Lipoprotein lipase is expressed in cultured Schwann cells and functions in lipid synthesis and utilization

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Abstract **We have previously demonstrated that lipoprotein lipase (LPL; triacylglycero-protein acylhydrolase, EC 3.1.1.34) is most likely expressed in the non-neuronal cells of the spinal cord, and glial cells may thus be the site of expression in the peripheral nervous system as well. We investigated the expression of LPL in cultured 1.17 cells, an immortalized rat sciatic nerve Schwann cell line. The 1.17 cells were shown to express LPL mRNA by reverse transcriptase-polymerase chain reaction analysis. The 1.17 Schwann cells demonstrated heparin-releasable lipolytic activity that was inhibited by the lipase inhibitor tetrahydrolipstatin in a dose-dependent manner. Preincubation of 1.17 cells with an anti-rat LPL antiserum reduced the heparin-releasable lipolytic activity to <10% of that measured in untreated cells. To investigate the role of LPL in Schwann cell lipid metabolism, 1.17 cells were incubated for up to 24 h with an emulsified [^{14}C]triolein substrate and the incorporation of [^{14}C]triolein radioactivity into various cellular lipids was examined in the presence of either anti-rat LPL antiserum or preimmune serum. Inhibiting LPL activity reduced the incorporation of ^{14}C into cellular polar lipids, diacylglycerol, and cholesteryl esters by >80% at 2 and 6 h after addition of the radiolabeled substrate. At 24 h, radioactivity in diacylglycerol and cholesteryl esters was similar in cells treated with anti-LPL antiserum or preimmune serum, whereas ^{14}C incorporation into polar lipids was still reduced by >60%. Separation of the polar lipids into individual lipid species revealed no specific changes in triolein-derived radioactivity incorporation across the phospholipid species examined. These results suggest that LPL-mediated hydrolysis of exogenous triacylglycerol is an important source of free fatty acids for the Schwann cell and thus may play a critical role in myelin biosynthesis in the peripheral nervous system.—**Huey, P. U., T. Marcell, G. C. Owens, J. Etienne, and R. H. Eckel. **Lipoprotein lipase is expressed in cultured Schwann cells and functions in lipid synthesis and utilization. *J. Lipid. Res.* 1998.** 39: **2135–2142.**

Supplementary key words myelin • phospholipids • triacylglycerol • peripheral nerve • lipolysis

Lipoprotein lipase (LPL; triacylglycero-protein acylhydrolase, EC 3.1.1.34) is a 56 kDa enzyme produced by several tissues in the body, including adipose tissue, muscle, heart, and the lactating mammary gland (for a review, see ref. 1). It is rate-limiting for the hydrolysis of triacylglycerol (TAG) contained in the neutral lipid core of circulating chylomicrons and very low density lipoproteins (VLDL). In skeletal and cardiac muscle, LPL-mediated hydrolysis of TAG produces mono- and diacylglycerol (MAG, DAG) and free fatty acids (FFA) for oxidation; in adipose tissue, the FFA produced by LPL activity are re-esterified into neutral lipids (triacylglycerol > > cholesteryl esters) and stored as fat. LPL is synthesized within the parenchymal cells of these tissues and is transported to the capillary endothelium, where it binds to heparan sulfate proteoglycans on the luminal surface and thus has access to circulating lipoproteins.

LPL has also been detected in various regions of the rat (2–4), rabbit (5), and guinea pig (6) brain. Cultured rat brain cells were shown to incorporate triolein-derived radioactivity into triacylglycerol, phosphatidylcholine, and sphingomyelin, suggesting a role for neuronal LPL in the provision of FFA for cellular lipid synthesis (2). We have also demonstrated the presence of LPL mRNA in the rat spinal cord, primarily in the white matter corresponding to myelinated nerve fibers (7). This finding suggested that glial cells also synthesize LPL in the central nervous system (CNS) and implicated LPL activity in the synthesis and maintenance of CNS myelin.

As proposed for myelinating glial cells of the CNS, Schwann cells in the peripheral nervous system (PNS) may express LPL in order to facilitate FFA utilization for myelin lipid biosynthesis. As a first step to investigate this possibility, we examined LPL expression and lipid metab-

Abbreviations: LPL, lipoprotein lipase; FFA, free fatty acid; DAG, diacylglycerol; TAG, triacylglycerol; PL, polar lipids; FC, free cholesterol; CE, cholesteryl esters; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; CNS, central nervous system; PNS, peripheral nervous system.

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olism in a cultured Schwann cell line, 1.17 Schwann cells (8). These cells were derived from neonatal rat sciatic nerve by chronic administration of glial growth factor (neuregulin) and forskolin; they express several Schwann cell-specific markers and retain the ability to envelop axons in co-culture with dorsal root ganglion neurons. Here we report the first examination of the expression and potential function of LPL in Schwann cells.

EXPERIMENTAL METHODS

Materials

[^{14}C]triolein (tri[1-^{14}C]oleoylglycerol, 100 μCi/ml) was purchased from Amersham (Arlington Heights, IL). Egg yolk l-α-lecithin was obtained from Calbiochem (San Diego, CA). Solvents for lipid extraction and thin-layer chromatography (HPLC grade) were obtained from Mallinckrodt. Neutral lipid standards for thin-layer chromatography were obtained from Supelco (Bellefonte, PA); polar lipid standards were purchased from Avanti Polar Lipids (Alabaster, AL). Reagents for polymerase chain reaction (PCR) amplification were purchased from Fisher Scientific. Cell culture medium and trypsin/EDTA solution were obtained from Gibco/BRL; all other cell culture reagents were purchased from Gemini Bioproducts (Calabasas, CA). Tetrahydrolipstatin (THL, Orlistat) was kindly provided by Dr. M. K. Meier and P. Weber, Pharmaceuticals Division, F. Hoffman-La Roche Ltd., Basel.

Cell culture

1.17 Schwann cells were routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 4 mm glutamine, 1% v/v penicillin/streptomycin (10,000 U/ml and 10,000 μg/ml, respectively). Cells were passaged by 0.25% trypsin/1 mm EDTA release and seeded at a density of approximately 5×10^6 cells per 100-mm dish prior to experiments.

RNA isolation and quantification of LPL mRNA

Total RNA was isolated from confluent cultures of 1.17 cells by extraction with TRIzol reagent (Gibco/BRL) and processing according to the manufacturer's instructions. Reverse transcription (RT) of 1 μg total RNA in 20 μl total volume was accomplished by random hexamer priming followed by incubation with 200 U Moloney murine leukemia virus reverse transcriptase (Gibco/BRL) for 1 h at 42°C. The RT reactions were diluted 1:10 with water, and 5 μl was subjected to duplex PCR amplification using primers specific for rat LPL (forward primer = 5′-GAG ATTTCTCTGTATGGCACA-3′ and reverse primer = 5′-CTGCAG ATGAGAAACTTTCTC-3′ (9, 10)) and a "competimer"/primer mix specific for 18S ribosomal RNA (QuantumRNA kit; Ambion, Inc., Austin TX). The ratio of 18S "competimers" to primers was adjusted according to the manufacturer's instructions such that the amount of 18S rRNA product was within the same range as that of LPL mRNA. Amplification was carried out using the following protocol: "hot start" at 95°C for 3 min followed by 32 cycles of 95°C for 18 s, 62°C for 20 s, and 72°C for 40 s, with a final elongation at 72°C for 7 min. Control experiments demonstrated that this protocol allowed quantification within the linear range of amplification of both LPL and 18S rRNA products. The PCR products were separated by electrophoresis through 2.5% agarose and visualized with ethidium bromide staining. A digital image of the gel was obtained with an AlphaImager2000 (Alpha Innotech Corp.) and analyzed for band pixel density using the SigmaGel program (Jandel Scientific).

LPL activity assay

Culture medium was aspirated and the cultures were rinsed three times with Krebs-Ringer phosphate (KRP) buffer. Cell-surface LPL activity was released by incubating the cultures with 15 μg/ml sodium heparin in KRP buffer for 5 min on ice with gentle rocking. Duplicate aliquots of the heparin-releasable fraction were assayed for LPL activity by incubation with a lecithin-emulsified [^{14}C]triolein substrate for 45 min at 37°C (11). Liberated ^{14}C-labeled FFA were quantified by scintillation counting of the aqueous phase after extraction of the reaction mixture. In experiments utilizing tetrahydrolipstatin (THL) to inhibit lipase activity, a THL stock solution (24 mm in DMSO) was serially diluted in DMSO and added to the assay tubes along with the [^{14}C]triolein substrate; the final concentration of DMSO in each tube was 2.5% (v/v).

Lipid biosynthesis assay

Confluent 1.17 Schwann cells in 100-mm dishes were preincubated with a 1:1000 dilution of goat anti-rat LPL antiserum (12) or goat preimmune serum in 8 ml culture medium for 1 h to inhibit LPL activity; control cultures received no antiserum. One hundred μl (=0.0625 μCi) of emulsified [^{14}C]triolein was then added to each dish as a source of exogenous triacylglycerol. Incorporation of [^{14}C]triolein-derived radioactivity into de novo synthesized lipids was assessed 2, 6, and 24 h later by rinsing the dishes three times with phosphate-buffered saline and extracting total cellular lipids with two rinses of 3 ml of hexane–isopropanol 3:2 (v/v). The lipid extracts were pooled, dried under nitrogen, redissolved in chloroform–methanol 2:1, and spotted onto Whatman PE SIL G flexible TLC plates. A neutral lipid standard mix containing 50 μg each of monoolein, diolein, triolein, free cholesterol, oleic acid, and cholesteryl oleate was spotted on each plate for band identification. The plates were developed in heptane–diethyl ether–glacial acetic acid 85:20:1.5 (v/v) until the solvent front was ~1 cm from the top of the plate. The separated ^{14}C-labeled lipids were detected by exposure of the TLC plates to a PhosphorImager screen for 24 h and analyzed using the ImageQuant software (Molecular Dynamics, Sunnyvale, CA). After PhosphorImager detection, the plate was exposed to iodine vapor to locate the lipid standards, and the positions of the bands corresponding to polar lipids (PL), diacylglycerol (DAG), free cholesterol (FC), triacylglycerol (TAG), and cholesteryl esters (CE) were recorded. The plate was then blanched of iodine staining by incubation in a 60°C oven, after which the individual lipid bands were cut from the TLC plate and transferred to scintillation vials containing 5 ml Ready Safe scintillation fluid (Beckman). Radioactivity in the lipid bands was quantified by scintillation counting in a Beckman LS 6000 liquid scintillation counter.

To further examine radiolabel incorporation into individual polar lipid classes, the monodimensional TLC separation method of Ruiz and Ochoa (13) was used. Whatman LK5D grooved glass-backed TLC plates were impregnated with 1 mm EDTA, pH 5.5, then washed in chloroform–methanol–water 60:40:10 overnight. Lipid extracts from Schwann cells incubated with either preimmune serum or anti-LPL antiserum for 2, 6, and 24 h were spotted onto the preabsorbent region of the plate. A polar lipid standard mix consisting of 25 nmol each dioleoylphosphatidylcholine, dioleoylphosphatidylserine, dioleoylphosphatidylethanolamine, brain sphingomyelin, and liver phosphatidylinositol was combined with the neutral lipid mix described above and spotted in a separate lane. The TLC plate was developed in chloroform–methanol–water 60:40:10 until the solvent front was 1 cm above the preabsorbent zone, then thoroughly dried with a heat gun directed toward the back of the plate. The lipids were separated by sequential development in chloroform–methanol–water 65:40:5

to 2 cm, ethyl acetate–2-propanol–ethanol–chloroform–methanol–0.25% KCl 35:5:20:22:15:9 in water to 5 cm, toluene–diethyl ether–ethanol 60:40:3 to 7.5 cm, n-heptane–diethyl ether 94:8 to 10.5 cm, and pure n-heptane to 12.5 cm, with thorough drying of the plate between each development step. The TLC plate was then exposed to a phosphor screen as described above, and relative radioactivity incorporation into the individual lipid classes was quantified by ImageQuant analysis. After exposure to the phosphor screen, the lipids and standards were visualized by charring with 10% cupric sulfate in 8% phosphoric acid.

RESULTS

Cultured 1.17 rat Schwann cells express LPL mRNA as demonstrated by reverse transcriptase/polymerase chain reaction (RT-PCR) analysis (**Fig. 1**). Although there was a tendency for the amount of LPL mRNA to increase with length of time in culture, the levels remained largely constant from preconfluence (day 5) until the cells were densely packed (day 18). This cell line also expresses cell-surface lipolytic activity that is first detectable at confluence, increases as the cultures become more highly packed, then levels off or decreases slightly (**Fig. 2**). The heparin-releasable lipolytic activity in 1.17 Schwann cells was effectively inhibited by tetrahydrolipstatin (THL) in a dose-dependent manner (**Fig. 3A**) and by a goat antiserum raised against rat adipose tissue LPL (Fig. 3B).

Incubation of 1.17 Schwann cells with emulsified [^{14}C]triolein resulted in labeling of several lipid classes as demonstrated by thin-layer chromatography of total cell

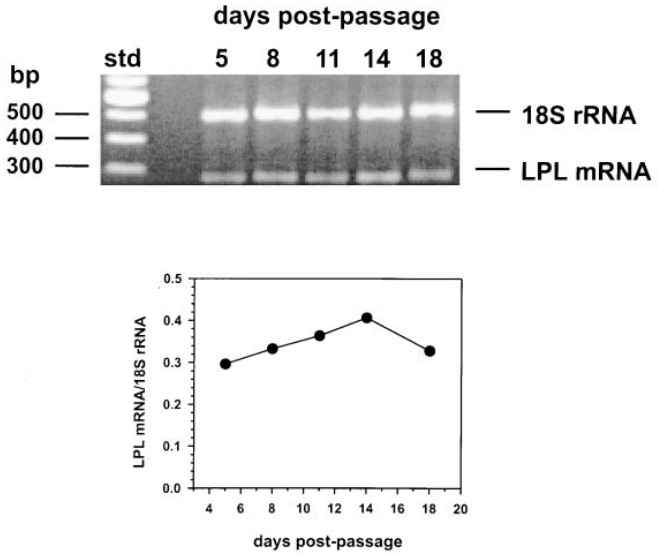

Fig. 1. Expression of LPL mRNA in 1.17 Schwann cells as a function of time in culture. 1 μg total RNA isolated from 1.17 Schwann cells on the indicated days post-passage was used as the template for first-strand synthesis using random hexamer primers and Moloney murine leukemia virus reverse transcriptase. Subsequent polymerase chain reaction was carried out using rat LPL-specific primers and an 18S rRNA "competimer"/primer mix as described in Experimental Methods. An image of the EtBr-stained gel is shown in the top panel; the bottom panel shows the ratio of the pixel density of the LPL band to that of the corresponding 18S band for each sample.

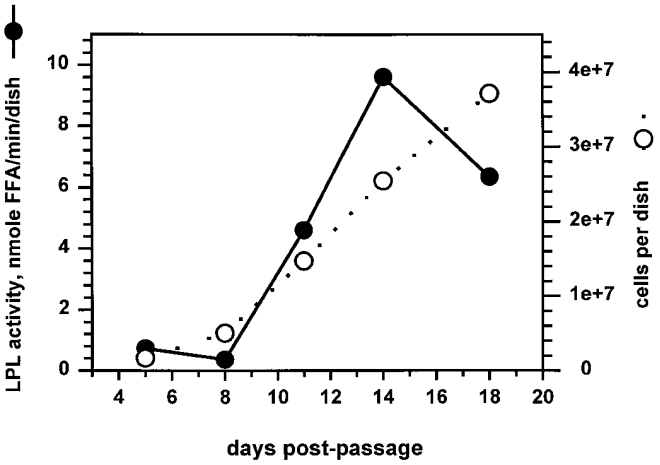

Fig. 2. LPL activity in 1.17 Schwann cells as a function of time in culture. Cell cultures were rinsed with Krebs-Ringer phosphate (KRP) buffer on the indicated days post-passage, and cell-surface LPL was released by incubation with 15 μg/ml sodium heparin in KRP for 5 min on ice. LPL activity (closed circles) was measured in the heparin-releasable fraction as described in Experimental Methods. Adherent cells were released with trypsin/EDTA and counted using a hemacytometer (open circles). Each point represents the average of duplicate dishes assayed at each time point.

lipids (**Fig. 4**). Polar lipids (PL) were the most highly labeled lipid class and were very efficiently labeled by 2 h after addition of the [^{14}C]triolein substrate. Substantial levels of cell-associated radiolabeled triacylglycerol (TAG) were also detected, probably representing primarily non-hydrolyzed [^{14}C]triolein substrate. Other radiolabeled lipids detected included diacylglycerols (DAG), cholesteryl esters (CE), and free cholesterol (FC). Two methods were evaluated for the quantification of [^{14}C]triolein radioactivity in cellular lipids: ImageQuant analysis after exposure of the TLC plate to a phosphor screen, and liquid scintillation counting of the individual lipid bands. Both methods revealed the same general pattern of lipid labeling (Fig. 4); however, PhosphorImager analysis proved to be more sensitive for quantification of the less highly labeled lipids and allowed easier isolation and more precise quantification of the closely spaced FC and DAG bands.

The role of LPL in the Schwann cell was addressed by assessing [^{14}C]triolein metabolism in 1.17 cells in which LPL activity was inhibited using the anti-rat LPL antiserum. Blocking LPL activity resulted in a decrease in the ability of Schwann cells to utilize [^{14}C]triolein-derived FFA for de novo synthesis of three of the lipid classes examined. The incorporation of [^{14}C]triolein radioactivity into PL (**Fig. 5A**) and CE (Fig. 5B) was reduced as early as 2 h following inhibition of LPL to 9.5% and 15%, respectively, of the radioactivity in cultures receiving preimmune serum and remained decreased to the same degree (9.7% and 14%, respectively) at 6 h. At 24 h, PL radioactivity in cultures receiving the anti-LPL antiserum was still reduced to 34% of control levels, while CE radioactivity in anti-LPL-treated cultures was equivalent to preimmune serum-treated control cultures. Incorporation of [^{14}C]tri-

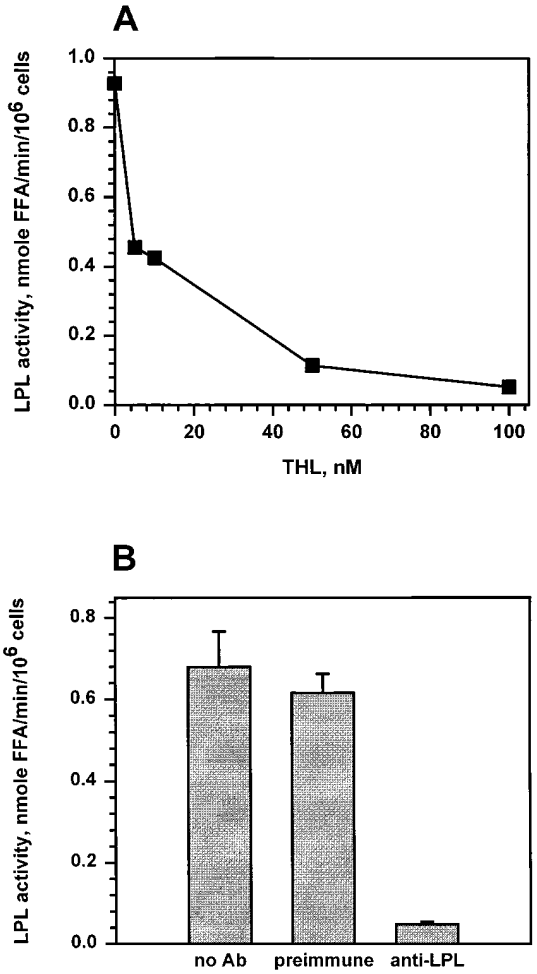

Fig. 3. Inhibition of 1.17 Schwann cell LPL activity by tetrahydrolipstatin (THL) and anti-LPL antiserum. (A) LPL activity was released from confluent 1.17 Schwann cells with 15 μg/ml sodium heparin in KRP buffer. Duplicate aliquots of the heparin-releasable fraction were assayed for LPL activity in the presence of increasing concentrations of THL. (B) Parallel cultures were incubated in culture medium alone ("no Ab") or culture medium containing 1:1000 dilutions of either goat preimmune serum or goat anti-rat LPL antiserum for 2 h prior to heparin release and assay of LPL activity. The average ± SD of triplicate dishes is shown.

olein radioactivity into FC was also reduced at 2 h after addition of the anti-LPL antiserum to 19% of the levels detected in cultures receiving preimmune serum (Fig. 5B); however, by 6 h, FC radioactivity in anti-LPL-treated cultures was similar to control levels. Conversely, levels of radiolabeled cell-associated TAG (Fig. 5A) and DAG (Fig. 5B) increased in response to inhibition of LPL activity. DAG radioactivity in anti-LPL antiserum-treated cultures was equivalent to that in preimmune serum-treated cultures at 2 h and increased to 260% of the control value by 24 h. TAG radioactivity in anti-LPL antiserum-treated cultures was increased to 130% of the control value at 2 h, 170% at 6 h, and 200% at 24 h. Very similar results were obtained from a duplicate experiment (data not shown).

The decreased incorporation of triolein-derived radioactivity into PL was examined by further separating the PL class into individual phospholipids. Sphingomyelin (SM) was poorly labeled over the 24-h time course, and no significant difference in incorporation of triolein-derived radioactivity was detected between cultures treated with either preimmune serum or anti-LPL antiserum (**Fig. 6**). Phosphatidylserine (PS) was more effectively labeled with the triolein emulsion, and radioactivity was reduced by 70% in cultures receiving anti-LPL for 24 h; however, no difference in PS radioactivity between cultures receiving anti-LPL versus preimmune serum was detected at the earlier 2 and 6 h time points. In contrast, robust labeling of cellular phosphatidylcholine (PC) with [^{14}C]triolein-derived radioactivity was observed at 2, 6, and 24 h, with inhibition of LPL activity significantly reducing radiolabel incorporation by 79% at 2 h, 81% at 6 h, and 59% at 24 h (Fig. 6). Similarly, incorporation of triolein-derived radioactivity into phosphatidylethanolamine (PE) and phosphatidylinositol (PI) was reduced by 71% at 2 h, 86% at 6 h, and 65% at 24 h after addition of anti-LPL antiserum.

DISCUSSION

Our results demonstrate that 1.17 Schwann cells express cell-surface LPL activity that can be inhibited by THL, a general inhibitor of several related mammalian lipase enzymes (14), and by an antiserum raised against rat adipose tissue LPL (12). Schwann cells incubated with lecithin-emulsified triolein, a substrate that mimics lipoprotein particles, efficiently incorporated triolein-derived radioactivity into cellular PL, indicating active synthesis of phospholipids and other polar lipids from the products of TAG hydrolysis. [^{14}C]triolein radioactivity was also found in cell-associated DAG, presumably as a direct product of TAG hydrolysis but also potentially synthesized de novo from [^{14}C]triolein-derived FFA; CE, indicating that FFA liberated by LPL activity are substrates for cholesterol esterification; and FC, suggesting that ^{14}C-labeled FFA liberated by LPL activity are available for cholesterol biosynthesis following oxidation to acetyl-CoA.

The ability of the anti-LPL antiserum to almost completely inhibit LPL activity was utilized to examine the role of LPL activity in Schwann cell lipid metabolism. Blocking LPL activity led to decreased incorporation of emulsified [^{14}C]triolein-derived radioactivity into cellular PL, FC, and CE, with PL biosynthesis being the most persistently affected. Separation of the PL into separate phospholipids indicated that PC and PE/PI are the most highly labeled PL species and are also those most affected by inhibiting LPL activity. These results support the hypothesis that LPL functions in Schwann cells to provide FFA for biosynthesis of these lipids. In contrast, cell-associated DAG and TAG radioactivity increased in response to LPL inhibition. The high TAG radioactivity (see Figs. 4 and 6A) almost certainly represents [^{14}C]triolein that remains associated with the cells even after rinsing; inhibiting LPL activity would be expected to block hydrolysis of this cell-associated substrate and prevent its uptake and subsequent utilization for lipid biosynthesis. Indeed, cell-

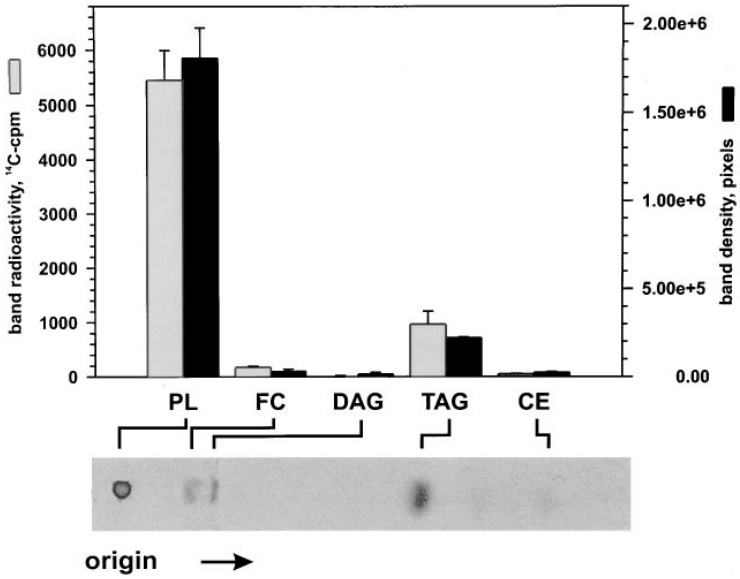

Fig. 4. Incorporation of [^{14}C]triolein radioactivity into 1.17 Schwann cell lipids. Confluent cultures were incubated with emulsified [^{14}C]triolein for 24 h, and total lipids were extracted from the cells and separated by thin-layer chromatography. Radioactivity in the indicated lipid bands was quantified by exposure to a phosphor screen (black bars) and by scintillation counting (grey bars). The average ± SD of triplicate dishes is shown. The bottom panel shows the migration of the lipid classes quantified. PL, polar lipids; DAG, diacylglycerol; FC, free cholesterol; TAG, triacylglycerol; CE, cholesteryl esters.

associated TAG radioactivity tended to increase over the incubation period (Fig. 5A), probably reflecting an increased cell density over the time course of the experiment. The observed accumulation in DAG radioactivity in Schwann cells treated with anti-LPL (Fig. 5B) suggests that some hydrolysis of the cell-associated [^{14}C]triolein can still occur in the absence of LPL activity.

The level of heparin-releasable activity in 1.17 Schwann cells at the maximum of the growth curve shown in Fig. 2 is roughly equivalent to the heparin-releasable LPL activity previously measured in undifferentiated 3T3-L1 preadipocyte-like fibroblasts; upon differentiation, 3T3-L1 cell-surface LPL activity increases to levels approximately 10 to 15-fold higher than those seen in these cultured Schwann cells (15, 16). The dependence upon culture density of LPL activity in differentiating 3T3-L1 cells is similar to that of the 1.17 Schwann cells in that heparin-releasable LPL activity was minimal when the cells were subconfluent, increased abruptly at 2–3 days post-confluence, then decreased somewhat but remained relatively stable between 4 and 7 days post-confluence (15, 16). In contrast, the patterns of LPL mRNA expression in 1.17 Schwann cells and 3T3-L1 cells are not alike. The amount of LPL mRNA in 1.17 Schwann cells is relatively constant with respect to length of time in culture (Fig. 1), whereas 3T3-L1 cells were shown to accumulate LPL mRNA and increase their rate of transcription of LPL during differentiation to adipocytes (16). Thus, the regulation of cell-surface LPL activity by 1.17 Schwann cells involves factors other than modification of steady state LPL mRNA levels.

One such factor may be contact inhibition of cell growth. Similar patterns of expression of two other enzymes involved in intracellular lipid metabolism, HMG-CoA reductase and acyl-CoA:cholesterol acyltransferase, were observed in cultured C6 glial cells (17). The increase in activities of these enzymes with increasing cell density was not related to LDL receptor activity, soluble factors, or extracellular matrix components and was therefore assumed to result from a cell interaction-dependent mechanism. However, expression of active cell-surface LPL by Schwann cells may depend, at least in part, on sufficient extracellular matrix development in order to anchor the lipase molecule effectively. LPL binds to heparan sulfate proteoglycans on cell surfaces, and this interaction is necessary for proper cell-surface localization and metabolism of LPL in adipocytes (18), aortic endothelial cells (19), and Chinese hamster ovary cells (20). Schwann cells normally deposit a basal lamina consisting of the glycoproteins laminin and tenascin as well as several types of collagen (21–23) and heparan sulfate proteoglycan (24). The development of the basal lamina correlates with the Schwann cells' ability to myelinate axons (25), and the 1.17 cell line used in these studies retains both the ability to secrete laminin and to myelinate dorsal root ganglion neurons (8). It is tempting to speculate that LPL expression in peripheral nerves in vivo may also correlate with basal lamina formation and thus with the ability of Schwann cells to myelinate axons.

These results obtained with cultured Schwann cells in vitro suggest the involvement of LPL in the process of lipid uptake and reutilization in Schwann cells of peripheral nerves in vivo. Several studies in the rat sciatic nerve have elucidated the role of non-neuronal cells in lipid metabolism after crush injury. The myelin sheath surrounding the axon degenerates and the released myelin lipids are absorbed by Schwann cells, leading to the accumulation of intracellular lipid droplets. Macrophages that infiltrate the injury site also scavenge myelin lipids as well as synthesize and secrete large amounts of apolipoprotein (apo) E (26–28); apoE synthesis by resident non-neuronal cells of the nerve is also stimulated in response to injury (29). In addition to apoE, apoA-I, apoA-IV, and apoD have also been identified in the regenerating nerve (30, 31). The accumulation of myelin lipids and apolipoproteins leads to the local synthesis of intraneurial lipoprotein particles (30, 32, 33) that are thought to provide lipids to cells of the regenerating nerve for remyelination. In support of this model, cultured Schwann cells and neurons have been shown to take up human serum LDL particles

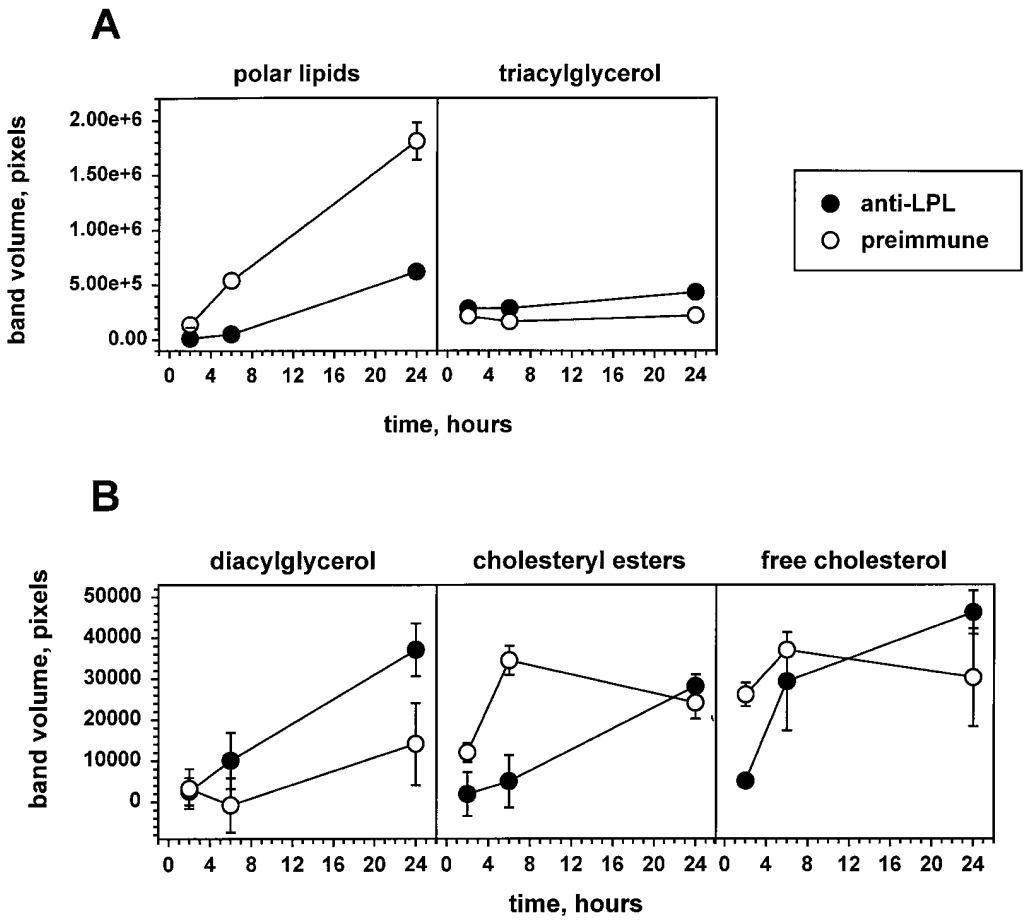

Fig. 5. Incorporation of [^{14}C]triolein radioactivity into 1.17 Schwann cell lipids. (A) Polar lipids and triacylglycerol; (B) diacylglycerol, cholesteryl esters, and free cholesterol. Confluent cultures were incubated with emulsified [^{14}C]triolein for 2, 6, or 24 h in the presence of 1:1000 goat anti-rat LPL antiserum (closed circles) or 1:1000 goat preimmune serum (open circles). After rinsing three times with phosphate-buffered saline, total lipids were extracted from the cells and separated by thin-layer chromatography. Radioactivity in the lipid classes was detected and quantified by exposure to a phosphor screen. Each point represents the average ± SD of the results from triplicate dishes. Lipid radioactivities in cultures incubated with preimmune serum were essentially identical to those in cells receiving no serum addition; the results obtained from the "no Ab" controls are omitted for clarity. Note the difference in the ordinate scales between panels (A) and (B).

(34, 35) and endoneurial lipoproteins isolated from injured rat sciatic nerves (35) by a mechanism involving the LDL receptor (28, 35–37). The expression of proteins involved in lipid transport is also evident in the CNS, where glial cells express the LDL receptor (17, 38, 39) as well as the LDL receptor-related protein (LRP) (40–42). The very low density lipoprotein (VLDL) receptor has also been detected in the rabbit (43) and mouse (44) brain, while several regions of the human brain express the apoE receptor 2 (45). The abundance of lipoprotein receptors in the CNS as well as the presence of LPL in the brain and spinal cord (2–7) illustrate the importance of lipoprotein catabolism in the normal maintenance of CNS myelin; however, as CNS neurons are not efficiently remyelinated after injury, the role of these proteins in the response to CNS injury may be quite different from that observed in the PNS.

The reutilization of cholesterol (28, 32, 46–48) and FFA (48, 49) for remyelination by Schwann cells of the injured peripheral nerve has been extensively studied, and it is likely that other myelin lipid components are recycled by lipoprotein-mediated transport within the injured nerve. Triacylglycerol, the LPL substrate used in these studies, is not a major component of normal nerve lipids; labeling nerves in vivo with [^{3}H]oleate resulted in >90% of the label being incorporated into phospholipids (49), a result very similar to the one reported here using [^{14}C]triolein (Fig. 4), with very little radioactivity appearing in TAG. However, crush injury to the [^{3}H]oleate-labeled nerve resulted in an increase in the appearance of radioactivity in CE and TAG, suggesting that FFA released by hydrolysis of myelin phospholipids is recycled into these lipids in response to injury (49). In addition to its ability to hydrolyze TAG, LPL possesses hydrolytic activity toward PC and PE (50). Thus, it is possible that LPL hydrolyzes myelin phospholipids in the injured nerve as well as TAG contained in the endoneurial lipoproteins formed in response to nerve injury.

In order to evaluate the role of LPL in the peripheral nerve in vivo, we have performed crush injuries to the rat

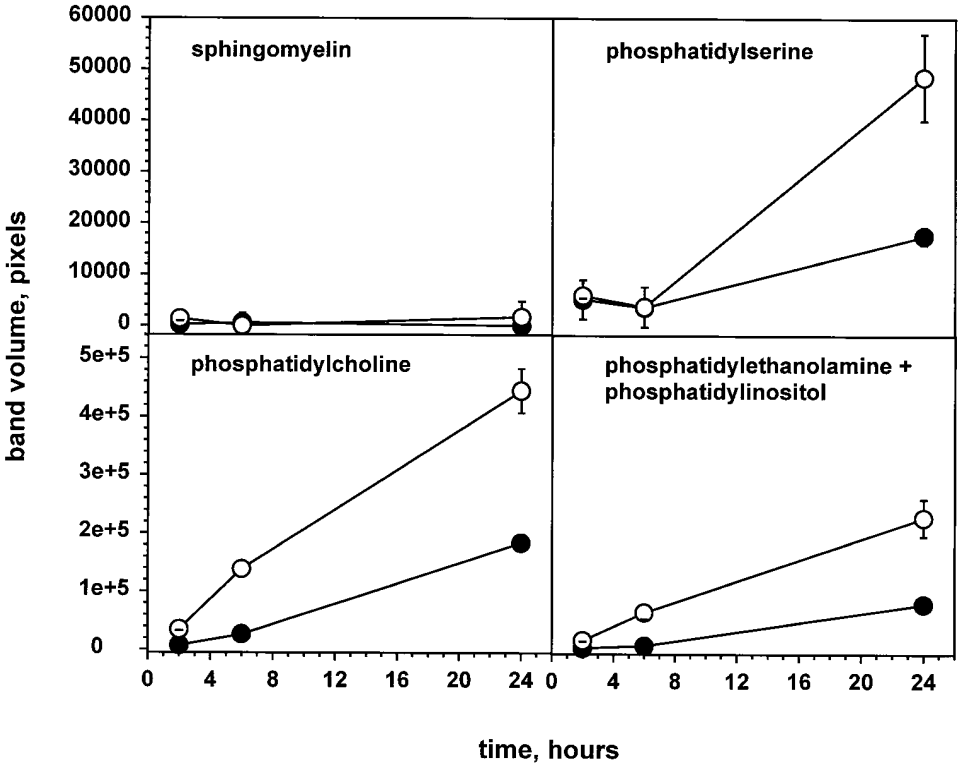

Fig. 6. Incorporation of [^{14}C]triolein radioactivity into 1.17 Schwann cell polar lipids. Confluent cultures were incubated with emulsified [^{14}C]triolein for 2, 6, and 24 h in the presence of either anti-LPL antiserum (closed circles) or preimmune serum (open circles). Total lipids were extracted from the cells and separated by thin-layer chromatography. Radioactivity in the indicated lipid bands was quantified by exposure to a phosphor screen and ImageQuant analysis. The data are expressed as the average ± SD of the results from triplicate dishes. Phosphatidylethanolamine and phosphatidylinositol were not sufficiently resolved to allow individual quantification and were therefore combined. Note the difference in the ordinate scales between the upper and lower plots.

sciatic nerve and measured LPL activity in both the injured and uninjured nerves as a function of time post-injury. Our results indicate that normal rat sciatic nerve expresses both LPL mRNA and activity, and that sciatic nerve LPL activity is up-regulated in response to crush injury (P. U. Huey, K. C. Waugh, J. Etienne, and R. H. Eckel, manuscript in preparation). This increase in sciatic nerve LPL activity is observed within 4 days post-injury, after which it gradually declines to the level of uninjured nerves by 4–5 weeks post-injury. This time course of LPL up-regulation is consistent with a role for LPL in hydrolyzing lipids from degenerating myelin and allowing their reutilization as the nerve recovers. These observations and the results reported here lead us to propose a model in which LPL in the peripheral nerve facilitates lipid hydrolysis to provide FFA for resynthesis of myelin lipids under circumstances in which Schwann cells are induced to myelinate neurons, e.g. in response to nerve injury.

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