Lipoprotein lipase (LPL) is a key enzyme involved in the metabolism of lipoproteins, providing tissues like adipose tissue or skeletal muscle with fatty acids. LPL is also expressed in the brain, fulfilling yet unknown functions. Using a neuroblastoma cell line transfected with a NEO- or a LPL-expression vector, we have developed a model to study the function of LPL in neurons exposed to native or copper-oxidized lipoproteins. The addition to the culture media of VLDL with 10 μM copper sulfate led to a significant reduction in the viability of NEO transfectants whereas LPL-transfectants were protected from this injury. In the presence of VLDL and CuSO4, LPL transfectants were even able to display significant neurite extension. This neurotogenic effect was also observed in LPL-transfectants exposed to native lipoproteins. However, addition of VLDL particles oxidized with CuSO4 prior to their addition to the culture media resulted in neurotoxic effects on LPL transfectants. These findings suggest that the presence of LPL in cultured neuronal cells modulates the physiological response of neurons following exposure to native or oxidized lipoproteins. LPL could thus play a key role in the differentiation of Neuro-2A cells and in the pathophysiological effects of oxidative stress in several neurodegenerative disorders.

Earlier studies have shown that mechanisms are present in the brain to ensure proper lipid transport and homeostasis (7,8). Various classes of lipoproteins have been found in the cerebrospinal fluid, most of them containing apolipoprotein E (apoE) as their major apolipoprotein (9,10). In humans, apoE is present in three different isoforms: E2, E3, and E4, each encoded by a different allele. In the brain, specific isoforms of apoE are associated with increased risk of developing Alzheimer’s disease (11) and poor outcome from brain injury (12). It has been postulated that apoE could be involved in the redistribution of cholesterol and phospholipids among neuronal cells involved in synaptogenic processes following brain injury (11). In vitro studies with neuronal cell lines have suggested that alternate apoE effects because of different apoE isoforms may be mediated by lipoprotein receptors, which are involved in the endocytosis of apoE-enriched lipoproteins by neurons (13,14). These multiligand receptors have been identified as important players in neurophysiological mechanisms involved in the long term potentiation of hippocampal cells (15) and in the differentiation of neuronal cell lines (16,17). Cerebrospinal fluid lipoproteins, which have a beneficial role in cholesterol homeostasis in the brain, are found to be susceptible to oxidative modifications. These oxidative processes could be associated with the development of neuropathologies or result from brain injuries (18–22). Several independent studies have described the cytotoxic potential of oxidized lipoproteins on different cell types, including neurons (23–25). The capacity of LPL to act as a ligand for lipoprotein receptors and to synergize with apoE in the uptake of lipoproteins by these receptors (26,27) leads us to hypothesize that LPL could play important physiological functions in neuronal cells and could thus be involved in the processing of native, as well as oxidized lipoproteins in the brain. In the present study, we show that LPL can modulate the physiological response of neuronal cells to native and oxidized triglyceride-rich lipoproteins.

**EXPERIMENTAL PROCEDURES**

**Amplification and Cloning of Human LPL cDNA from Lymphocyte RNA and Construction of a LPL Expression Vector—**Human lymphocytes were isolated by centrifugation over a Ficoll-Paque gradient (Amersham Biosciences) according to the manufacturer’s instructions. Total RNA from these cells was extracted with TRIZOL reagent (Invitrogen), and 1 μg was reverse transcribed with EXPAND reverse transcriptase primed with an oligo(dT)12 primer (Roche Molecular Diagnostics). Polymerase chain reaction amplification of LPL cDNA was carried out using primers LPL-ExtA (5′-GAAAGCTGCCCACTTCTA-3′) and LPL-ExtB (5′-CTTATTTTACTCTGCGCTC-3′). Each primer was added to the reaction mixture at a final concentration of 1 μM. Other components of the reaction consisted of deoxynucleotides (1 mM each), 1× Taq reaction buffer, and 1 unit of Taq polymerase (Amersham Biosciences) in a final volume of 100 μl. The reaction mixtures were denatured at 94 °C for 5 min. PCR amplification was then performed using 30 successive cycles of primer annealing (57 °C/1 min), extension (72 °C/1 min), and denaturation (94 °C/1 min). This PCR amplification gave a single DNA fragment of 1894 bp containing the coding sequence of the
human LPL gene, including the signal peptide. This fragment was then ligated in pGEM-T vector (Promega) and the identity of the cDNA insert in the resulting construct, pGEM-LPL, was confirmed by sequencing. To prepare a LPL expression vector, the pGEM-LPL plasmid was digested with restriction enzyme, NotI (New England Biolabs Ltd., Mississauga, Canada), and the resulting 1900-bp fragment containing the LPL cDNA was purified using a QIAQUICK gel extraction kit according to the manufacturer’s instructions (Qiagen, Mississauga, Canada). The purified cDNA fragment was ligated into a pcDNA3.1 (+) vector (Invitrogen) previously digested with NotI. The resulting constructs were transformed and amplified in Escherichia coli DH5α cells and the colonies were screened for a plasmid containing the LPL cDNA inserted in the sense orientation. This plasmid, pcDNA-LPL, was purified with a Plasmid Maxi kit (Qiagen, Ottawa, Canada) and then transfected into Neuro-2A cells. Neuro-2A cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). They were grown and transfected at 37 °C under a 5% CO2 atmosphere in Dulbecco’s modified Eagle’s medium/F-12 media containing 10% fetal bovine serum. For experimental protocols requiring Neuro-2A cells cultured in serum- and lipoprotein-free media (N2 media, see Ref. 28), the cells were incubated in Dulbecco’s modified Eagle’s medium/F-12 10% fetal bovine serum media. During the next 8 h, the medium/F-12 10% fetal bovine serum media, see Ref. 28), the cells were incubated in Dulbecco’s modified Eagle’s medium/F-12 containing the following additives: 5 μg/ml insulin, 100 μg/ml transferrin, 20 nM progesterone, 100 μM putrescin, and 30 nM selenium. All media, serum, and supplements were from Sigma. Neuro-2A cells were transiently transfected using calcium phosphate-precipitated plasmid DNA. Briefly, the cells were rinsed with PBS (phosphate-buffered saline), trypsinized, counted, and 1 × 106 cells were seeded in 100-mm cell culture Petri dishes (Falcon-BD, Oakville, Canada) containing 10 ml of Dulbecco’s modified Eagle’s medium/F-12 supplemented with 10% fetal bovine serum. After 20 h of incubation at 37 °C, the cell culture media were changed and the cells were incubated at 37 °C for 2–4 h more before the addition of the calcium/phosphate-precipitated DNA. Either pcDNA-LPL vector or control pcDNA-Neo vector were precipitated by the calcium/phosphate procedure (28) and aliquots of the precipitates containing 20 μg of DNA were added to each individual 100-mm culture plate. The cells were incubated with the precipitate for 4 h after which the precipitate was removed by aspiration of the PBS before addition of 10 ml of fresh Dulbecco’s modified Eagle’s medium/F-12 10% fetal bovine serum media. During the next 8 h, the cells were harvested by trypsinization and counted. In all the experiments, 200,000 cells were seeded in 9.5-cm2 well from cell culture grade multwell plates (Falcon-BD, Oakville, Canada).

**Purification of Human Very Low Density Lipoprotein (VLDL)—** All human volunteers were normolipemic individuals who were previously characterized as homoyzogotes for the apoE3 allele at the Lipid Research Centre according to Hixson and Vernier (29). Human VLDL (d < 1.006 g/ml) was isolated by ultracentrifugation from fasting plasma. The purity of VLDL was verified by gel electrophoresis using a combi-frac column (Beckman Instruments, Inc., Fullerton, CA). All lipoprotein preparations were determined using specific antibodies against the LPL protein, with a normal distribution, Student’s t test and standard ANOVA were done to identify significant differences across experimental groups. For sets of data with a non-normal distribution, the Wilcoxon/Kruskal-Wallis and Spearman’s nonparametric test was used to assess the degree of correlation between the lipoprotein concentrations and the observed neurite lengths.

**RESULTS**

**LPL Activity in Media of Cultured Neuro-2A Transfectants—** The amplified LPL cDNA used in this study contained a single base change at position 1536 (G/A) of the human LPL mRNA (GenBank™ accession number M15856) (35). This constituted a silent polymorphism and did not affect the amino acid sequence of the LPL protein. Calcium/phosphate transfection of Neuro-2A cells with pcDNA-LPL vector resulted in secretion of variable levels of heparin-releasable LPL activity, ranging from 25 to 390 μmol of free fatty acids released per hour, with a mean activity of 132.0 μmol ± 57.2 (mean ± S.E., n = 6). As determined by β-galactosidase staining (data not shown), we consistently observed that ~30% of the cell population was transfected. The heparin-releasable LPL activity secreted by control NEO transfectants was low at 3 ± 1 μmol of free fatty acids released per hour (mean ± S.E., n = 6).
In all experiments \((n = 6)\), a large proportion of the secreted enzyme remained closely associated with the cell surface, as only \(13.4 \pm 1.9\%\) of total LPL activity was released spontaneously into the culture media, in the absence of added heparin or VLDL. The remaining bound LPL activity was released upon addition of heparin to the media. Interestingly, we observed that VLDL was almost as effective as heparin in releasing LPL from the cell surface. Thus, heparin released a mean LPL enzyme remained closely associated with the cell surface, as

### Table I

| Lipid and lipoprotein composition of human plasma and VLDL used in the present studies | n | Concentration* |
|---|---|---|
| **Plasma lipids (millimoles/liter)** | | |
| Cholesterol | 5 | 4.50 ± 0.28 |
| Triglycerides | 5 | 1.30 ± 0.17 |
| Phospholipids | 3 | 2.48 ± 0.07 |
| **VLDL lipids (millimoles/liter)** | | |
| Cholesterol | 5 | 1.96 ± 0.13 |
| Triglycerides | 5 | 4.54 ± 0.23 |
| Phospholipids | 5 | 1.49 ± 0.08 |
| **VLDL apoproteins (milligrams/liter)** | | |
| ApoE | 5 | 40.50 ± 2.89 |
| ApoB | 5 | 450 ± 30 |
| ApoC-II | 5 | 52.67 ± 1.24 |
| ApoC-III | 5 | 209.33 ± 12.61 |

* Mean ± S.E.

**Effects of VLDL and CuSO_4 on the Morphology and Differentiation of LPL- and Control NEO-transfected Cells**—To determine the impact of LPL expression on cell morphology and differentiation following exposure to lipoproteins, LPL and control NEO transfectants were incubated in the presence of VLDL. VLDL particles were isolated from five apoE 3/3 normolipidemic donors. Cholesterol, triglyceride, and phospholipid concentrations of donor plasma and VLDL particles as well as the apolipoprotein composition of VLDL were determined and are shown in Table I. The effect of VLDL oxidation on cell differentiation and survival was also evaluated following the addition of CuSO_4, a pro-oxidative agent, to the culture media containing native VLDL. Representative photomicrographs of NEO- and LPL-transfected Neuro-2A cells exposed to VLDL with or without CuSO_4 are shown in Fig. 1. There were no significant morphological changes visible between LPL and control NEO transfectants incubated in the N2 media alone (Fig. 1, A versus E), indicating that LPL expression by itself was not sufficient to trigger pathways leading to phenotypic differentiation of Neuro-2A cells. Similarly, addition of CuSO_4 alone to the N2 media (Fig. 1, C and G) did not induce any significant morphological changes in either the LPL or NEO transfectants. However, following the addition of VLDL to the culture media (Fig. 1, B and F), striking differences were observed in the morphology of the LPL-transfected cells as compared with NEO transfectants. In LPL-secreting cells (Fig. 1F), addition of VLDL markedly stimulated the extension of neurites and induced a significant enlargement of cell volume. Oil Red O staining demonstrated the presence of intracellular vesicles filled with lipids probably resulting from endocytosis of lipoproteins (Fig. 1F). No such lipid vesicles were detected in LPL transfectants cultured in N2 media alone (Fig. 1E), or in NEO transfectants incubated with or without VLDL (Fig. 1, B and A). In the presence of VLDL and 10 \(\mu\)M CuSO_4, there was a marked reduction in the number of NEO transfectants (Fig. 1D). However, the LPL transfectants were not affected by CuSO_4 (Fig. 1H) and the cells continued to extend neurites as in the case of LPL transfectants incubated with VLDL alone (Fig. 1F).

![Fig. 1](http://www.jbc.org/)

FIG. 1. Photomicrographs of Neuro-2A cells transiently transfected with LPL or control NEO expression vectors, following exposure to human VLDL with or without CuSO_4. Neuro-2A cells transfected with NEO (panels A–D) or LPL (panels E–H) expression vectors were incubated at 37 °C for 48 h in N2 media alone (A and E), N2 media supplemented with 40 \(\mu\)g/ml VLDL (B and F), N2 media containing 10 \(\mu\)M CuSO_4 (C and G), or N2 media containing 40 \(\mu\)g/ml VLDL and 10 \(\mu\)M CuSO_4 (D and H). The cells were then rinsed with PBS, fixed with paraformaldehyde, and stained with Oil Red O, as described under “Experimental Procedures.” Photomicrographs of representative microscope fields were taken with a ×20 objective. Scale bar shown in the bottom left of panel A equals 25 \(\mu\)m.
volume, observed in LPL transfectants (Fig. 2B). As shown earlier (Fig. 1F), this cell enlargement was concomitant with the intracellular accumulation of lipid vesicles. In control NEO cells, addition of VLDL had no effect on neurite length and cell size, but it significantly increased the number of neurites per cell (Fig. 2C). In contrast, the number of neurites per cell was not affected by VLDL in LPL-transfected cells. Following simultaneous addition of VLDL and 10 μM CuSO₄ to the culture media, the number of neurites per NEO cell (Fig. 2C) decreased considerably whereas in LPL-transfected cells incubated with VLDL, the addition of CuSO₄ had no significant effect either on the neurite extension (Fig. 2A) or the increase in cell size (Fig. 2B) or the number of neurites per cell (Fig. 2C), as compared with those observed with VLDL alone.

In view of the observation that lipid uptake by Neuro-2A cells leads to neurite extension, it was of interest to ascertain which of the principal components of the VLDL may be responsible for this effect. As neurons are not normally exposed to substantial concentrations of VLDL, identification of the key components of VLDL that mediate neurite extension might point to other potential lipid or lipoprotein molecules that may be physiologically relevant in neurite extension. With this in mind, we performed Pearson's correlation analyses between the main VLDL components and the neurite length observed in LPL transfectants across seven independent experiments. Using this strategy, we found that three of the VLDL constituents (triglycerides, cholesterol, and phospholipids) showed strong and significant degrees of correlation with neurite lengths observed in these experiments (Table II). No correlation was found for apoE, suggesting that LPL may well serve the purpose of ligand for lipid receptors on the neurons (55).

**Effects of VLDL and CuSO₄ on the Survival of Neuro-2A Transfectants and the Production of Peroxidation Products in the Incubation Media—**To better understand the vulnerability of Neuro-2A upon exposure to VLDL and CuSO₄, the viability of Neuro-2A transfectants and the accumulation of lipoperoxidation products in the culture media were measured. The degree of cell survival in Neuro-2A transfectants was evaluated by the XTT reduction assay, and the accumulation of peroxidation products was measured by the levels of TBARS in the culture media. Addition of VLDL alone to LPL and NEO transfectants did not affect cell survival, as both cell types exhibited a 100% survival rate at each of the VLDL concentrations tested, ranging from 0 to 40 μg/ml (Fig. 3A). Addition of CuSO₄ (10 μM) to N2 media containing 0–20 μg/ml VLDL also had no effect on the survival of the two neuronal transfectants. However, when the VLDL concentration was elevated to 40 μg/ml (in the presence of Cu²⁺), this caused a 72% reduction in the viability of NEO transfectants whereas LPL transfectants remained fully viable even under these conditions (Fig. 3B).

The degree of oxidation of VLDL particles was measured by determining the production of TBARS in the culture media following incubation with increasing concentrations of VLDL and 10 μM CuSO₄. In the absence of neuronal cells, incubation of N2 media with 40 μg/ml VLDL plus 10 μM CuSO₄ induced a 2-fold production of TBARS as compared with N2 media without VLDL (Fig. 4A). At concentrations of VLDL lower than 40 μg/ml, there were no significant changes in the level of TBARS under the conditions used in these experiments. To examine possible interactions between neuronal survival and the formation of lipid oxidation products in the surrounding medium, we then measured the levels of TBARS in the culture media when LPL and NEO transfectants were incubated with 40 μg/ml VLDL in the presence or absence of 10 μM CuSO₄. VLDL alone had no effect on either cell type, but NEO transfectants incubated in the presence of VLDL and CuSO₄ accumulated high levels of peroxidation products in their environment. Media obtained from LPL transfectants showed no such increase in TBARS (Fig. 4B), indicating that the production and/or accumulation of TBARS in the culture media was inhibited by LPL expression in Neuro-2A cells.

**Effect of Preoxidized VLDL on the Survival of Neuro-2A Transfectants—**To determine whether LPL expression in Neuro-2A cells interferes with the oxidation of ambient VLDL, or protects the cells from the insult of oxidized lipoproteins, LPL and NEO transfectants were incubated in the presence of VLDL previously oxidized with 10 μM CuSO₄ for 3, 6, or 24 h. As expected, incubation of VLDL with 10 μM CuSO₄ stimulated
Several studies have indicated that LPL is not only involved in the metabolism of circulating triglyceride-rich lipoproteins but also in the production of TBARS. When compared with control VLDL (0.65 ± 0.0009), the levels of TBARS reached 0.86 ± 0.019, 1.38 ± 0.027, and 2.50 ± 0.069 nmol of malonaldehyde equivalents/mg of protein (mean ± S.E., n = 2) after 3, 6, and 24 h of preoxidation, respectively.

The LPL transfectant cells were found to be more sensitive than their NEO counterparts to oxidative damage induced by preoxidized VLDL (Fig. 5). After an 18-h incubation with 3-h preoxidized VLDL, the viability of the LPL transfectants was reduced to only 37% of control (0-h incubation time point). Under the same conditions, the survival of NEO cells was only slightly reduced (3%). Preoxidation of VLDL for longer periods (up to 24 h) did not lead to significant further reductions in the viability of LPL transfectants. However, a slight but not statistically significant reduction in cell viabiliy was observed in NEO cells upon incubation with 24-h preoxidized VLDL. Exposure of NEO and LPL transfectants for longer periods of time (48 h) to preoxidized VLDL eventually led to massive cell death.

**TABLE II**

| Transfectants | VLDL | n | Cholesterol | Triglycerides | Phospholipids | ApoE |
|---------------|------|---|-------------|--------------|--------------|------|
|               | µg/ml|   |             |              |              |      |
| LPL           | 40   | 7 | 0.927       | 0.782        | 0.927        | −0.441 |
| NEO           | 40   | 7 | 0.491       | 0.709        | 0.491        | −0.530 |
|               |      |   | (p < 0.0026)| (p < 0.0378) | (p < 0.0026) |      |

*NS, not significant.

**DISCUSSION**

The LPL and the control NEO transfectants were incubated in the presence of 40 µg/ml VLDL.

Spearman's nonparametric correlation analysis was performed, as indicated under “Experimental Procedures.” The LPL and the control NEO transfectants were incubated for longer periods of time to preoxidized VLDL eventually led to massive cell death.

**Fig. 3.** Effects of VLDL and CuSO4 on the survival of Neuro-2A cells transiently transfected with LPL or control NEO expression vectors. Neuro-2A transfectants were incubated at 37 °C for 48 h in N2 media, with or without VLDL and CuSO4, as indicated. The culture media were aspirated and replaced with the XTT reagent. Cells were incubated for an additional 18 h prior to optical density measurements for viability, as indicated under “Experimental Procedures.” All measurements were made in triplicate. Data are mean ± S.E. and represent the percentage of cell survival following a given treatment as compared with the mean cell survival of NEO transfectants incubated in N2 media alone across five independent experiments. In experiments carried out without CuSO4 (panel A, n = three independent experiments), significance levels were determined using Student’s t test (**, p < 0.005). When media containing CuSO4 were used (panel B, n = five independent experiments), significance levels were determined with an ANOVA test to identify significant mean differences between LPL and NEO transfectants (***, p < 0.0001).

**Fig. 4.** Levels of TBARS in culture media containing VLDL and CuSO4, incubated without (A) and with (B) Neuro-2A transfectants. Panel A, VLDL was added to the N2 media at increasing concentrations (0–40 µg/ml) and then incubated with 10 µM CuSO4 at 37 °C for 48 h. Panel B, Neuro-2A transfectants were incubated at 37 °C for 48 h in N2 media containing VLDL (40 µg/ml) and/or CuSO4 (10 µM), as indicated. Levels of TBARS were determined in the culture media and expressed as the percentage of increase compared with the levels of TBARS found in the control media without VLDL and CuSO4. Data are mean ± S.D. of three (panel A) or four (panel B) independent experiments performed in triplicate. In panel A levels of significance (**, p < 0.0001) were determined using an ANOVA test for comparing mean differences across all experimental groups. In panel B levels of significance (**, p < 0.0209) were determined using the Wilcoxon two-group test.

in both populations, as only 3% of LPL transfectants and 3.5% of NEO transfectants survived under these conditions (data obtained from a single experiment performed in triplicate).
LPL Modulation of Neuronal Cell Survival and Differentiation

but may play other important physiological roles in the lipid homeostasis of the brain. First, abundant expression of LPL mRNA and protein have been demonstrated in various regions of the brain of several mammalian species (4, 39). This enzyme is expressed in neuronal cells in locations not necessarily associated with vascular structures, the normal site of LPL activity. Furthermore, LPL has been known as a ligand for receptors of the LDL receptor family, some of which are critical in various neurophysiological functions such as long-term potentiation of neuronal cells in the hippocampus (40, 41). Moreover, LPL has been identified, in addition to other ligands for LDL receptors, in the neuritic plaques present in the brains of patients suffering from Alzheimer’s disease (42). More recently, mutant forms of the LPL protein have been associated with the incidence of the Alzheimer’s disease type of dementia (43).

Although VLDL are not usually found in the central nervous system, they were used in this study as they mimic the physiological effects of cerebrospinal fluid lipoproteins on the phenotypical differentiation of Neuro-2A cells (58). Moreover, as suggested recently by Huey et al. (54), plasma lipoproteins are likely to influence damaged peripheral nervous tissue and may eventually come in contact with degenerating nerve cells. Our results show that following exposure to VLDL, LPL-transfected cells are able to accumulate large amounts of lipids resulting in a significant increase in their cell size (Fig. 1). It is of interest to note that even though only 30% of the cells were effectively transfected and thus secreted LPL, this morphological change was observed in the entire cell population. We propose that this redistribution to the whole population is accomplished by the ability of VLDL to stimulate the release of LPL, anchored to the LPL-positive cell surfaces through heparan sulfate proteoglycans, into the culture medium, thus bringing the released LPL into contact with all other cells in the culture medium.

Our findings also suggest that the VLDL particles interact with both the cell surface and the LPL and are subsequently taken up by a massive endocytotic mechanism. This LPL-stimulated endocytosis is reminiscent of the hepatic clearance of lipoprotein remnants where LPL and apolipoproteins, especially apoE, act as ligands for both the cell surface heparan sulfate proteoglycans and members of the LDL receptor family (26, 27, 44–46). Previous studies have clearly demonstrated the presence of heparan sulfate proteoglycans and LDL receptor family members on the surface of Neuro-2A cells (38). Moreover, our results show an intimate association of secreted LPL activity with the cell surface of Neuro-2A. Thus, the bridging function of LPL, which is independent of its catalytic activity, may be an important physiological mechanism for the uptake of lipoproteins by this cell line. Alternatively, the classical pathway of LPL-mediated hydrolysis of triglycerides may also lead to the massive intracellular accumulation of free fatty acids as observed in transgenic mice overexpressing human LPL in the muscle (47, 48).

It has been demonstrated that following injury, cells of the peripheral and central nervous systems are able to synthesize large amounts of apoE (49, 50). Subsequent to Wallerian degeneration of the nerve fibers, this apoE could be used to assemble lipoprotein particles containing lipids from degenerating cells and myelin sheath. These apoE-rich particles could then be rapidly taken up by viable cells and the lipids recycled effectively. As proposed by Poirier et al. (51), this model could be important for providing surviving cells with building blocks necessary to sustain the increase in plasma membrane surface during synaptogenesis. Work by Mauch et al. (52) and de Chaves et al. (53) have clearly underlined the importance of lipids for axonal elongation and synaptogenesis. Recently, Huey et al. (54) have suggested that LPL might be involved in the scavenging of cellular debris following sciatric nerve injury. It is of considerable interest to note that LPL-expressing cells, in contrast to control NEO cells, are able to significantly extend their neurites following the addition of VLDL to the culture media (Figs. 1 and 2), and that this neurite extension strongly correlates with the cholesterol, triglyceride, and phospholipid content of the lipoprotein, but not with apoE (Table II). As suggested previously by de Chaves et al. (53), these data indicate that, the delivery of lipids to neurons is not necessarily dependent upon apoE and could be fulfilled by other ligands for LDL receptor such as LPL. Some of these lipids (triglycerides) could mediate the interactions of VLDL with LPL whereas others (cholesterol and phospholipids) could be used as plasma membrane constituents during the increase in cell size and neurite elongation observed in the LPL transfectants. An alternative pathway to explain the observed role of LPL in neurite development could depend on the binding of LPL to a cell receptor resulting in the activation of an intracellular signaling pathway leading to neuronal differentiation. Such LPL-activated signaling pathways have already been described in other cell types (55) and have underlined the importance of lipoprotein receptors in neuronal cell signaling (56, 57). In any case, lipids are important to sustain the large increase in plasma membrane surface associated with differentiation of LPL transfectants.

Several other lipoprotein-dependent neuritogenic processes have been described in the last few years (13, 16, 17, 58, 59). Most of them involve the heparan sulfate proteoglycan-low density lipoprotein-related protein-mediated endocytosis of lipoproteins and are affected by the apoE isofrom present on the lipoprotein particle. ApoE, a 33-kDa polypeptide, most com-
monly occurs in three major isoforms encoded by three alleles found at the same genetic loci, apoE2, E3, or E4. This apoE genotype is also known to influence the likelihood of developing Alzheimer’s disease (60). After endocytosis, selective intracellular accumulation of apoE2 and E3 occurs, because of their ability to escape the lysosomal degradation pathway following endocytosis (61). They are found to associate with the microtubules where they are presumed to act as scaffolding molecules, thereby stabilizing the cytoskeleton and thus sustaining neurite outgrowth. On the other hand, the accumulation of apoE4 in the same cells leads to destabilization of the microtubules and eventually results in the retraction of existing neurites (62). In addition to their role in neurotogenesis, apoE isoforms also (a) influence the cytotoxicity of amyloid-β peptides (63, 64), (b) interact with other cytoskeletal components, such as the microtubule-associated protein Tau (65), and (c) exhibit potential antioxidant properties (64, 66). Even though the apoE-3 isoform content of the VLDL used in our experiments did not influence the neurite length on LPL transfectants (Table II), it will be of interest to investigate the impact of LPL expression on neurite extension when neuronal cells are challenged with other apoE isoforms, especially apoE4.

VLDL, while in the process of undergoing oxidation in the culture medium by CuSO4, had deleterious effects on the mitochondrial content of the VLDL used in our experiments did not influence the neurite length on LPL transfectants (Table II), it will be of interest to investigate the impact of LPL expression on neurite extension when neuronal cells are challenged with other apoE isoforms, especially apoE4.

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