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Secretion of Lecithin:Cholesterol Acyltransferase by Brain Neuroglial Cell Lines

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The ability of different human and rat brain cell lines (neuronal and gliomal) to secrete lecithin:cholesterol acyltransferase (LCAT) was examined. Of these, the strongly secreting human gliomal (U343 and U251) cell lines were selected for a detailed study of enzymatic and structural properties of the secreted LCAT. Both plasma- and brain-derived enzymes are inhibited by DTNB (90%) and are activated by apolipoprotein A-I. LCAT mRNA was measured in these cell lines at levels similar to that found in HepG2 cells. In contrast, apoA-I, apoE, and apoD mRNAs were undetectable in these cell lines. The presence of functional LCAT secreted by cultured nerve cells provides an *in vitro* model to study the expression and function of LCAT in the absence of others factors of plasma cholesterol metabolism.

Key Words: **LCAT; nerve cells.**

The major part of cholesteryl ester in plasma is formed by lecithin:cholesterol acyltransferase (LCAT), (EC.2.3.1.43.). This enzyme is an integral part of several HDL species (1) and catalyses the transfer of an acyl group from lecithin to the 3-hydroxyl group of cholesterol (2). LCAT is secreted from the liver as well as by the human hepatoma cell line, HepG2 (3, 4). More recently LCAT mRNA was identified in brain, and subsequently by in situ hybridization (5, 6). However, the properties of brain LCAT activity have not been characterized. Cerebrospinal fluid contains LCAT (7) although the enzyme is relatively inactive and represents 20 fold less than the activity found in plasma. In the nervous system, cholesterol is obtained both by synthesis (8-10) and through receptor mediated uptake of cholesterol-carrying lipoproteins (11-13). In plasma, LCAT plays an important role in regulating cell membrane cholesterol content (14). However the plasma LCAT pool is probably not accessible to nerve tissue, because of the blood brain barrier (15). LCAT derived from nerve cells may play this role in the cerebral extracellular space. Here, we provide evidence that LCAT is synthesized and secreted extracellularly by neuroglial cells.

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Abbreviations: ApoA-I, apolipoprotein A-I; LCAT, lecithin:cholesterol acyltransferase; GFAP, glial fibrillary acidic protein.

MATERIALS AND METHODS

Cell cultures. Brain cell lines, SH-SY-SY, SK-N-MC, SK-N-SH, Kelly, NLF, NGP, MCN-1-1, LAN-1, LNA-2 and LNA-5 were kindly provided by Dr. Michael Bishop (UCSF). Mouse Neuro2A, rat RT4B and human glioma/astrocytoma U251 and U343-MGa-C12:6 were kindly provided by Dr. Francois Besnard (National Institute of Health). Cells were grown in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine and 5 μg/ml gentamycin at 37°C in a 5% CO_2 atmosphere. To determine LCAT activity, the cells were washed 6 times with phosphate-buffered saline (PBS) and then cultured 36 h in the same medium where the fetal calf serum was replaced by Ultroser G (2%) (IBF, France). After removing the medium, the cells were washed twice with PBS, then dissolved in 0.1 N NaOH and sonicated two times for 30 seconds. Protein concentrations were determined according to Lowry (16).

Assay of LCAT activity. LCAT activity was determined as the rate of synthesis of labeled cholesteryl esters from [1,2,^{3}H-cholesterol] (New England Nuclear, Boston, MA); specific activity 60 Ci/mmol in benzene. The medium from cultured brain cell lines was assayed using lecithin-[^{3}H]-cholesterol single walled vesicles (17). The liposomes were activated for 1 h with apolipoprotein A-I (18). Each assay contained 5 μg cholesterol (SA: 1.2 10^5 dpm/ug), 40 μg egg lecithin, 5 μg of apoA-I (Sigma Chemical Co., St Louis, MO) in 50 μl with an equal volume of recrystallized human albumin (15%) and 200 μl of cell culture medium. Incubation was for up to 120 min at 37°C. The reaction was stopped in ice water and lipids extracted with 800 μl of chloroform-methanol (1/1, v/v). Labelled cholesteryl ester generated by LCAT was separated from unesterified cholesterol by

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TABLE I

Properties of Brain and Plasma Derived LCAT

	LCAT activity (%)		
	Control	With DTNB	Without apoA-I
U343	100	10.8 ± 4.8	21.7 ± 5.2
U251	100	9.1 ± 3.7	22.5 ± 4.2
Plasma[a]	100	7.7 ± 0.9	18.4 ± 2.2

Note. Control values are expressed relative to the activity measured as described in Table I and represent for U343 and U251 9.0 + 2.4 and 7.0 + 1.9 pmol.CE ml^{-1} h^{-1} per mg cell protein respectively. The values are the mean + SD of 3 experiments where each assay was performed in duplicate.

[a] Purified plasma LCAT was incubated in presence of Ultroser (2%) as described under Materials and Methods.

thin-layer chromatography on silica gel layers on plastic sheets (Merck) developed in hexane/diethyl ether/acetic acid (83/16/1) (19). Radioactivity was determined by liquid scintillation spectrometry. Production of labeled cholesteryl ester was shown to be linear during the assay.

Purification and identification of LCAT protein. LCAT was purified from human plasma by ultracentrifugation followed by sequential chromatography on phenylagarose, DEAE-cellulose and hydroxyapatite (20). LCAT was also purified from cell media as previously described (21) using a column (7x0.5 cm) of phenylagarose (Pharmacia) equilibrated with 3 M NaCl, 1 mM EDTA and 10 mM Tris pH 7.4. The medium from 3xT150 flasks was added to the column. After washing with 20 ml Tris/EDTA (10 mM/1 mM), pH 7.4, LCAT was eluted with distilled water. These fractions were immediately tested for LCAT activity and the most active fractions were pooled, frozen at −70°C and lyophilized overnight. The protein was then dissolved in Laemmli buffer, boiled 5 min and fractionated on a SDS/10% polyacrylamide minigel (Novex, Encinitas, CA) at 125 V for 2 h at room temperature. The separated proteins were transferred overnight onto nitrocellulose as solid phase (Sartorius, West Coast Scientific, Hayward, CA) (22). Immunodetection of LCAT was carried out by using anti-LCAT site-directed antibody raised in rabbits against LCAT residues 165-183; and then with ^{125}I-labeled goat antibody to rabbit IgG (1).

mRNA isolation and analysis. RNAs were extracted from nerve cells and human hepatoma cells (HepG2) using 3 confluent T175 flasks for each cell line. The mRNA was prepared by oligo d(T)-cellulose chromatography (K-1593-oZ; Invitrogen, San Diego, CA). Poly(A^+)mRNA of human liver was from Clontech (Palo Alto, CA). The poly(A^+)mRNA was precipitated and stored at −70°C with 0.1 volume of 2 M sodium acetate and 2 volumes of 100% ethanol. The RNA was precipitated by centrifugation, and then resuspended in 1XMEA (20 mM MOPS; 1 nM EDTA; 5 mM NaOAc), 50% formamide, 2.2 M formaldehyde. An aliquot was heated at 65°C for 5 min, loading dye was added and then it was run in 1% agarose-formaldehyde gel. The RNA was transfered to Nylon membrane (Sigma) and the RNA was crosslinked 2-4 min with U.V light. Poly(A^+)mRNA from human liver was processed identically. RNA blots were hybridized with [^{32}P]-labeled human LCAT, apolipoprotein A-I, E, D and GFAP cDNAs. The full-length human LCAT cDNA was isolated from HepG2 cells as described (23). The probe for apoA-I and apoE were derived from clones pAI-113 and pE-301 respectively (American Type Culture Collection, ATCC 57024 and 57020). The 600 bp PstI fragment corresponding to human apoA-I amino acids 94-243 was used to probe apo-AI mRNA. The 291 Bp PstI fragment coding for amino acid 155-252 of human apoE was purified. The full length ApoD and GFAP cDNAs were kindly provided by Dr. Dennis Drayna (Genentech, South San Francisco) (24) and Dr. Michael Brenner (NIH) (25), respectively. All probes were labeled with [^{32}P]dCTP by random priming. The mRNAs were prehybridized at 65°C with the [^{32}P]-labelled probes in a solution containing 1 M NaCl, 1% SDS, 10% Dextran sulfate, 100 μg/ml salmon sperm DNA. Blots were washed at 65°C with 2X SSPE for 1 h and 1 h with 0.2X SSPE.

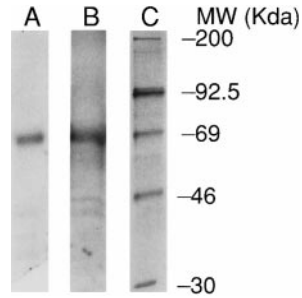

FIG. 1. Western immunoblotting of purified plasma LCAT (A) and LCAT secreted by U251 cells (B). The LCAT was partially purified from cell media as described under Materials and Methods and separated on a 10% SDS-PAGE mini-gel that was transferred and immunoblotted with anti-LCAT antibody (1). Gels were calibrated (lane C) with the following ^{14}C-methylated standards (Amersham): myosin (200 kDa), phosphorylase b (92.5 kDa), bovine serum albumin (69 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and lysozyme (14.3 kDa).

RESULTS

Origin and properties of neuronal LCAT activity. The possible secretion of LCAT activity was first screened in the culture medium of 25 neuronal and gliomal cell lines of human and rodent origins. Activity was detected in about two thirds of these cell lines at a

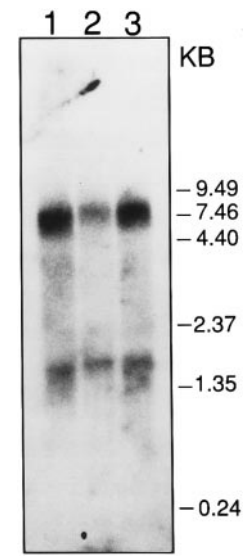

FIG. 2. Northern blotting of cell line mRNA. Poly(A+) mRNA were isolated from HepG2 cells (1), U251 (2) and U343 (3). 15 μg of samples were run on a 1% agarose-formaldehyde gel blotted to a nitrocellulose filter and probed with LCAT DNA which had been labelled as described under Materials and Methods.

TABLE II

Distribution of LCAT, apoA-I, apoD, apoE and GFAP mRNAs in the Nerve Cell Lines and Liver

mRNA	U343	U251	Liver
LCAT	+	+	+
apoA-I	−	−	+
apoD	−	−	+
apoE	−	−	+
GFAP	+	+	−

Note. mRNAs were extracted and hybridized with the different probes as described under Materials and Methods.

mean level similar to those found in the medium of human hepatoblastoma cells (HepG2) (not shown). Two human gliomal (U343 and U251) cell lines which secreted relatively high levels of LCAT, were further characterized. The LCAT activity represented about 8 pmoles cholesterol esterified ml^{-1} h^{-1} per mg of cell protein after 36 h incubation time. As shown in Table I, LCAT activity in each line was inhibited by about 90% in the presence of the sulfhydryl inhibitor (DTNB), a potent inhibitor of LCAT activity (26). LCAT activity was also activated by apoA-I in each medium, as is the case for plasma LCAT (18). These data show that the enzymatic properties of neural cell LCAT are similar to those of the plasma LCAT.

Synthesis and secretion of LCAT by U251 cells. Cultured astrocytes U251 were incubated 24 hours in serum free medium. LCAT from the medium was purified by phenylagarose chromatography and characterized by western immunoblotting (Fig. 1). The apparent molecular weight of the secreted LCAT was about 67,000, similar from that of the plasma LCAT (19). Gliomal cells secreted LCAT with properties similar to that of the plasma (27).

Analysis of LCAT, apoA-I, E and D mRNAs. To detect the presence of LCAT, apoA-I, apoE and apoD mRNAs, poly(A^+) mRNAs were prepared from nerve cells and HepG2 cells and analyzed by hybridization with the respective [^{32}P] labelled cDNAs. Blots probed with human LCAT-cDNA (Fig. 2) showed two major bands in each cell lines, one corresponding to 1.5 Kb, the predicted size of LCAT mRNA and a larger of 7.5 Kb also observed in HepG2 mRNA (28). Similar amount of LCAT mRNA signal was found in HepG2 and brain cell lines. No detectable apoA-I, apoE and apoD mRNAs were found in the nerve cells (Table II), in contrast to HepG2 cell mRNA. Finally, blot probed with human GFAP-cDNA showed that U343 and U251 cell line were derived from gliomas cells.

CONCLUDING REMARKS

The properties and synthesis of LCAT have been studied in considerable detail in hepatoma cell lines, but far less is known about LCAT in extra hepatic tissues. LCAT action in brain may be important for function of the central nervous system, since several patients with LCAT deficiency showed signs of hearing loss and sensory impairments (30). Another study reported the importance of this enzyme in the esterification of pregnenolone and dehydroepiandrosterone (31). LCAT may be very active in the cerebrospinal fluid using these steroids as substrate. Further investigations will be needed to understand the physiological significance of this enzyme in the metabolism of HDL in the extravascular fluid.

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