Molecular Identification of a Novel Mammalian Brain Isoform of Acyl-CoA:Lysophospholipid Acylation Activity, LPEAT2

Jingsong Cao1*, Dandan Shan1, Tracy Revett1, Dongmei Li1, Leeying Wu3, Wei Liu3, James F. Tobin3, and Ruth E. Gimeno2*

From the Departments of 1Cardiovascular and Metabolic Diseases and 2Bioinformatics, Wyeth Research, Cambridge, Massachusetts 02140

Acyl-CoA-dependent lysophospholipid acyltransferases play an important role in attaining the appropriate molecular species of phospholipids. A number of genes encoding these activities were recently identified. It has become clear that multiple genes can encode one enzymatic activity and that a given gene may encode multiple activities. Here we report the identification of a gene encoding a mammalian acyl-CoA-dependent lysophospholipid acyltransferase with prominent activity toward ethanolamine-containing lysophospholipids, which we termed acyl-CoA:lysophosphatidylethanolamine acyltransferase 2, LPEAT2 (previously annotated as AYTL3 or AGPAT7). LPEAT2 is predominantly expressed in brain, coinciding with an enrichment of phosphatidylethanolamine in this tissue. Ectopic expression of LPEAT2 in mammalian HEK293T cells led to a dramatic increase (up to 9-fold) in LPEAT activity when compared with cells transfected with empty vector or an unrelated acyltransferase. LPEAT2 also exhibited significant acyl-CoA-dependent acyltransferase activity toward 1-O-alkenyl-lysophosphatidylethanolamine, lysophosphatidylglycerol, 1-O-alkyl-lysophosphatidylcholine, lysophosphatidylserine, and lysophosphatidylethanolamine but lacked appreciable acylating activity toward phosphatidylcholine, lysophosphatidylserine, and lysophosphatidylglycerol. LPEAT2 also exhibited significant acyl-CoA-dependent acyltransferase activity toward 1-O-alkenyl-lysophosphatidylethanolamine, lysophosphatidylglycerol, 1-O-alkyl-lysophosphatidylcholine, lysophosphatidylserine, and lysophosphatidylethanolamine but lacked appreciable acylating activity toward phosphatidylcholine, lysophosphatidylserine, and lysophosphatidylglycerol. LPEAT2 also exhibited significant acyl-CoA-dependent acyltransferase activity toward 1-O-alkenyl-lysophosphatidylethanolamine, lysophosphatidylglycerol, 1-O-alkyl-lysophosphatidylcholine, lysophosphatidylserine, and lysophosphatidylethanolamine but lacked appreciable acylating activity toward phosphatidylcholine, lysophosphatidylserine, and lysophosphatidylglycerol.

Phospholipids are the major constituents of biological membranes, playing important roles in multiple cellular processes including maintenance of the cellular permeability barrier, regulation of the activities of proteins associated with the membrane, and regulation of intracellular signaling by serving as precursors of signaling molecules (1, 2). The biosynthetic pathways for the major phospholipids have been well established (1, 3). Starting with phosphatidic acid, there are two major pathways for de novo formation of phospholipids: the CDP-choline/CDP-ethanolamine pathway, in which phosphatidic acid is dephosphorylated to form diacylglycerol (DAG)3 followed by transfer of choline or ethanolamine from their CDP conjugates to DAG, and the CDP-DAG pathway, in which phosphatidic acid is activated to form CDP-DAG followed by formation of the phospholipids (Fig. 1). Phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) are mainly formed through the former pathway, whereas the latter one leads to formation of phosphatidylinositol (PtdIns), phosphatidylglycerol (PtdGly), and cardiolipin. PtdSer is synthesized by head group exchange between PtdCho or PtdEtn and serine. A hepatocyte-specific PtdEtn N-methylntransferase converting PtdEtn to PtdCho was reported to play a critical role in maintaining a correct ratio of PtdCho to PtdEtn as well as in lipoprotein homeostasis (4). In addition to the common diacylglycerolphospholipids, significant amounts of two other classes of glycerophospholipids exist in eukaryotic membranes: 1-alkyl-glycerophospholipids and 1-alkenyl-glycerophospholipids (or plasmalogens). These ether-containing phospholipids are usually present in a tissue-specific manner in vivo to exert specific functions; for instance, high levels of 1-alkyl-phospholipids in inflammatory

3 The abbreviations used are: DAG, diacylglycerol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; 1-alkenyl-phosphatidylethanolamine; 1-alkenyl-PtdEtn; PtdIns, phosphatidylinositol; PtdGly, phosphatidylglycerol; PAF, platelet-activating factor; GPAT, acyl-CoA:glycerol-3-phosphate acyltransferase; LPCAT, acyl-CoA:lysophosphatidylcholine acyltransferase; LysoPAPAT, lysoplatelet-activating factor acyltransferase; AGPAT, acyl-CoA:1-acylglycerol 3-phosphate acyltransferase; LPAAT, acyl-CoA:lyso-phosphatidic acid acyltransferase; LPEAT, acyl-CoA:lysophosphatidylethanolamine acyltransferase; DGAT, acyl-CoA:diacylglycerol acyltransferase; LPLAT, acyl-CoA:lysophosphatidylcholine acyltransferase; MBOAT, membrane-bound O-acyltransferase; LysoPtdGly, lysophosphatidylglycerol; LysoPAPAT, 1-O-alkyl-lysophosphatidylcholine; siRNA, small interfering RNA; PtdSer, phosphatidylserine; ER, endoplasmic reticulum; LysoPtdEtn, lysophosphatidylethanolamine; LysoPtdGly, lysophosphatidylglycerol; LysoPtdCho, lysophosphatidylcholine.
cells are important for the formation of PAF, and 1-alkenylphospholipids (plasmalogens) are enriched in the nervous system, where they are major lipid components of the myelin sheath (2). After de novo formation, phospholipids undergo rapid remodeling, catalyzed by phospholipase (e.g., phospholipase A2) and lysophospholipid acyltransferases (LPLATs; Fig. 1) (5–7). This remodeling process serves to maintain and adjust the appropriate distribution of acyl groups in particular membranes and also generates many bioactive lipids in particular tissues or cells, for example PAF in inflammatory cells as above.

EXPERIMENTAL PROCEDURES

Materials—Unless stated otherwise, all lipids and acyl-CoAs were purchased from Avanti Polar Lipids (Alabaster, AL) or Sigma. Origins or species of lysophospholipids are as follows: lysophosphatidylcholine (Sigma, from bovine brain, contains primarily palmitic, stearic, and oleic acids); lysophosphatidylinositol (Sigma, from egg yolk, contains primarily palmitic or stearic acids); lysophosphatidylglycerol (Avanti Polar Lipids, from porcine brain, mainly contains 1-alkenyl-LPE with stearic acyl); 1-O-palmitoyl-sn-3-phosphocholine (LysoPAF, Sigma); lysophosphatidylinositol (Sigma, 1-palmitoyl); lysophosphatidylglycerol (Avanti Polar Lipids, 1-stearoyl); lysophosphatidylinositol (Sigma, form soybean, contains primarily palmitic and stearic acids); and lysophosphatic acids (Sigma, 1-oleoyl). [14C]acetyl-CoA (C2:0), [14C]maltoglycerol (Avanti Polar Lipids, from porcine brain, mainly contains 1-alkenyl-LPE with stearic acyl); 1-O-palmitoyl-sn-3-phosphocholine (LysoPAF, Sigma); lysophosphatidylinositol (Sigma, 1-palmitoyl); lysophosphatidylglycerol (Avanti Polar Lipids, 1-stearoyl); lysophosphatidylinositol (Sigma, form soybean, contains primarily palmitic and stearic acids); and lysophosphatic acids (Sigma, 1-oleoyl). [14C]acetyl-CoA (C2:0), [14C]maltoglycerol (Avanti Polar Lipids, from porcine brain, mainly contains 1-alkenyl-LPE with stearic acyl); 1-O-palmitoyl-sn-3-phosphocholine (LysoPAF, Sigma); lysophosphatidylinositol (Sigma, 1-palmitoyl); lysophosphatidylglycerol (Avanti Polar Lipids, 1-stearoyl); lysophosphatidylinositol (Sigma, form soybean, contains primarily palmitic and stearic acids); and lysophosphatic acids (Sigma, 1-oleoyl). [14C]acetyl-CoA (C2:0), [14C]maltoglycerol (Avanti Polar Lipids, from porcine brain, mainly contains 1-alkenyl-LPE with stearic acyl); 1-O-palmitoyl-sn-3-phosphocholine (LysoPAF, Sigma); lysophosphatidylinositol (Sigma, 1-palmitoyl); lysophosphatidylglycerol (Avanti Polar Lipids, 1-stearoyl); lysophosphatidylinositol (Sigma, form soybean, contains primarily palmitic and stearic acids); and lysophosphatic acids (Sigma, 1-oleoyl).
the cells were harvested in cold phosphate-buffered saline, and the cell pellets were either used immediately or stored at −70 °C. To express LPEAT2 in a bacterial system, N-terminally FLAG-tagged LPEAT2 cDNA in pcDNA3.1/V5-His was subcloned into EcoRI and NotI sites of the pET21a vector (Novagen) to ensure an in-frame translation of full-length protein. The plasmid was transformed into bacterial strain BL21 (DE3) (Invitrogen). The cells were grown in LB medium at 37 °C to an A600 of 0.6, followed by a 12-h incubation in the presence of 0.4 mM isopropyl β-D-thiogalactopyranoside. The cells were harvested in cold phosphate-buffered saline, and cell pellets were stored at −70 °C. Mammalian and bacterial cells were disrupted by probe sonication with predetermined settings. The resulting cell lysate was used for Western analysis or activity assay after determination of total protein with a Bio-Rad protein assay.

In Vitro Acyltransferase Activity Assays—LPLAT activity was determined by measuring the incorporation of [14C]-labeled acyl moieties of acyl-CoA or acetyl group from acetyl-CoA (acyl donor) into phospholipids in the presence of corresponding lysophospholipids (acyl acceptor) as previously described (11, 16). Briefly, the reaction mixture contained 80 mM Tris/HCl, pH 7.5, 100 or 200 μM each lysophospholipid, 50 μM [14C]-acyl-CoA (50 mCi/mmol) or [14C]acyetyl-CoA (50 mCi/mmol), and cell lysate (containing 50 μg of total protein) in a total volume of 50 μl. The reaction was terminated by adding 375 μl of chloroform:methanol (2:1, v/v) after incubation at room temperature for 15 min. The lipids were extracted by vigorously vortexing for 10 min after the addition of 0.1 ml of 0.9% KCl. Phase separation was achieved by a brief spin. The organic phase (bottom) was then transferred to a fresh glass tube, dried under N2 steam, and visualized by exposure to a PhosphorImager screen. Under some circumstances, quantification was achieved by a brief spin. The organic phase (bottom) was then transferred to a fresh glass tube, dried under N2 steam, and visualized by exposure to a PhosphorImager screen. The enzymatic products were identified with authentic standards by exposure to I2 vapor. Under some circumstances, quantification was achieved by exposure to I2 vapor. The resulting cell lysate was used for Western analysis or activity assay after determination of total protein with a Bio-Rad protein assay.

Quantitative Reverse Transcription-PCR Analysis—TaqMan real time quantitative PCR was performed using an ABI Prism 7900 sequence detector (PE Applied Biosystems, Foster City, CA) with 18 S as internal control, as described (16). Gene-specific primers were obtained from Applied Biosystems (Mm01336600_g1 and Hs00405723_m1 for mouse and human LPEAT2, respectively). Relative expression was determined by the Ct method (Applied Biosystems).

siRNA-mediated Knockdown of Gene Expression—HEK293T cells cultured in 6-well plate with 2 ml of medium were transfected with 50 pmol (final concentration, 25 μM) of control siRNA (Ambion catalog number 4613) or two LPEAT2-specific siRNA duplexes purchased from Ambion (siLPEAT2–1, identification number 217054; siLPEAT2–2, identification number 217053). 48 h after transfection, LPEAT2 mRNA was measured by quantitative PCR, and lysophospholipid acyltransferase activities were determined as described above.

Statistical Analysis—Statistical significance was determined by Student’s t test.

RESULTS

Identification and Cloning of the Human LPEAT2 Gene—Using a seed alignment sequence derived from the previously described glycerolipid acyltransferase motif (PF00553) (19), we have previously generated a comprehensive list of human, mouse, and rat genes containing the glycerolipid acyltransferase motif (PF00553) (16). Our list contained a subfamily of several orphan acyltransferases, two of which were recently deorphaned and shown to encode enzymes mediating the formation of PtdCho, 1-O-alkyl-PtdCho, and PAF in lung alveolar type II cells and inflammatory cells via the remodeling pathway, respectively (12–14). The four signature motifs important for acyltransferase catalytic activity are present at N-terminal acyltransferase domain (Fig. 2, boxed letters). This gene shares a high degree of homology with LPCAT1 and LysoPAFAT/LPCAT2 (34 and 43% identity, respectively), and analysis of transcriptional profiling data suggested that it was highly expressed in brain and inflammatory cells, suggesting that it might function as a lysophospholipid acyltransferase in these tissues. A full-length cDNA was obtained and shown to encode a 524-amino acid protein with a predicted molecular mass of 57.2 kDa. The four signature motifs important for acyltransferase catalytic activity are present at N-terminal acyltransferase domain (Fig. 2, boxed letters). Although we found no Ca2+ binding EF hand motif in contrast to LPCAT1 and LysoPAFAT/LPCAT2, several residues important for Ca2+ binding within an EF hand motif were conserved (Fig. 2, red letters). The protein is predicted to contain one transmembrane domain (Fig. 2, amino acids 40–62, indicated
LPEAT2 Is a Lysophospholipid Acyltransferase

A.

B.

![Graph showing tissue distribution of human and mouse LPEAT2 mRNA levels](image)

**FIGURE 3.** Tissue distribution of human (A) and mouse (B) LPEAT2 mRNA detected by quantitative PCR. LPEAT2 expression levels were normalized to 18 S rRNA by the Ct method as recommended by Applied Biosystems. The data are expressed as the means ± S.D. (n = 4–6).

with red bars above the sequence). A mouse ortholog of LPEAT2 was identified based on genomic data base searching, sharing 93% amino acid identity with the human gene (data not shown).

**Tissue Distribution of LPEAT2 Transcripts in Mouse and Human**—As queried using several transcriptional profiling databases, transcripts of human LPEAT2 were predominantly detected in whole brain and different brain regions; significant levels were also noticed in inflammatory tissues and cells (data not shown). Reassessment of tissue distribution of human and mouse LPEAT2 mRNA by TaqMan real time reverse transcription-PCR confirmed abundant expression in brain in both species (Fig. 3). Human and mouse LPEAT2 transcripts were also expressed in many other tissues, albeit at much lower levels when compared with brain.

Notably, LPEAT2 mRNA expression levels were very low in liver and lung in both species, suggesting that LPEAT2 does not play a major role in phospholipid formation in these tissues with active phospholipid remodeling.

**Lysophospholipid Acyltransferase Activity of LPEAT2**—To determine the enzymatic activities of LPEAT2, we overexpressed N-terminally FLAG-tagged hLPEAT2 in HEK293 cells. Western blot analysis showed an apparent molecular mass of ~60 kDa for FLAG-hLPEAT2 (Fig. 4A), consistent with a predicted value of 58.3 kDa, suggesting an absence of major post-translational modifications. Lysates from cells overexpressing LPEAT2 were tested for acyl-CoA-dependent acyltransferase activities toward a variety of lysophospholipids. Considering the predominant expression of LPEAT2 in brain, a tissue where PtdEtn is enriched, we hypothesized that LPEAT2 might encode a protein with LPEAT activity. Indeed, lysates from cells overexpressing LPEAT2 showed a dramatic ~9-fold increase in the formation of radiolabeled PtdEtn compared with cells transfected with empty vector or hDGAT1 (Fig. 4B, lane 3 versus lanes 1 and 2). No appreciable PtdEtn formation was observed in samples lacking exogenously added LysoPtdEtn, demonstrating that the PtdEtn formed was derived from LysoPtdEtn (Fig. 4B, lane 4). As expected, overexpression of DGAT1 led to a significant (~30-fold) increase in DGAT but not LPEAT activity, and no increase in DGAT activity was detected from the cells overexpressing recombinant LPEAT2 (Fig. 4C). In addition to PtdEtn, overexpression of LPEAT2 also greatly (4–7-fold) enhanced formation of PtdGly (Fig. 4D) and alkyl-PtdCho (Fig. 4E) from LysoPtdGly and LysoPAF (1-O-alkyl-LysoPtdCho), respectively. Formation of PtdCho and PtdSer was also moderately (~2–3-fold) increased (Fig. 4F). In contrast, no significant increase in lysosphatidylinositol or lysosphatidic acid acyltransferase activity was found in LPEAT2-overexpressing cells as compared with control cells (Fig. 4G). Specific activities calculated from Fig. 4 (B–G) are shown in Fig. 4H. During our studies, we noticed that LysoPtdEtn appears to be less soluble than other lysophospholipid substrates, which may contribute to a lower apparent specific activity for LysoPtdEtn compared with other substrates. Although the background levels of acyltransferase activities in our experiments are in line with those observed by Chen et al. (12), our values are lower than the ones reported by Nakanishi et al. (13) and Shindou et al. (14), possibly reflecting differences in the cell lines used, protein expression levels, and assay conditions. In separate experiments, overexpression of LPEAT2 resulted in no increase in GPAT or LysoPAF acyltransferase activity (data not shown). These data show that LPEAT2 encodes a lysophospholipid acyltransferase that can utilize a subset of lysophospholipids as substrates and, importantly, can catalyze the formation of PtdEtn.

The high levels of endogenous lysophospholipid acyltransferase activities observed in mammalian cells (Fig. 4H; see also Refs. 11–14 and 16) may make it difficult to conclusively define the substrate specificity of LPEAT2. Similar to other investigators (12, 14, 20, 21), we chose a mammalian expression system for the initial evaluation of enzyme activity to maximize the chances of expressing functional protein. Recently, Soupene et al. (22) reported that murine lysosphospholipid acyltransferases expressed in Escherichia coli show significant enzymatic activity above a much lower background. We expressed LPEAT2 in E. coli and assessed enzymatic activities. Similar to our findings in mammalian cells, we detected a significant increase in acyltransferase activities toward LysoPtdEtn, 1-alkenyl-LysoPtdEtn, LysoPAF,
LPEAT2 Is a Lysophospholipid Aciyltransferase

and LysoPtdCho but not LysoPtdGly and lysophosphatidylinositol in E. coli lysates heterogeneously overexpressing LPEAT2 (supplemental Fig. S1B). However, although the expression levels of recombinant LPEAT2 in E. coli were much higher than in mammalian HEK293 cells (supplemental Fig. S1A), the specific activities measured (expressed as nmol product formed per min per mg of protein) were at least 10-fold lower than those observed in mammalian cells (Fig. 4H and supplemental Fig. S1B), suggesting a unique advantage of the mammalian expression system in preserving the functional activity of recombinant LPEAT2. Another approach to overcome the high levels of endogenous background activities is to purify the recombinant protein in an active form from its crude preparation. We found that the activities of LPEAT2 were very sensitive to inactivation by detergent (see, for example, supplemental Fig. S2), making purification of this membrane-associated protein challenging. To date, we have not yet identified the purification conditions that allow us to retain the activity of LPEAT2. It is likely that the catalytic activity of LPEAT2 requires its presence in an intact membrane, similar to other ER-associated acyltransferases (23).
**LPEAT2 Is a Lysophospholipid Acyltransferase**

![Diagram](image)

**FIGURE 5.** Acyltransferase activity of LPEAT2 toward 1-acyl-LysoPtdEtn and 1-alkenyl-LysoPtdEtn (A) and LysoPtdGly and 1-alkyl-PtdCho (B). The acyltransferase activities were conducted in the presence of 14C-labeled oleoyl-CoA or arachidonoyl-CoA and each lysophospholipid acceptor as indicated. The data are representative of two independent experiments with similar results. 1-alkenyl-PtdEtn, 1-alkenyl-phosphatidylethanolamine; 1-alkenyl-LysoPtdEtn, 1-alkenyl-lysophosphatidylethanolamine.

**FIGURE 6.** Acyl-CoA preference of the LPEAT (A) and 1-alkenyl-LPEAT activity (B) and kinetic analysis (C and D) of LPEAT activity in LPEAT2. In A and B, assays were conducted with a variety of 14C-labeled acyl-CoAs (50 μM) and LysoPtdEtn (A) or 1-alkenyl-LysoPtdEtn (B) (each at 200 μM). In C and D, substrate concentration dependence of LysoPtdEtn (C) or Oleoyl-CoA (D) was determined with the indicated concentrations of LysoPtdEtn or [14C]oleoyl-CoA in the presence of 50 μM of [14C]oleoyl-CoA or 200 μM of LysoPtdEtn, respectively. The data are representative of two independent experiments with similar results.

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**1-Alkenyl-LysoPtdEtn Acyltransferase Activity of LPEAT2**—PtdEtn and other phospholipids are usually composed of three subclasses: diacyl, alkylacyl, and alkenylacyl, among which the diacyl form is the most common one in most tissues (referred as PtdEtn in this study). In addition to PtdEtn, mammalian brain and nervous system are enriched particularly with 1-alkenyl-PtdEtn being called ethanolamine plasmalogen (24, 25). To investigate whether LPEAT2 catalyzes the remodeling of ethanolamine plasmalogen as it does with PtdEtn, we carried out acyltransferase reactions with 1-alkenyl-LysoPtdEtn as acyl acceptor. As shown in Fig. 5A, when oleoyl-CoA was used as acyl donor, LPEAT2 ectopically expressed in 293T cells led to a dramatic increase (~7.8-fold compared with background) in the formation of radiolabeled 1-alkenyl-PtdEtn, which is comparable with (and even greater than) the formation of PtdEtn (~5.3-fold). Overexpression of LPEAT2 also resulted in a modest increase (~2.4- or 2.0-fold) in incorporation of arachidonoyl into 1-acyl- or 1-alkenyl-PtdEtn. However, preference of this brain-enriched polyunsaturated fatty acyl-CoA over oleoyl-CoA by LPEAT2 was not observed (Fig. 5A). Similarly, the acyltransferase activity of LPEAT2 toward other lysophospholipids such as LysoPtdGly and 1-alkyl-PtdCho also preferred oleoyl-CoA over arachidonoyl-CoA (Fig. 5B).

**Acyl-CoA Preference and Kinetic Analysis of LPEAT2**—We next examined the LPEAT and 1-alkenyl-LPEAT activities of LPEAT2 toward a broad range of acyl-CoA species. As shown in Fig. 6A (LPEAT) and 6B (1-alkenyl-LPEAT), LPEAT2 showed a preference for long chain (C16:0, C18:0, and C18:1) over short (C2:0 and C3:0), medium (C8:0), or very long chain (C20:4) fatty acyl-CoAs. No preference for unsaturated over saturated fatty acyl-CoAs was observed (Fig. 6A and B). To fur-
ther characterize the enzymatic activity of LPEAT2, we examined its activity at different concentrations of acyl-CoA donor and acceptor, using oleoyl-CoA and LysoPtdEtn as substrates. As shown in Fig. 6C, incorporation of the oleoyl group of oleoyl-CoA into PtdEtn increased in a concentration-dependent manner when LysoPtdEtn was varied (up to 400 μM) and oleoyl-CoA was held constant (50 μM). The calculated maximal velocity of this reaction was 260 pmol/min/mg protein with the apparent Km for LysoPtdEtn being ~80 μM. When LysoPtdEtn was held constant (200 μM), LPEAT2 activity increased with increasing concentrations of oleoyl-CoA up to 50 μM, at which the activity appeared to reach a plateau (~280 pmol/min/mg of protein) and was inhibited by further increases of oleoyl-CoA (Fig. 6D). The apparent Km for oleoyl-CoA was ~20 μM. Importantly, the activity of LPEAT2 was higher in LPEAT2-expressing cell lysates as compared with control lysates at all concentration tested.

Despite the presence of amino acids conserved with the Ca2+-binding domain of LPCAT1 and LysoPafAT/LPCAT2, and the reported Ca2+ dependence of LysoPafAT/LPCAT2 (one of the two closest homologs for LPEAT2), LPEAT as well as other lysophospholipid acyltransferase activities of LPEAT2 were not affected by the addition of exogenous Ca2+ or Mg2+ (data not shown).

Subcellular Localization of LPEAT2—To examine the subcellular localization of LPEAT2, we overexpressed FLAG-tagged LPEAT2 in HEK293T cells as well as in Neural 2A cells, a neuronal cell line. By immunofluorescence, LPEAT2 in both cell lines displayed an asymmetrically perinuclear pattern (Fig. 7, a, d, g, and j) that was distinct from the staining of a mitochondrial marker (MitoTracker Red CMXRsos) (Fig. 7, b and h) and antibody specific for calnexin (panels e and k), respectively. Merged pictures (panels c, f, i, and l) of the left two corresponding images were shown in the right panels. The experiments were repeated three times with similar results. Bar, 20 μm.
LPEAT2 Is a Lysophospholipid Acyltransferase

some, but not all, of the LPEAT and 1-alkenyl-LPEAT activity in HEK293 cells. Interestingly, LPGAT, LysoPAFAT, LPCAT, and lysophosphatidylinositol acyltransferase activity was not affected by LPEAT2 knockdown (Fig. 8B), suggesting that these activities of LPEAT2 are not important contributors to the endogenous acyltransferase activity for each of these lysophospholipids in HEK293 cells.

DISCUSSION

The present study identifies AGPAT7, an orphan member of the glycerolipid acyltransferase family with no known enzymatic activity, as an acyl-CoA-dependent lysophospholipid acyltransferase with prominent activity acylating ethanolamine-containing lysophospholipids (i.e. 1-acyl-LysoPtdEtn and 1-alkenyl-LysoPtdEtn). During the revision of this manuscript, Hishikawa et al. (26) reported identification of a distinct mouse LPEAT1, which belongs to a totally different acyltransferase family, termed membrane-bound O-acyltransferases (MOBAs) and was previously annotated as MBOAT1. They have renamed the MBOAT1 gene LPEAT1. Because our data identify AGPAT7 as a second enzyme showing prominent LPEAT activity, we renamed this gene LPEAT2. LPEAT2, when overexpressed in mammalian cells, shows significant and substantial acyltransferase activity toward multiple lysophospholipid substrates, namely 1-acyl-LysoPtdEtn, 1-alkenyl-LysoPtdEtn, LysoPtdGly, and LysoPAF, and also conferred less pronounced activity toward lysophosphatidylserine and LysoPtdCho. Importantly, LPEAT2 is predominantly expressed in the brain, a tissue with known high levels of PtdEtn, suggesting that the primary physiological function of LPEAT2 may be remodeling of PtdEtn.

Similar to its closest homologs, LPCAT1 and LysoPAFAT/LPCAT2 (12–14), LPEAT2 can acylate multiple, but distinct lysophospholipids. However, we found significant differences between the activities of LPEAT2 and its homologs; unlike LPCAT1 and LysoPAF/LPCAT2, which show a substantial (3–9-fold) increase in LysoPtdCho acyltransferase activity when overexpressed in mammalian cells (12–14), LPEAT2 overexpression resulted in only a small (~2-fold) increase using LysoPtdCho as a substrate. In contrast, although LPCAT1 overexpression resulted in only a modest (2-fold) (12, 13) increase in LysoPtdEtn acyltransferase activity, LPEAT2 overexpression resulted in a 5–9-fold increase using LysoPtdEtn as a substrate. Furthermore, unlike LysoPAF/LPCAT2 (14), LPEAT2 lacks significant LysoPAF acetyltransferase activity. Finally, LPEAT2 showed no preference for saturated (palmitoyl-CoA) over unsaturated acyl-CoA (oleoyl-CoA), in contrast to what has been reported for LPCAT1 (12, 13). As expected from the absence of a canonical EF hand motif, the activity of LPEAT2 was not modulated by the presence of calcium or magnesium. Despite its previous annotation as AGPAT7, LPEAT2 did not confer acyltransferase activity against acyl-glycerol-3-phosphate. In addition, no acyltransferase activity was detected against other precursors in neutral lipid/phospholipids biosynthesis, such as glycerol-3-phosphate and diacylglycerol, suggesting that LPEAT2 specifically acts in phospholipid remodeling rather than de novo synthesis of neutral lipids or phospholipids.

LPEAT2 also facilitates the acylation of several other lysophospholipids. Like LPCAT1 (13), LPEAT2 shows significant levels of LPGAT activity. An additional acyltransferase catalyzing formation of PtdGly via the remodeling pathway, LPGAT1, has also been recently identified (15). Despite the presence of the conserved glycerolipid acyltransferase motif, LPGAT1 does not share appreciable homology with LPEAT2. The physiological significance of the LPGAT activity in LPEAT2, LPCAT1, and LPGAT1 remains to be investigated. LPEAT2 also shows significant levels of LysoPAF acyl (but not lyso-PAF acetyl) transferase activity. Because LPEAT2 is also highly expressed in inflammatory cells (from transcriptional profiling, data not shown), the 1-O-alkyl-LysoPtdCho-acylating activity could be of physiological significance, possibly regulating cellular levels of arachidonic acid and its derivatives. Recognition of multiple structurally similar substrates is not unusual in lipid metabolic enzymes. For example, DGAT1, an enzyme catalyzing conversion of diacylglycerol to triacylglycerol, also facilitates the synthesis of diacylglycerol, waxes, and retinyl esters (27), and members of DGAT2 protein family can acylate both wax alcohols and DAG (28).

During the preparation of this manuscript, Soupene et al. (22) independently reported AGPAT7 as a close homolog of LPCAT1 and LysoPAFAT/LPCAT2. They overexpressed mouse AGPAT7/LPEAT2 in E. coli and found a weak LPCAT activity and no detectable LPEAT activity; other phospholipid substrates were not tested. We expressed human LPEAT2 in E. coli and found increased acyltransferase activities toward a variety of substrates, including LysoPtdEtn and 1-alkenyl-LysoPtdEtn. However, despite the robust expression levels of LPEAT2 protein in E. coli, in our hands the specific activities observed in E. coli are substantially less than those found in the mammalian system, raising questions about whether the expressed LPEAT2 protein is correctly folded in E. coli. Altered phospholipid composition in bacterial compared with mammalian membranes may also affect both the activity of membrane-associated proteins and the presentation of phospholipid substrates. Although the differences between our findings and Soupene et al. (22) may reflect differences between human and mouse LPEAT2, it is also possible that the use of E. coli versus mammalian expression system could contribute to these differences.

To begin to investigate the function of LPEAT2 in the context of a cell, we used small interfering RNA-mediated knockdown of LPEAT2 in HEK293 cells. Depletion of LPEAT2 resulted in a significant decrease in LAPET and 1-alkenyl-LPEAT activity but did not affect LPGAT and LysoPAFAT activity, suggesting that LPEAT2 is primarily an important contributor to LysoPtdEtn acyltransferase activity in this cell type. The maximum decrease in LPEAT and 1-alkenyl-LPEAT activity observed was ~25% and ~50% respectively, compared with a more than 50% decrease in LPEAT2 transcripts, suggesting the existence of additional mammalian LPEATs. A new class of mammalian lysophospholipid acyltransferases, some of which show LPEAT activity, were recently identified (26, 29). These enzymes are members of the MBOAT family, mammalian homologs of which include previously identified acyl-CoA:cholesterol acyltransferases 1 and 2 and DGAT1, as well as several other uncharacterized proteins. It is possible that MBOAT family member LPEAT1 or another, not yet characterized
member of this family contributes to the remaining LPEAT activity in HEK293 cells depleted of LPEAT2.

In addition to distinct substrate preferences, LPEAT2, LPCAT1, and LysoPAFAT/LPCAT2 also exhibit distinct tissue distribution patterns, suggesting distinct functions in vivo. LPCAT1, highly expressed in lung alveolar type II cells, was proposed to be an enzyme catalyzing the formation of dipalmitoyl-PtdCho, a major component of lung surfactant (12, 13). LysoPAFAT/LPCAT2, highly expressed in inflammatory cells, may play important roles in inflammation and other cellular functions elicited by PAF (14). Here we show that LPEAT2 is predominantly expressed in brain, a tissue that is highly enriched in PtdEtn, suggesting a possible role for LPEAT2 in modulating brain phospholipid composition. Thus, three isoforms of lysophospholipid acyltransferases, with sequence homology to each other, are expressed in a tissue-specific manner and play important roles in attaining specific phospholipid species to meet the distinct functional needs of target tissues. The tissue distribution of LPEAT2 is also distinct from MBOAT1/LPEAT1 or MBOAT5/LPCAT3, which are most highly expressed in nonbrain tissues (26, 29). In the nervous system, ethanolamine phospholipids are major constituents of the myelin sheath, which are produced by oligodendrocytes (centrally) or Schwann’s cells (peripherally), increasing signal transmission speed along the axon. It will be interesting to determine the role of LPEAT2 in formation and function of myelin sheaths and in the pathogenesis of demyelinating diseases, such as multiple sclerosis. PtdEtn has also been implicated in the pathogenesis of other neurological disorders. For example, decreased PtdEtn, as well as PtdCho, levels are associated with Alzheimer disease, and modification of membrane phospholipids has been suggested to be useful in restoring brain function in this disease (25, 30–32). Molecular identification of LPEAT2 as a phospholipid-remodeling enzyme highly expressed in brain opens the door to examine the role of this enzyme in neuronal development and in the pathology of central nervous system-related diseases.

In summary, we have identified a human gene encoding an acyl-CoA-dependent lysophospholipid acyltransferase, which we termed LPEAT2. Although LPEAT2 possesses substantial acyltransferase activity toward multiple lysophospholipid substrates, we decided to name it LPEAT2 based on its predominant expression in brain where PtdEtn is enriched compared with other phospholipids and based on our finding that LPEAT2 depletion in mammalian HEK293 cells selectively reduces LPEAT and 1-alkenyl-LPEAT activity. Importantly, LPEAT2 is the first example of a brain mammalian lysophospholipid acyltransferase that has substantial LPEAT activity. Given the predominant expression of LPEAT2 in brain and the significant enrichment of PtdEtn in this tissue, LPEAT2 may play an important role in phospholipid remodeling and PtdEtn enrichment in the brain.

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