INHIBITION OF ACYL COENZYME A:LYSOLECITHIN ACYLTRANSFERASES BY LOCAL ANESTHETICS, DETERGENTS AND INHIBITORS OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES*

W. Thomas Shier

Cell Biology Laboratory, The Salk Institute for Biological Studies,
Post Office Box 1809, San Diego, California 92112

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SUMMARY

Acyl coenzyme A:lysolecithin acyltransferase plays a major role in regulating the amount of lysolecithin in cell membranes. The acyltransferase activity in microsomal preparations from rat liver, rat heart and rabbit gastric mucosa is inhibited by a series of tertiary amine local anesthetics, detergents, and some inhibitors of cyclic nucleotide phosphodiesterases. Aspirin and indomethacin cause elevated lysolecithin/lecithin ratios in the stomachs of mice after oral administration. Inhibition of acyltransferase activity in microsomal preparations by local anesthetics correlates with reported anesthetic potencies at approximately 1/100 reported therapeutic dosages. In BHK-13 cells acyltransferase activity is inhibited at 1/3 to 1/10 the concentrations that have been reported to cause alterations in the mobility and topography of cell surface receptors.

Acyl coenzyme A:lysolecithin acyltransferase (EC 2.3.1.23) is a widespread microsomal enzyme activity (1,2) that catalyses the transfer of predominantly unsaturated fatty acids from their coenzyme A esters to lysolecithin (lysophosphatidylcholine) to produce lecithin (phosphatidylcholine). Since this reaction is not a part of the normal pathway for the de novo synthesis of lecithin, the primary role of this enzyme may be in phospholipid turnover (2) as part of the phosphoglyceride deacylation-reacylation cycle (Fig. 1), in which it removes the lysolecithin produced by widespread endogenous phospholipase A2 activity (1,2). Recent results from this (3,4) and other laboratories (5) have suggested a potential role for lysolecithin in the regulation of membrane-associated enzymes. At physiological concentrations lysolecithin stimulates guanylate cyclase and inhibits adenylate cyclase in membrane preparations from 3T3 mouse fibroblasts (3), rat heart (4) and neuroblastoma cells (5). At a similar lysolecithin-to-protein ratio it stimulates glycosyltransferase activity in rat liver microsomes (6).

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THE PHOSPHOGLYCERIDE DEACYLATION-REACYLATION CYCLE

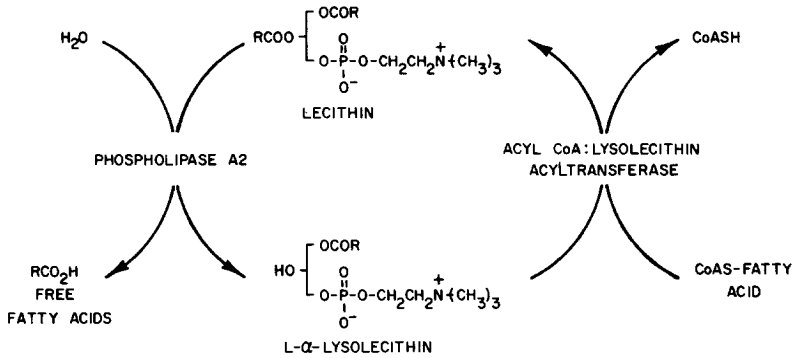


Fig. 1. The phosphoglyceride deacylation-reacylation cycle.

Increased levels of lysolecithin in affected tissues or in plasma has been associated with several disease states including gastric ulceration (7), experimental atherosclerosis (8), multiple sclerosis (9), and hepatoma (10). For example, Johnson (7) has observed a greater than 10-fold elevation of lysolecithin levels in the night gastric juice from gastric ulcer patients. Gastric lysolecithin is presumably produced as a result of duodenal reflux which introduces lysolecithin produced by the action of pancreatic phospholipase. Davenport (11), who showed that lysolecithin destroys the gastric barrier and alters the fluxes of Na^+ and H^+ through canine stomach walls, has suggested this destruction as a mechanism for the induction of gastric ulceration.

MATERIALS

Dipyridamole was the gift of Societa Italiana Medicinali Scandicci, prostaglandins the gifts of Upjohn Co., and chlorpromazine the gift of Smith Kline and French. Lidocaine, dibucaine, phenacaine, pramoxine and proaine amide were obtained from Pfaltz and Bauer as the hydrochlorides. Other chemicals were obtained from Sigma and radiochemicals from New England Nuclear. Triton X-100 and Tween 80 were assigned molecular weights of 603 and 1329 daltons, respectively.

[Choline methyl-^{14}C]-lysolecithin was prepared from [choline methyl-^{14}C]-lecithin by treatment with phospholipase A2 (*Crotalus adamanteus* venom, Worthington) according to the method of Wells and Hanahan (12).

Sprague Dawley rats were obtained from Simenson Laboratories. BHK-13 baby hampster kidney cells were obtained from Dr. W. Eckhart and grown in a humidified CO_2 incubator in Dulbecco-Vogt's modified Eagle's medium

Table 1. Inhibition of oleoyl coenzyme A:lysolecithin acyltransferase in microsomal preparations by local anesthetics.

Inhibitor	Apparent inhibition constant (K_I) (μM)		
	Rat liver	Rat heart	Rabbit gastric mucosa
Lidocaine hydrochloride	1700	1600	760
Dibucaine hydrochloride	16	17	8.4
Tetracaine hydrochloride	58	75	38
Procaine hydrochloride	1000	2000	2300
Quinidine hydrosulfate	17	5.3	ND
Procaine amide hydrochloride	650	420	ND
Pramoxine hydrochloride	24	34	ND
Phenacaine hydrochloride	230	190	ND

ND = not determined

containing 10% calf serum. Balb/c mice were obtained from a colony maintained at The Salk Institute.

Acyltransferase assay *in vitro*. The method of Lands and Hart (13) was used to prepare microsomes from rat liver, rat heart and rabbit gastric mucosa. The oleoyl coenzyme A:palmitoyl-L-α-lysolecithin acyltransferase activity was measured according to the method of Lands and Hart (13) using an assay mixture containing 0.2 mg enzyme protein per ml, 20 μM oleoyl coenzyme A, palmitoyl L-α-lysolecithin in the concentration range 3 to 20 μM, and 1 mM 5,5'-dithio-*bis*-(2-nitrobenzoate) as the coloragen (413 nm). Inhibition constants (K_I) were calculated using double reciprocal plots.

RESULTS

Acyltransferase activity *in vitro*. The acyltransferase activities in microsomal preparations exhibited approximately ideal Michealis-Menton kinetics for non-competitive inhibition in the presence of non-amphipathic inhibitors such as theophyline, but in the presence of amphipathic inhibitors the kinetics deviated significantly from ideality (substrate inhibition at high substrate concentrations). Apparent K_m values of 24.5 μM, 25.8 μM and 23.3 μM were observed for palmitoyl L-α-lysolecithin with microsomal preparations from rat liver, rat heart and rabbit gastric mucosa, respectively. The acyltransferase activities in these preparations, when assayed under similar conditions, were inhibited by similar concentrations of the same group of agents, including local anesthetics (Table 1), detergents and a group of agents that have been reported to inhibit cyclic nucleotide phosphodiesterases in various tissues (14,15) (Table 2).

Table 2. Inhibition of oleoyl coenzyme A:lysolecithin acyltransferases in microsomal preparations by other agents.

Inhibitor class	Inhibitor	Apparent inhibition constant (K_I) (μM)		
		Rat liver	Rat heart	Rabbit gastric mucosa
Inhibitors of cyclic nucleotide phospho-diesterases	Theophylline	32,000	21,000	10,000
	Caffeine	61,000	76,000	48,000
	1-Methyl-3-isobutylxanthine	2,100	2,900	1,000
	Aspirin	33,000	22,000	84,000
	Acetaminophen	9,600	20,000	8,900
	Indomethacin	170	110	190
	Chlorpromazine hydrochloride	48	280	9.6
	Dipyridamole hydrochloride	120	200	120
	Quinacrine dihydrochloride	26	39	24
Detergents	Sodium dodecylsulfate	55	26	5
	Tween 80	6.4	4.7	1.3
	Triton X-100	4.9	4.4	47

An extensive series of agents that have been reported to affect cyclic nucleotide metabolism in various experimental systems were tested for their effect on rat liver acyltransferase activity. No activators were found. The following agents did not affect acyltransferase activity in the indicated concentration range: prostaglandins E_1, E_2, and $F_{2\alpha}$ (10^{-4} to 10^{-9} M); insulin and glucagon (10^{-4} to 10^{-7} M); 3':5'-cyclic AMP, 3':5'-cyclic GMP, L-epinephrine, L-norepinephrine, D,L-isoproterenol, serotonin, L-dopamine, histamine, atropine, acetylcholine, carbamylcholine, methacholine, succinylcholine, putrescine, spermidine, and spermine (10^{-3} to 10^{-7} M); adenosine and Ca^{++} (10^{-2} to 10^{-7} M); K^+ and F^- (10^{-1} to 10^{-7} M); and concanavalin A and trypsin (0.1 to 0.001 mg/ml). Corticosteriods (hydrocortisone, cortisone, dexamethasone, prednisolone and prednisone) and cytochalasin B inhibited acyltransferase activity in these preparations but only at concentrations several orders of magnitude higher than those reported for other activities.

Acyltransferase activity *in vivo*. Except in those tissues in which exchange with lipoproteins or the action of other enzymes plays a significant role in removing lysolecithin, inhibition of acyltransferase activity in tissues would be expected to lead to increased levels of lysolecithin as a result

Table 3. Effect of acyltransferase inhibitors on the lysolecithin/lecithin ratio in the phospholipids of mouse stomach.

Treatment	Dosage[a] (mg/kg)	Lysolecithin/lecithin ratio in treated tissue	Significance[b] with respect to saline control
Saline	-	0.094 ± 0.013	-
Aspirin	200	0.302 ± 0.029	P <0.01
Indomethacin	25	0.229 ± 0.006	P <0.01

[a] Groups of 4 Balb/c mice were radiolabelled in the choline lipids with 5 μCi of [methyl-^{14}C]-choline administered by stomach tube. One week later the drugs dissolved in saline with careful neutralization were administered by stomach tube to groups of 4 mice. The same dose was administered again 3 hours later and after an additional 2 hours the animals were sacrificed, and the stomachs were removed, homogenized and extracted with 5 ml of chloroform: methanol 2:1. Polar materials were removed by extraction with 1 ml of saline. The lower phase was separated and evaporated. The residue was mixed with carrier lipids and fractionated by thin layer chromatography on silica gel in chloroform:methanol:water 65:35:5. The lecithin and lysolecithin spots were visualized with iodine vapor, scraped from the plate and the radioactivity determined by liquid scintillation counting.

[b] Student's T test

of endogenous phospholipase A2 activity. No significant lysolecithinase or lysolecithin:lysolecithin acyltransferase activities were detected in any preparation tested in this study. Elevated lysolecithin/lecithin ratios were observed in the stomachs of mice (Table 3) that were administered aspirin and indomethacin at levels required to inhibit the inflammatory response in mice (16).

The effect of tertiary amine local anesthetics on acyltransferase activity in cultured cells were studied in confluent BHK-13 cells. The increase in the ratio of lecithin to lysolecithin radioactivity is linear with cell protein in growing cells and with time for at least one hour. No significant amount of radiolabelled glycerophosphorylcholine was produced by these cells, indicating no significant amount of lysolecithinase or lysolecithin:lysolecithin acyltransferase activity. Each local anesthetic listed in Table 4, when added to the culture medium at a concentration approximately equal to the K_i values observed with rat heart and liver microsomal preparations, caused a significant (P <0.01) inhibition of the conversion of [^{14}C]-lysolecithin to [^{14}C]-lecithin.

DISCUSSION

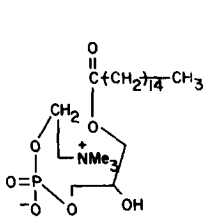
The similarity of both the apparent K_m values and the apparent K_i

Table 4. Inhibition of acyltransferase activity in BHK-13 cells by local anesthetics.

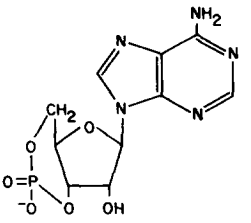
Inhibitor	Concentration in medium (M)	Lecithin/lysolecithin ratio after 45 min[a]
None	-	1.19 ± 0.02
Lidocaine hydrochloride	1.6×10^{-3}	0.55 ± 0.03
Dibucaine hydrochloride	1.6×10^{-5}	0.17 ± 0.01
Tetracaine hydrochloride	7.5×10^{-5}	0.80 ± 0.04
Procaine hydrochloride	2.0×10^{-3}	0.54 ± 0.01
Quinidine hydrosulfate	1.7×10^{-5}	0.54 ± 0.03
Pramoxine hydrochloride	3.5×10^{-5}	0.72 ± 0.06
Phenacaine hydrochloride	2.0×10^{-4}	0.64 ± 0.03

[a] BHK-13 cells were grown to confluence in 3.5cm dishes, washed on the dish twice with cold Tris-saline buffer (0.15 M NaCl, 0.025 M Tris-HCl, pH 7.2; TBS) and incubated with occasional swirling at 4°C for 15 min in 1 ml of TBS containing palmitoyl-[^{14}C-methyl,choline]-lysolecithin (1 µg/ml) and sodium oleate (1 µg/ml). The solution containing any unabsorbed lipid was removed, the cells were washed twice with TBS, and 1 ml of Dulbecco modified Eagle's medium with or without an acyltransferase inhibitor was added. The dishes were incubated 45 min at 37°C in a humidified CO_2 incubator. The medium was removed, the cells were washed twice with TBS and scraped off the dishes in 1 ml of 0.14 M sodium chloride containing 0.01 M Tris and 0.2% EDTA at pH 7.3. The radioactive lipids in the cell suspensions were extracted with 5 ml of chloroform:methanol 2:1, and analyzed as described in Table 3.

values for various inhibitors with the enzyme preparations in Tables 1 and 2 suggests that the acyltransferases in these tissues are very similar, at least in the neighborhood of the active site. Inhibition of acyltransferase activity by inhibitors of cyclic nucleotide phosphodiesterase may reflect the limited structural similarity between lysolecithin and cyclic nucleotides (see Fig. 2). Similar substrate binding structures may have been produced on the two types of enzymes by convergent evolution and these binding structures may both interact with the same inhibitor structures. The use of cyclic nucleotide phosphodiesterase inhibitors has been suggested (14), as a possible rationale for the treatment of diseases associated with lowered cyclic AMP levels (e.g., hypertension, asthma, psoriasis and possibly cancer [17]). The results presented above suggest that this approach may be counterproductive if the relative sensitivities of the phosphodiesterases and acyltransferases in the target tissue lead to increased lysolecithin levels, which in turn may lead to decreased adenylate cyclase activity (3,4,5).



PALMITOYL L-α-LYSOLECITHIN



3':5'-CYCLIC AMP

Fig. 2. The structures of palmitoyl L-α-lysolecithin and 3':5'-cyclic AMP in conformations that reveal structural homology.

Elevated lysolecithin/lecithin ratios in mouse stomachs following oral administration of aspirin (Table 3) suggests a mechanism by which inhibition of acyltransferase activity in the gastric mucosa could be involved in the development of gastric ulceration associated with prolonged use of aspirin at high dosages. If the production of lysolecithin in the stomach as a result of duodenal reflux is an important factor in the development of the disease, as suggested by Davenport (11), inhibition of acyltransferase activity would be expected to limit the ability of the gastric mucosa to resist the lytic effects of duodenal lysolecithin.

Inhibition of acyltransferase activity with a resulting increase in the level of lysolecithin in cell membranes may account for some of the actions of local anesthetics in biological systems. The observed K_i values for the inhibition of acyltransferase activity are approximately 100 times lower than therapeutic concentrations (19), and they correlate with anesthetic potencies reported by Blaustein and Goldman (20) (correlation coefficient, $r = 0.92$, 0.90, and 0.90 for the preparations from rat liver, rat heart and rabbit gastric mucosa, respectively; overall significance, $P < 0.005$). The action of local anesthetics on nerves has been attributed to the closure of sodium channels in the cell membrane (21). The composition of these sodium channels is unknown (21), but lysolecithin accumulating in the membranes of nerve cells could interact with either protein or lipid components of sodium channels in a manner that could result in closure of the channels. The observed K_i values for the inhibition of acyltransferases are 3- to 10-fold lower than the concentrations used to cause dissolution of the cytoskeleton of cultured cells (22). Lysolecithin accumulating in cells treated with tertiary amine local anesthetics could conceivably promote dissolution of the cytoskeleton directly by virtue of its detergent proper-

ties, or by elevating intracellular Ca^{++} by increasing the permeability of membranes.

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