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ACYLTRANSFERASE-CATALYZED TRANSFER OF ARACHIDONIC ACID TO LYSOPHOSPHOLIPIDS IN RAT PANCREATIC ACINI

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The conversion of 2-lysophospholipids into corresponding phospholipids via acyl-CoA acyltransferase was demonstrated in homogenates of rat pancreatic acini. Arachidonic acid was greatly preferred over stearic acid as the acyl donor. Lysophosphatidylinositol and lysophosphatidylcholine acyltransferases were distributed in subcellular fractions of acinar homogenates with specific activity highest in the fractions known to contain secretory organelles and mitochondria. The distribution of lysophosphatidylinositol acyltransferase paralleled that of a mitochondrial marker (succinate cytochrome C reductase). These findings extend the evidence implicating arachidonate release and reincorporation into phospholipids as a link in the pathway that culminates in pancreatic secretion.

Recent studies have implicated arachidonic acid derived from phosphatidylinositol breakdown and turnover in the secretory process of the exocrine pancreas (1-4). A calcium-dependent activation of phospholipase A_2 degrades phosphatidylinositol in certain secretory organs (4-6). This deacylation reaction together with subsequent reacylation of LPI appears to be involved in the mechanism regulating secretion (7). A primary as well as a zymogen-derived form of phospholipase A_2 exists in the rat pancreas (8,9), and the activation of phospholipase A_2 with the resulting release of non-esterfied arachidonate from position 2 of phospholipid is presumed to be responsible for catalyzing the deacylation reaction. In addition, the pigeon pancreas possesses the enzymatic potential for acylating LPI, as well as LPC, with CoA-activated oleic acid (10).

In light of the apparent importance of arachidonic acid metabolism in pancreatic function, the present study was undertaken to demonstrate the acylation

Abbreviations: LPC, lyosphophatidylcholine; LPI, lysophosphatidylinositol; LPS, lysophosphatidylserine; LPE, lysophosphatidylethanolamine.

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of 1-acyl-sn-glycero-3-phospholipids by arachidonic acid in rat pancreatic acini. The relative rates of incorporation of arachidonate into various lysophospholipids catalyzed by this reaction were determined, and preliminary studies on the subcellular location of acyltransferase activities are reported.

MATERIALS AND METHODS

Materials. (1-^{14}C)-Arachidonic acid (58 Ci/mol) was obtained from Amersham/ Searle Corp. and (1-^{14}C)-stearic acid (52 Ci/mol) was purchased from New England Nuclear. All biochemicals, phospholipid standards, and lysophospholipids were purchased from Sigma Chemical Co. with the exception of LPI which was purchased from Avanti Polar Lipids Inc.

Methods. Isolated pancreatic acini were prepared from starved male rats as described in detail elsewhere (4). The acini isolated from a single rat were homogenized in 5ml of 0.3M sucrose containing 0.2mg/ml soybean trypsin inhibitor (11). The homogenate was then centrifuged at 125g for 15 min to sediment debris and nuclei, and the pellet was discarded. The supernatant was centrifuged at 1500g for 15 min. The pellet was resuspended in 10ml of fresh sucrose, centrifuged at 13000g, and resuspended in 600µl sucrose plus trypsin inhibitor. Granule-enriched and mitochondrial-enriched fractions were obtained in certain experiments by sequential centrifugation at 1500g and 13000g for 15 min. The top brownish layer of mitochondria of the 1500 pellet was removed from the underlying white pellet of granules by aspiration using a Pasteur pipet, and the supernatant was centrifuged at 13000g to yield a mitochondrial-enriched fraction. The resultant supernatant was centrifuged at 105,000g for 60 min to obtain a microsomal pellet. Following all of these procedures, which were carried out at 5°C, all pellets were resuspended in 600µl of 0.3M sucrose containing trypsin inhibitor.

Samples from homogenate and cell fractions were used for determinations of amylase (12) and succinate cytochrome C reductase activities (13) and total protein (14). Acyltranferase activity was determined in 100µl of tissue suspension contained in 200µl of cocktail plus lysophospholipid (20µM) and radioactive fatty acid (0.2µCi). The cocktail was composed of Tris-HCl (30mM-pH 8.3); ATP (6mM); Coenzyme A (100µM); Cleland's Reagent (1mM); $MgCl_2$ (6mM); EGTA (1mM). Incubations were generally carried out for 5 min and terminated by adding 3 ml of chloroform/methanol (1:2 v/v). The phospholipids were extracted and separated by thin layer chromatography as previously described (4). Silica gel scrapings of spots corresponding to authentic lipid standards were transferred to 7 ml polyethylene minivials and counted by liquid scintillation spectrometry. The amount of fatty acid incorporated into each phospholipid was calculated from the specific activity of each added fatty acid, corrected for the amount of fatty acid incorporated into endogenous lysophospholipids generated during the incubation.

RESULTS

Upon addition of lysophospholipids as acceptor molecules to homogenate of pancreatic acini, arachidonate incorporation into corresponding phosphoglycerides was enhanced (Fig. 1). The greatest enhancement of acylation activity was observed when LPC and LPI were the acceptor molecules. Acylating activity observed with LPS and LPE was only 10-20% of that observed with LPC or LPI. Enzymic acylation was linear for the initial 30 min of incubation when either

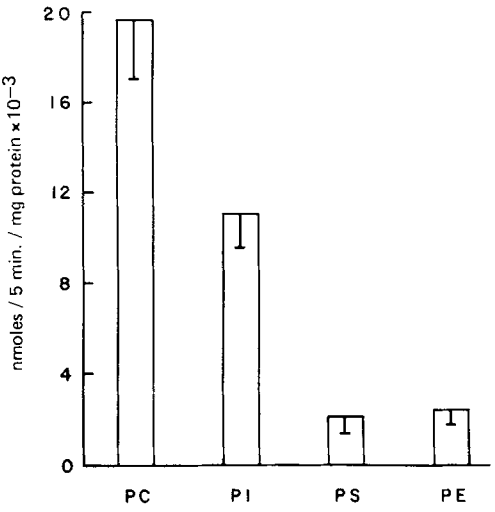

Fig. 1. Acylation of exogenous lysophospholipids by pancreatic acini. LPC, LPI, LPS, or LPE (20 μM) were incubated with 0.2 μCi ^{14}C-arachidonic acid for 5 min at 37°C with homogenate of rat pancreatic acini. Each vertical bar represents the amount of corresponding phospholipid formed. All values are the mean (± S.E.M.) of at least 4 different determinations.

LPC or LPI was the acceptor molecule, and 20μM of the lysophospholipid gave maximal rates of arachidonate incorporation (data not shown). When an equivalent amount of radiolabeled stearate was used as substrate, acylating activity in homogenate was only 2% of that when arachidonate was the donor molecule, and no selectivity for LPI or LPC was observed.

Acylation of added LPC and LPI using arachidonate was highest in the 13000g fraction, representing more than a 30-fold enrichment over homogenate (Fig. 2). Acylation of LPC by the microsomal fraction was only 50% of that detected in the homogenate and acylation of added LPI was virtually undetectable in this fraction. There was essentially no acylating activity in the soluble fraction.

In some experiments additional differential centrifugation procedures were carried out according to established techniques (15) in an attempt to separate secretory granules from mitochondria. While only a partial separation of these two cellular organelles was attained as determined by marker enzymes (Table 1), the ratio of the acylation rate in the 1500 and 13000g fractions using LPI as the substrate (0.50) closely paralleled the ratio of succinate cytochrome C reductase activity (0.63), a marker for mitochondria. Moreover, there was a

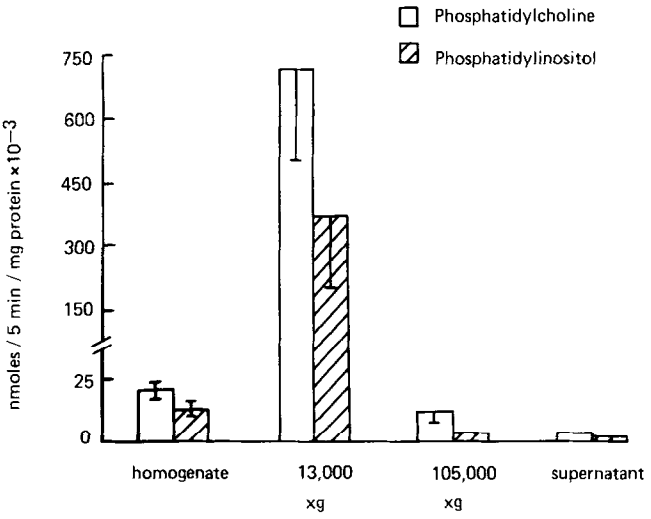

Fig. 2. Distribution of acyltransferases in subcellular fractions of pancreatic acini. Subcellular fractions of homogenate were incubated with ^{14}C-arachidonate plus either LPC or LPI as described in Materials and Methods. Each vertical bar represents the amount of corresponding phospholipid formed. All values are the mean (± S.E.M.) of at least 4 different determinations.

high correlation between acylation of LPI and succinate cytochrome C reductase activity (r=0.98) and a high inverse correlation between acylation of LPI and amylase activity (r= -0.92). By contrast, there was no correlation between acylation of LPC and either of the marker enzymes ($r < 0.5$), although the ratio of the rate of acylation of LPC in the 1500 and 13000g fractions

Table 1. The relative distribution of marker enzymes and acylation of LPC and LPI in pancreatic acini

	Specific Enzyme Activity			
	1500 g fraction		13000 g fraction	
Succinate Cyt. C Reductase [a]	1.2 ± 0.3		1.9 ± 0.3	
Amylase [b]	28 ± 4		10 ± 2	
	LPC	LPI	LPC	LPI
Acyl CoA Transferase [c]	400 ± 35	81 ± 30	190 ± 45	180 ± 25

Values represent mean specific enzyme activity (per mg protein) (± S.E.M.) derived from at least 4 different determinations. [a] moles cytochrome C reduced/min; [b] maltose equivalents formed/3 min; [c] nmoles arachidonate incorporated/5 min x 10^{-3}.

(2.1) closely approximated the ratio of amylase activity (2.8), a marker for secretory granules.

DISCUSSION

Changes in phospholipid metabolism can be effected by the deacylation-reacylation pathway that brings about the non-random distribution of fatty acids in phospholipids (16). In the exocrine pancreas, for example, the molecular species of phosphatidylinositol is 1-stearoyl, 2-arachidonyl-sn-glycero-phosphorylinositol (17). Our previous studies have demonstrated the selective action of various secretagogues to promote the incorporation of arachidonic acid into primarily phosphatidylinositol (4,7). The present investigation provides direct evidence that the rat exocrine pancreas possesses the enzymatic machinery to rapidly and selectively incorporate arachidonic acid into lysophospholipids by demonstrating the existence of an active, membrane-bound acyl CoA: 1-acyl-sn-glycero-3-phospholipid acyltransferase.

The relative selectivity of the unsaturated arachidonic acid as the acyl donor over the saturated stearic acid is in harmony with previous findings that pancreatic secretagogues are unable to promote the incorporation of stearate into acinar phospholipids (4). Moreover, the preferential incorporation of arachidonic acid into LPC and LPI is concordant with findings in other systems (16,18,19). On the other hand, pancreatic secretagogues effectively promote incorporation of arachidonic acid into phosphatidylinositol, but not into phosphatidylcholine, in intact acini (4). This implies that at least part of selectivity of the (arachidonyl) phosphatidylinositol turnover observed during the secretory response of the exocrine pancreas, and perhaps of other secretory cells (4,7), may be ascribed both to the preference of a phospholipase A_2 for phosphatidylinositol (2,4), as well as the avidity of LPI for arachidonic acid (18,19).

Acylation occurred predominantly in the 13,000g fraction containing principally secretory organelles and mitochondria. LPC acyltransferase activity was also observed in the microsomal fraction in agreement with previous findings in brain (20,21), although LPI acyltransferase activity was virtually absent in

this fraction derived from pancreatic acini. The high correlation between LPI acyltransferase and succinate cytochrome C reductase activities connotes a mitochondrial localization of this particular acyltransferase; whereas, the distribution of LPC acyltransferase remains to be determined. The physiological implication of these findings awaits density gradient centrifugation studies to ascertain more definitively the cellular location of these two apparently distinct acyltransferases. Nevertheless, these acyltransferases probably play an important role in the stimulus-induced turnover of arachidonic acid in position 2 of phospholipids in the exocrine pancreas.

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