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Hippocampal lipoprotein lipase regulates energy balance in rodents

Alexandre Picard [1], Claude Rouch [1,2], Nadim Kassis [1,2], Valentine S. Moullé [1], Sophie Croizier [3], Raphaël G. Denis [3], Julien Castel [1,2], Nicolas Coant [1], Kathryn Davis [4], Deborah J. Clegg [4], Stephen C. Benoit [5], Vincent Prévot [3], Sébastien Bouret [3,6], Serge Luquet [1,2], Hervé Le Stunff [1], Céline Cruciani-Guglielmacci [1], Christophe Magnan [1,*]

ABSTRACT

Brain lipid sensing is necessary to regulate energy balance. Lipoprotein lipase (LPL) may play a role in this process. We tested if hippocampal LPL regulated energy homeostasis in rodents by specifically attenuating LPL activity in the hippocampus of rats and mice, either by infusing a pharmacological inhibitor (tyloxapol), or using a genetic approach (adeno-associated virus expressing Cre-GFP injected into $Lpl^{lox/lox}$ mice). Decreased LPL activity by either method led to increased body weight gain due to decreased locomotor activity and energy expenditure, concomitant with increased parasympathetic tone (unchanged food intake). Decreased LPL activity in both models was associated with increased *de novo* ceramide synthesis and neurogenesis in the hippocampus, while intrahippocampal infusion of *de novo* ceramide synthesis inhibitor myriocin completely prevented body weight gain. We conclude that hippocampal lipid sensing might represent a core mechanism for energy homeostasis regulation through *de novo* ceramide synthesis.

Keywords Lipid sensing; Obesity; Ceramides; Parasympathetic nervous system; Energy expenditure

1. INTRODUCTION

The central nervous system (CNS) is a key player in the regulation of energy balance in mammals [1,2]. This process involves a combination of signals arising from the periphery, including hormones and nutrients, which are detected by specialized areas like the hypothalamus and brainstem [3–5]. Since the work of Oomura et al. [6], there is a growing amount of evidence to suggest that hypothalamic fatty acid sensing plays a role in the regulation of energy balance, including insulin secretion and action, hepatic glucose production and food intake [7–10]. However the molecular mechanisms involved in this fatty acid sensing are still a matter of debate [8]. Postprandial triglycerides (TG)-enriched particles are abundant lipid species hydrolyzed by the lipoprotein lipase (LPL), and recent studies have highlighted a role for neuronal LPL-mediated hydrolysis of TG particles in the regulation energy balance [11,12].

Otherwise, other areas beside the hypothalamus have been shown to be involved in the regulation of energy homeostasis. Among them, the hippocampus has also been described as a potential site for the regulation of feeding behavior and body weight homeostasis [13,14]. For example, hippocampal lesions are associated with increased body weight and food intake in *ad libitum* fed rats [15]. Hunger and satiety circulating signals such as leptin or ghrelin were also shown to bind to hippocampus cells to regulate food intake in rats [16]. Moreover the orexigenic peptide ghrelin was shown to modulate hippocampal dendritic spine and memory acquisition [17]. Studies in both rodents and humans have reported that high fat/refined sugar diets impair hippocampal function [18]. Interestingly, LPL is highly expressed in the hippocampus [19,20] suggesting a potential role for this enzyme in body weight regulation.

The present work was aimed at studying whether TG hydrolysis by LPL specifically in the dorsal hippocampus could represent a physiologically relevant mechanism in energy homeostasis and body weight regulation. To this end, two different species and two experimental approaches were used to locally decrease LPL activity in the hippocampus: (1) the infusion of tyloxapol, an inhibitor of LPL activity, into the hippocampus of rats and (2) the bilateral injection of an adeno-associated viral vector expressing a Cre-GFP fusion protein (AAV Cre-GFP) into the hippocampus of $Lpl^{lox/lox}$ mice, leading to the specific deletion of the *Lpl* gene in the hippocampus of these mice ("LPL Hip−/−"). We observed using both techniques that hippocampal LPL inhibition led to both decreased locomotor activity and energy expenditure and ultimately induced body weight gain. Importantly,

[1]Université Paris Diderot, Sorbonne Paris Cité, BFA, EAC 4413 CNRS, Case courrier 7126, 4, rue Marie Andrée Lagroua Weill-Hallé, F-75205 Paris Cedex 13, France [2]Centre National de la Recherche Scientifique-CNRS EAC 4413, F-75205 Paris, France [3]Jean-Pierre Aubert Research Center, Development and Plasticity of the Postnatal Brain, INSERM U837, 59045 Lille Cedex, France [4]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA [5]Department of Psychiatry, University of Cincinnati, Cincinnati, OH, USA [6]The Saban Research Institute, Neuroscience Program, Children's Hospital Los Angeles, University of Southern California, Los Angeles, USA

*Corresponding author. Fax: +33 1 57 27 77 96. Email: christophe.magnan@univ-paris-diderot.fr (C. Magnan).

Abbreviations: LPL, lipoprotein lipase; CNS, central nervous system; TG, triglycerides; AAV, adeno-associated virus; GFP, green fluorescent protein; ANS, autonomic nervous system; RQ, respiratory quotient; SPT, serine palmitoyltransferase; CERS, ceramide synthase; SMPD1, acid sphingomyelin phosphodiesterase 1; SPHK1, sphingosine kinase 1

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food intake remained unchanged while the parasympathetic nervous system activity was increased suggesting a change in autonomic nervous system (ANS)-mediated peripheral energy handling. Inhibition of hippocampal LPL activity led to increased *de novo* ceramide biosynthesis, and a concomitant increase in neurogenesis, while, conversely, local infusion of myriocin – a potent inhibitor of *de novo* ceramide biosynthesis – completely prevented the metabolic changes in both mice and rats. Taken collectively these results highlight for the first time to our knowledge that nutritional lipid detection by LPL within hippocampus controls energy balance through *de novo* ceramide synthesis pathway.

2. MATERIAL AND METHODS

The experimental protocol was approved by the institutional animal care and use committee of the Paris Diderot University (CEEA40).

2.1. Animal models

Two-month-old male Wistar rats (225–250 g, Charles River, l'Arbresle, France) and two-month-old *Lpl*$^{lox/lox}$ mice (Jackson laboratory, strain B6.129S4-Lpl^{tm1ljg}/J, no. 006503) were used. Littermates Lpl$^{+/+}$ mice were used as controls. They were housed individually in stainless steel cages in a room maintained at 22±1 °C with lights on from 7 a.m. to 7 p.m. They were given a standard laboratory diet (proteins 19.4%; carbohydrates 59.5%; lipids 4.6%; vitamins and minerals 16.5%) and water *ad libitum*.

2.2. Solutions

Osmotic minipumps (Alzet® model 2004) were used to chronically infuse different solutions specifically into the hippocampus, through a catheter connected to a depth-adjustable cannula (Alzet® brain infusion kit 1 for rats and Alzet® brain infusion kit 3 for mice). The vehicle solution consisted of saline (Lavoisier). Tyloxapol solution (10 μg/day) was prepared by diluting 25 μL of tyloxapol (Sigma Aldrich # T8761) with 16.6 ml of saline. Myriocin solution (100 nM) was prepared by diluting a myriocin mother solution (Sigma Aldrich # M117; 2 mg/ml in methanol) 50,000 times in saline.

2.3. Viral production

An adeno-associated virus, AAV Cre-GFP, was used in order to induce genetic recombination within the hippocampus in *Lpl*$^{lox/lox}$ mice. The plasmid CBA.nls myc Cre.eGFP expressing the myc-nls-Cre-GFP fusion protein was kindly provided by Richard Palmiter (University of Washington, Seattle, USA). Adeno-associated viruses of the serotype 2/9 (AAV2/9) (6×10^{11} vg/ml and 1.7×10^{8} pi/μl) were produced by the viral production facility of the UMR INSERM 1089 (Nantes, France).

2.4. Surgical procedures

Both rats and mice were anesthetized with isoflurane and received a 10 μg/kg i.p. administration of xylazine. They were then placed on a stereotaxic frame. In rats, osmotic minipumps containing either vehicle, tyloxapol, myriocin, or both tyloxapol and myriocin were inserted subcutaneously and connected to cannula implanted within the hippocampus (*X*: −3.5 mm; *Y*: −4.5 mm; *Z*: −3.5 mm; [21]). In *Lpl*$^{+/+}$ and *Lpl*$^{lox/lox}$ littermate mice, 1 μl of 2/9 AAV Cre-GFP was injected per side (~6×10^{8} particles/μl at a rate of 0.20 μL/min for 5 min) bilaterally into the hippocampus (*X*: ±1 mm; *Y*: −2.06 mm; *Z*: −1.55 mm; [22]) to induce genetic recombination in floxed animals. After viral injection, mice were implanted with osmotic minipumps containing myriocin or vehicle.

2.5. Measurement of body weight and body composition

In rats, body weight was measured daily between 9 and 10 a.m. In mice, body weight was measured weekly at the same time. For both, body mass composition (lean tissue mass, fat mass, free water and total water content) was analyzed before the indirect calorimetry studies using an Echo Medical Systems EchoMRI 100 (Whole Body Composition Analyzers, EchoMRI, Houston, USA) according to manufacturer's instructions [23]. Briefly, awake animals were weighed before they were placed in a mouse holder and inserted in the MRI analyzer. Readings of body composition were obtained within 1 min. Body composition was expressed as a percentage of body weight.

2.6. Spontaneous alternation plus-maze task

Both vehicle and tyloxapol-infused rats were used for memory testing. Animals were extensively handled for one week prior to testing. Animals were placed into the center the center of the maze and allowed to explore freely for 20 min while arm entries were recorded. Performance scores were calculated as described previously [24].

2.7. Indirect calorimetry

Indirect calorimetry studies were performed in both rats and mice at day 14 during 5 days; this period corresponded to the beginning of increased body weight gain compared to controls. Animals were analyzed for whole energy expenditure (kcal/h), oxygen consumption and carbon dioxide production ($\dot{V}O_2$ and $\dot{V}CO_2$, where $\dot{V}$ is the volume), respiratory quotient ($RQ=\dot{V}CO_2/\dot{V}O_2$), food intake (g) and locomotor activity (counts/hour) using calorimetric cages with bedding, food and water (Labmaster, TSE Systems GmbH, Bad Homburg, Germany). Gas ratio was determined using an indirect open-circuit calorimeter [25,26], which monitored O_2 and CO_2 concentrations by volume at the inlet ports of a tide cage with an airflow of 0.4 L/min, with regular comparisons to an empty reference cage. Whole energy expenditure was calculated according to the Weir equation, using respiratory gas exchange measurements [27]. The flow was previously calibrated with a O_2 and CO_2 mixture of known concentrations (Air Liquide, S.A. France). Animals were individually housed in a cage with lights on from 7 a.m. to 7 p.m. and an ambient temperature of 22±±1 °C. All animals were acclimated to their cages for 48 h before experimental measurements. Data regarding food and water consumption were collected every 40 min, and all ambulatory movements recorded during the entire experiment, with the aid of an automated online measurement system combining highly sensitive feeding and drinking sensors and an infrared beam-based activity monitoring system. Gas and movement detection sensors were operational during both light and dark phases, allowing for continuous recording. Animals were monitored for body weight and composition at the beginning and end of the experiment. Data analysis was carried out with Excel XP using extracted raw values of $\dot{V}O_2$ consumption, $\dot{V}CO_2$ production (in ml/h), and energy expenditure (kcal/h). Subsequently, each value was expressed either by whole lean tissue mass extracted from the EchoMRI analysis.

2.8. Tissue collection

Brain tissues were dissected following the Glowinski and Iversen technique [28] at day 28; total hippocampus, adjacent cortex and hypothalamus were immediately frozen.

2.9. LPL activity assay

Heparin-releasable LPL activity was assayed in brain regions using a Roar LPL activity assay kit (RB-LPL, Roar Biomedical, Inc.). Briefly, tissues at day 28, then were lysed in 500 μL of assay buffer (150 mM

