyte membranes from one CF and one control subject.

Comparison of CF and control PEMT. The reproducibility of this assay from day to day for the same individual was assesed. Maximum coefficients of variation (SD/mean of at least three determinations on separate days for two to five normal subjects) were as follows: for lymphocyte enzyme K_m, 10%; lymphocyte specific activity, 25%; granulocyte enzyme K_m, 11%; granulocyte specific activity, 17%.

Like the larger groups of CF patients reported previously (4, 34, 35), these CF subjects had reduced cAMP response to isoproterenol (10^{-5} M) compared to healthy controls (Table 6). However, when CF subjects were compared with controls, no consistent differences in PEMT could be demonstrated. Means for specific activity did not differ between the groups, nor could consistent trends be demonstrated (Tables 7 and 8). The apparent K_m for SAM was lower in CF lymphocyte membranes than in

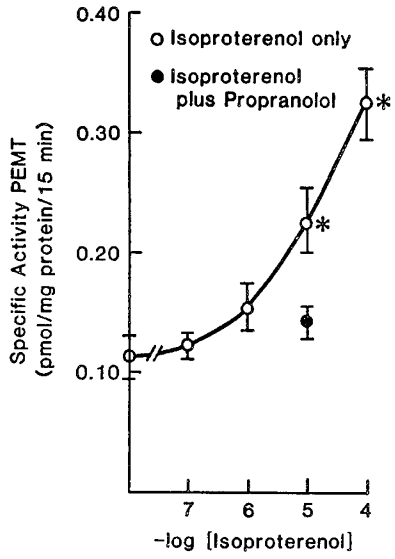

Fig. 7. Response of PEMT to isoproterenol in human granulocyte membranes. *Curve* shows the mean of six experiments; bars are SEM. *Asterisks* indicate significant ($p < 0.05$) differences from baseline.

Table 5. *Granulocyte PEMT responses*

Addition(s)	*n*	% Baseline*
Isoproterenol 10^{-5} M	6	197†
Isoproterenol 10^{-5} M	3	(86)‡
+ GMPPNP 10^{-4} M		(240)§
Epinephrine 10^{-5} M	3	143†
Norepinephrine 10^{-5} M	3	120†
Isoproterenol 10^{-5} M	6	132
+ Propranolol 10^{-5} M		
Propranolol 10^{-5} M	6	110
Prostaglandin E_1 10^{-6} M	3	78†
cAMP 10^{-5} M	4	78
cAMP 10^{-5} M + IBMX 10^{-4} M	2	113
GMPPNP 10^{-4} M	3	123
IBMX 10^{-4} M	2	65
fMLP 10^{-9} M	3	108
fMLP 10^{-6} M	3	85
Serum-treated zymosan	4	110

* Baseline values were determined with 4 μM SAM, after 15 min incubation at 37° C.

† Significantly different from baseline, paired *t* test ($p < 0.05$).

‡ Percent of control with isoproterenol alone.

§ Percent of control with GMPPNP alone.

Table 6. *Isoproterenol (10^{-5} M)-stimulated cAMP production (% control)*

CF subjects			Controls		
No.	Lymphocyte	Granulocyte	No.	Lymphocyte	Granulocyte
1	210	200	1a	440	180
			1b	330	260
2	141	100	2a	391	160
			2b	718	545
3	122	170	3a	310	202
			3b	560	260
4	119	131	4a	280	310
			4b	365	300
	123 ± 79*	150 ± 44*		424 ± 148	277 ± 121

* Different from control $p < 0.05$.

Table 7. *PEMT activity of lymphocytes*

CF subjects			Controls		
No.	K_m (μM)	V_{max} (pmol/mg/15 min)	No.	K_m (μM)	V_{max} (pmol/mg/15 min)
1	4.9	0.45	1a	7.2	1.46
			1b	10.6	0.72
2	3.3	0.55	2a	4.5	0.44
			2b	12.9	0.19
3	4.4	0.19	3a	7.3	0.41
			3b	3.6	0.35
4	1.7	0.87	4a	4.1	0.40
			4b	10.4	0.47
	3.6 ± 1.2	0.52 ± 0.24		7.6 ± 3.2	0.56 ± 0.37

Table 8. *PEMT activity of granulocytes*

CF Subjects			Controls		
No.	K_m (μM)	V_{max} (pmol/mg/15 min)	No.	K_m (μM)	V_{max} (pmol/mg/15 min)
1	3.0	0.22	1a	2.6	0.25
			1b	4.5	0.11
2	4.1	0.66	2a	2.6	0.15
			2b	3.9	0.22
3	8.3	0.30	3a	5.2	0.14
			3b	6.9	0.47
4	3.4	0.09	4a	5.5	0.22
			4b	1.7	0.52
	4.7 ± 2.1	0.32 ± 0.21		4.1 ± 1.6	0.26 ± 0.14

the controls ($p = 0.051$), but in granulocyte membranes, the K_m for SAM was the same for CF and control subjects.

DISCUSSION

Human lymphocyte membranes contain PEMT activity with apparent K_m for SAM of 7.01 μM, and specific activity at least 0.57 pmol/mg/15 min (values are not corrected for recovery). Human granulocyte membranes also contain PEMT activity, with apparent K_m for SAM 4.4 μM and specific activity at least 0.54 pmol/mg/15 min. These values are comparable to those reported for plasma membrane PEMT in human erythrocytes, mast cells, and cultured HeLa cells (6–13), but are considerably lower than PEMT activity in rat liver or adrenal microsomes (16, 18–20). Nevertheless, the enzyme studied herein produces bona fide PM, as identified in three different thin-layer chromatography system (Table 2) and by chemical analysis of its hydrolytic products (Fig. 3), and PM is produced only in the presence of active (not heat denatured) enzyme (Fig. 2). This activity has other properties expected of enzyme-catalyzed reaction, including pH dependence, inhibition by a competitor of SAM (S-adenosylhomocysteine) (Fig. 2), and time and substrate-concentration dependence (Figs. 2, 5, and 6). However, enzyme activity is low, and it is difficult to imagine that the minor changes in lipid composition which could be produced by this system substantially affect the membrane's physical properties (14). However, local rearrangement of lipids about specific proteins could alter their function. This hypothesis is supported by data which indicate that maximal stimulation of phospholipid methylation in C6 astrocytoma cells by β-adrenergic agonists and diazepam analogs is additive, suggesting that these two receptors do not activate the same pool of enzyme (21).

The results reported herein differ somewhat from those of Niwa *et al.* (22, 23), who found that nearly all the chloroform-extractable counts in lymphocyte and granulocyte membrane methylation assays were in methylated phospholipids. In our assays, fewer than 50% of the chloroform-extractable counts are in bona fide product: most travel at or near the solvent front, and some of them can be generated even in the absence of tissue

(Fig. 1). Thus it was necessary to chromatograph every sample to identify the product and to subtract the counts present in a boiled control in order to assess enzyme activity accurately. Others have also observed the same phenomenon in phospholipid methylation assays. For example, Laychock (24) reported that, in rat pancreatic islets, only 15% of lipid-extractable counts were recovered in methylated phospholipid product. Recently, nonenzymatic methylation of phosphatidylethanolamine under conditions comparable to those used here has been demonstrated (25). Thus, although tedious, separation of product for each assay tube appears to be prudent. Results obtained in other ways may be suspect.

Although early reports in intact mouse lymphocytes suggested that concanavalin A stimulates phospholipid methylation and may be required for lymphocyte transformation (26), in the present study PEMT was not stimulated by concanavalin A in human lymphocyte membranes. This finding is in general accord with those of Niwa *et al.* (22, 23), who find minimal stimulation of phospholipid methylation by concanavalin A in sonicates of human lymphocytes, and with the results of others who find no response of human lymphocyte phospholipid methylation to concanavalin A (27, 28).

Niwa *et al.* (22, 23) report that opsonized zymosan stimulates neutrophil phospholipid methylation, but such stimulation was not observed in the present study. Differences in preparative methods, assay conditions, and product identification may account for the different results. In particular, Niwa's assay conditions favored formation of PC, and relatively little PM. Inhibition of turnover of methylated phospholipids in neutrophils by fMLP was reported (29). However, no effect on synthesis was observed. In the present study. methylated phospholipid synthesis was not affected by fMLP, in agreement with prior results.

In tissues in which the coupling of the β-adrenergic receptor to adenylate cyclase is facilitated by phospholipid methylation, β-adrenergic agonists stimulate phospholipid methylation (6, 7, 30). In human lymphocyte and granulocyte membranes, stimulation of PEMT by β-adrenergic agents is dose dependent (Figs. 5 and 7), propranolol inhibitable, has order of potency of agonists typical of β_2-adrenergic systems (isoproterenol> epinephrine>norepinephrine) (Tables 4 and 5), and occurs under conditions under which production of cAMP was not expected (no substrate available, no guanine nucleotides added). Exogenous guanine nucleotides did not enhance the ability of isoproterenol to stimulate PEMT. Moreover, PEMT activity was not stimulated by addition of cAMP (Tables 4 and 5). Stimulation of PEMT by isoproterenol may be a non-cAMP mediated β-adrenergic effect, like inhibition of magnesium transport (31) and the initial stages of desensitization, the uncoupling of receptor and cyclase. Although these membrane preparations contain many subcellular fractions, stimulation of PEMT by isoproterenol but not cAMP suggests that at least some of the enzyme is in the plasma membrane. In tissues in which phospholipid methylation occurs in the microsome (*e.g.* liver), it is stimulated by cAMP, whereas in preparations in which PEMT is thought to be associated with the plasma membrane, there is no stimulation by cAMP (32).

Therefore, PEMT occurs in human lymphocyte and granulocyte (plasma) membranes and is stimulated by β-adrenergic agents. The β_2-adrenergic system in CF leukocytes fails to increase cellular production of cAMP to the same extent as in normal leukocytes (4, 33, 34; Table 6). CF heterozygotes have intermediate response (4, 33). Although a similar coupling defect does not appear to be the cause of the reduced β-adrenergic responses in some other CF tissues (35, 36), the findings in leukocytes have been reproduced in two separate laboratories and, importantly, occur in asymptomatic parents as well (4, 32). This leukocyte receptor-cyclase coupling defect might reflect a more fundamental inherited membrane or regulatory abnormality in CF. The presence of PEMT in leukocyte membranes, and its stimulation by the β_2-adrenergic system, suggests that lymphocyte and granulocyte membranes are a convenient system in which to test the hypothesis that in CF, PEMT activity is reduced.

This hypothesis is attractive because PEMT has been reported to facilitate coupling of β-adrenergic receptors to adenylate cyclase (6–9), and in lymphocytes and granulocytes from CF patients such coupling appears to be impaired (4). In addition, PEMT stimulates human erythrocyte calcium ATPase (10), and the activity of this enzyme is reduced in CF (3). Also, PEMT, along with PC-forming enzyme, appears to be required for stimulus secretion coupling (12, 13), which may be disordered in CF (5). In addition, Markovac and Erickson (37) report that variation in PEMT activity in mice is genetically determined. Thus, PEMT may be an important genetically controlled regulatory membrane enzyme, and its activity in CF is of interest.

However, no reduction in PEMT activity was found in CF in lymphocyte or granulocyte membranes. In lymphocytes, the K_m for SAM was somewhat reduced in CF preparations, but this is inconsistent with the hypothesis that overall PEMT activity is reduced in CF, and the K_m for SAM for PEMT is normal in granulocytes. It is possible that the crude membranes prepared from CF patients and controls contain different proportions of plasma membrane or different amounts of endogenous substrate (PE), thereby altering the apparent specific activity for PEMT. However, membranes prepared in the same way display the CF β-adrenergic defect. If reduced PEMT activity accounts for the impaired coupling, it should be manifest in these preparations. Because it was not, reduced PEMT activity will not explain the diverse disorders of membrane function in CF.

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