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Lipoprotein lipase is an important modulator of lipid uptake and storage in hypothalamic neurons

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ABSTRACT

LPL is the rate-limiting enzyme for uptake of TG-derived FFA in peripheral tissues, and the enzyme is expressed in the brain and CNS. We previously created a mouse which lacks neuronal LPL. This animal becomes obese on a standard chow, and we observed reduced lipid uptake in the hypothalamus at 3 months preceding obesity. In our present study, we replicated the animal phenotype in an immortalized mouse hypothalamic cell line (N41) to examine how LPL affects expression of AgRP as well as entry and storage of lipids into neurons. We show that LPL is able to modulate levels of the orexigenic peptide AgRP. LPL also exerts effects on lipid uptake into culture neurons, and that uptake of neutral lipid can be enhanced even by mutant LPL lacking catalytic activity. N41 cells also accumulate neutral lipid in droplets, and this is at least in part regulated by LPL. These data in addition to those published in mice with neuron-specific deletion of LPL suggest that neuronal LPL is an important regulator of lipid homeostasis in neurons and that alterations in LPL levels may have important effects on systemic metabolism and neuronal lipid biology.

1. Introduction

Lipoprotein lipase (LPL) catalyzes the release of free fatty acids (FFA) from triglyceride (TG)-rich lipoproteins and is the rate-limiting enzyme for delivery of lipids in peripheral tissues such as skeletal muscle, heart, and adipose tissue [1,2]. Normally found anchored to the GPIHBP1 on the luminal vascular endothelium, LPL is expressed in peripheral tissues that store neutral lipids or utilize FFA for energy. LPL is also found in both the central and peripheral nervous systems, although its exact role in these systems is still being elucidated [3–5]. The role of LPL in the brain has become increasingly relevant in recent years as links to regulation of energy homeostasis, Alzheimer's disease, and cognitive development have been identified [6–8]. Additionally, lipids are vital to the developing brain and are important substrates for membrane remodeling [9,10]. Whether LPL is directly involved in providing these lipids remains to be seen.

In a previous study we generated a mouse with neuron-specific LPL deletion (NEXLPL$^{-/-}$) to examine LPL's role in neuronal regulation of metabolism and energy homeostasis [6]. This mouse develops obesity by 6 months on a standard chow diet, with AgRP orexigenic peptide levels increased >3-fold at 3 months before obesity onset. Preceding obesity, we also observed reduced uptake of lipoprotein-derived FFA and reduced levels of n-3 long chain polyunsaturated fatty acids (LC-PUFA) in the hypothalamus. We speculate that deletion of neuronal LPL disrupts a major energy homeostasis signaling pathway, with increased AgRP levels underlying the obesity development.

In the current study, we used an immortalized mouse hypothalamic cell line (N41) to probe how LPL regulates neuronal AgRP, lipid uptake, and accumulation. N41 cells represent a clonal population of mouse hypothalamic neurons. These immortalized clonal populations have been published extensively due to their specific expression of relevant markers [11–15]. These commercially-available cells were chosen for our work due to extensive knowledge of the markers that are expressed in this cell line. Importantly for this work N41 cells express AgRP and LPL. We herein show that LPL is directly involved in neutral lipid transport into cultured hypothalamic neurons and that N41 cells store neutral lipid in

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droplet-like structures.

2. Materials and methods

2.1. DNA constructs

Human LPL cDNA [16] was subcloned into a murine stem-cell retrovirus (MSCV 2.2) vector (*Eco*RI/*Xho*I) directly preceding IRES-GFP. The mouse LPL cDNA construct was synthesized by GeneWiz (San Diego, CA) and subcloned into the MSCV 2.2 vector. Catalytically-inactive mLPL cDNA (Gly188Glu) was obtained from Keyclone Technologies (Cincinnati, OH). C-terminal 6xHistidine-tagged wild-type and mutant mLPL were created by PCR mutagenesis in pUC57 plasmid and subcloned into MSCV 2.2. Retroviral packaging was done with the pCL-Eco packaging plasmid and pseudotyped with VSV-G. LPL and control shRNA constructs for stable knockdown of LPL were obtained from the University of Colorado Functional Genomics Core.

2.2. Cell culture

mHypoE41 (N41) immortalized mouse hypothalamic neurons were purchased from CELLutions Biosystems (Winnipeg, MB). N41 cells were grown in DMEM containing 1000 mg/L glucose and 10% FBS at 37 °C in the presence of 5% CO_2. For serum-starving, cells were switched to DMEM containing no FBS for 12–16 h. The same lot of FBS (#A83DOOE) from Gemini Biotech was used for all experiments (lot# A83D). All viral packaging cells were grown in 4500 mg/L glucose supplemented with 10% FBS in a 5% CO_2 environment.

2.3. Viral packaging and transduction

To produce retrovirus for stable overexpression of LPL, 6 ug of control or LPL MSCV vector was transfected into Phoenix 293T cells along with 3 ug pCL-Eco and 2 ug VSV-G using 33 uL Lipofectamine 2000 (Life Technologies). Virus was harvested after 48 h of packaging in a total volume of 6 mL. 1 mL of viral particles was applied to N41 cells overnight. For stable knockdown of LPL, 12 ug of control or LPL shRNA lentiviral vector was transfected into HEK 293FT cells along with 10.8 ug pΔ8.9 and 1.2 ug VSV-G using 45 uL Lipofectamine 2000. Virus was packaged for 48 h in 6 mL media, and two rounds of transduction were performed. Stably-transduced cells were then selected for 3 days by growth in medium containing 5 ug/mL puromycin.

2.4. LPL-targeting siRNA/shRNA constructs

LPL was knocked down in N41 neurons using either siRNA or shRNA targeting. All LPL and control siRNAs were purchased from Ambion (Life Technologies). Control and LPL-targeting siRNA sequences can be found in the Supplemental materials section. For siRNA transfection, siRNA was transfected at a concentration of 35 nM with 2 uL/mL RNAiMax Lipofectamine (Life Technologies). Media was changed 16 h after transfection, and cells were allowed to grow 24 h before harvest. Sequences for control and LPL-targeting shRNAs can be found in the Supplemental materials section.

2.5. Measurement of LPL enzymatic activity

Heparin-released LPL enzymatic activity was measured using a phospholipid/^{3}H-triolein substrate with human serum as a source of ApoC2 as described previously [17].

2.6. Lipid uptake assay

To assess whether N41 cells can take up lipid derived from triglycerides and whether various apolipoproteins have an effect on uptake, an assay was performed by preparing a synthetic phospholipid/TG emulsion containing a ^{3}H triolein tracer. The emulsion was prepared by combining and sonicating the following: 5 mg ^{1}H triolein, 0.25 mg L-phosphatidylcholine, a trace amount of ^{3}H triolein (12.5 uCi total) tracer (Perkin Elmer, NET431001MC), 0.9 mL water, 2 mL 1 M tris–HCl (pH 8.0), 800 uL FFA-free BSA (MP Biomedical), and 300 uL KRP. After sonication, the substrate was diluted into DMEM to a final concentration of 85 uM triolein. For cells receiving ApoC2, purified human ApoC2 (MyBioSource) was added to a final concentration of 5 ug/mL. For cells receiving ApoE3 or ApoE4, the recombinant ApoE (MyBioSource) was added to the final diluted substrate at a concentration of 8 ug/mL. Substrate with or without apolipoproteins was applied to serum-starved cells for 2 h. Cells were washed with 0.1% FFA-free BSA and lysed in 1 mL of RIPA buffer. 800 uL of each lysis fraction was subjected to scintillation counting, while the rest was used for protein normalization.

2.7. Analysis of neutral lipid stores in N41 neurons

For qualitative analysis of lipid droplets in N41 cultures, live cells were stained for 5 min with AdipoRed (Lonza) and observed with fluorescent microscopy. For quantitative analysis of neutral lipid content in N41 neurons, coherent anti-stokes Raman spectroscopy (CARS) was employed. The CARS microscope was interfaced with two laser excitation beams. The pump beam was generated from a Ti:sapphire laser (Mira-900, Coherent). The Stokes beam was derived from a Nd:vanadate laser (PicoTrain, HighQ Lasers). The lasers were electronically synchronized at 76 MHz, and the pump and Stokes beams were spatially overlapped on a dichroic mirror (1000dcxr, Chroma) and directed to the laser scanning-microscope. The scanning system included two computer controlled galvanometric mirrors (Fluoview 300, Olympus) and a telescopic lens pair, which projects the excitation beams onto the back aperture of a 60X, 0.75 NA Olympus objective. Signal generated in the sample was detected in the forward direction, captured by a condenser, filtered by a bandpass filter (650 nm, Semrock), and detected by a photomultiplier tube (R3896, Hamamatsu). The images were recorded with FLUOVIEW software (Olympus) and ImageJ was used for data analysis.

2.8. Gene expression analysis

RNA was extracted from N41 cultures using the RNeasy Plus Mini it (Qiagen). For semi-quantitative RT-PCR, the One Step RT-PCR kit (Qiagen) was used in combination with an 18S competimer set (Ambion) for normalization.

2.9. Statistics

Variance is presented as SEM. Student *t*-tests were used to compare differences among groups. $P < 0.01$ was considered to be statistically-significant.

3. Results

3.1. LPL activity is present in N41 neurons and affects AgRP expression

We first determined whether LPL activity could be detected in cultured N41 neurons. Initial experiments lead to the observation that both heparin-releasable (extracellular) as well as intracellular