Research Article

EASL JOURNAL OF HEPATOLOGY

Vagus nerve contributes to the development of steatohepatitis and obesity in phosphatidylethanolamine *N*-methyltransferase deficient mice

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Background & Aims: Phosphatidylethanolamine *N*-methyltransferase (PEMT), a liver enriched enzyme, is responsible for approximately one third of hepatic phosphatidylcholine biosynthesis. When fed a high-fat diet (HFD), $Pemt^{-/-}$ mice are protected from HF-induced obesity; however, they develop steatohepatitis. The vagus nerve relays signals between liver and brain that regulate peripheral adiposity and pancreas function. Here we explore a possible role of the hepatic branch of the vagus nerve in the development of diet induced obesity and steatohepatitis in $Pemt^{-/-}$ mice.

Methods: 8-week old $Pemt^{-/-}$ and $Pemt^{+/+}$ mice were subjected to hepatic vagotomy (HV) or capsaicin treatment, which selectively disrupts afferent nerves, and were compared to sham-operated or vehicle-treatment, respectively. After surgery, mice were fed a HFD for 10 weeks.

Results: HV abolished the protection against the HFD-induced obesity and glucose intolerance in $Pemt^{-/-}$ mice. HV normalized phospholipid content and prevented steatohepatitis in $Pemt^{-/-}$ mice. Moreover, HV increased the hepatic anti-inflammatory cytokine interleukin-10, reduced chemokine monocyte chemotactic protein-1 and the ER stress marker C/EBP homologous protein. Furthermore, HV normalized the expression of mitochondrial electron transport chain proteins and of proteins involved in fatty acid synthesis, acetyl-CoA carboxylase and fatty acid synthase in $Pemt^{-/-}$ mice. However, disruption of the hepatic afferent vagus nerve by capsaicin failed to reverse either the protection against the HFD-induced obesity or the development of HF-induced steatohepatitis in $Pemt^{-/-}$ mice.

Conclusions: Neuronal signals via the hepatic vagus nerve contribute to the development of steatohepatitis and protection against obesity in HFD fed $Pemt^{-/-}$ mice.

Keywords: Hepatic vagotomy; Capsaicin; PEMT; Obesity; Steatohepatitis.
Received 28 August 2013; received in revised form 18 September 2014; accepted 18 November 2014; available online 27 November 2014

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Abbreviations: PEMT, phosphatidylethanolamine *N*-methyltransferase; HFD, high-fat diet; HV, hepatic vagotomy; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PC, phosphatidylcholine; PE, phosphatidylethanolamine; IR, insulin resistance; CT, CTP:phosphocholine cytidylyltransferase; VLDL, low density lipoprotein; DIO, diet-induced obesity; CE, cholesteryl ester; TG, triacylglycerol; TNFα, tumor necrosis factor α; IL, interleukin; UCP2, uncoupling protein 2; CD36, cluster of differentiation 36; PPAR, peroxisome proliferator-activated receptor.

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) is rapidly rising throughout the world. In the United States, it is estimated that 70% of obese and diabetic patients have NAFLD [1]. NAFLD includes a wide spectrum of hepatic diseases, from steatosis (lipid accumulation) to non-alcoholic steatohepatitis (NASH; steatosis with inflammation) and eventually end-stage liver failure [2–5]. Currently, it is unclear how steatosis progresses into non-alcoholic steatohepatitis (NASH). Recent studies [6,7] by us and others have linked NAFLD with aberrant hepatic phospholipid levels. The amount and ratio of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are essential for membrane integrity. Decreased hepatic PC and decreased ratio of hepatic PC/PE induces steatosis in mice and has been observed in NASH patients [7]. In mice, the PC/PE ratio is a strong predictor of liver function and survival rate after partial hepatectomy [8].

In the liver, PC is synthesized via both the CDP-choline pathway and the PEMT pathway [9,10]. Under normal conditions, the CDP-choline pathway accounts for ~70% hepatic PC biosynthesis, and the remaining 30% is synthesized via the PEMT pathway [10]. The rate-limiting reaction in the CDP-choline pathway is catalyzed by CTP:phosphocholine cytidylyltransferase (CT) [11].

CT is encoded by two genes, *Pcyt1a* and *Pcyt1b*, and CTα (the product of *Pcyt1a*) is the predominant isoform in the liver [12,13]. Inhibition of either the CDP-choline (by liver-specific deletion of CTα) or PEMT pathway results in steatosis [14]. This is due to decreased hepatic PC production resulting in impaired very low density lipoprotein (VLDL) secretion. When fed the HFD for 10 weeks, mice lacking PEMT (*Pemt*$^{-/-}$ mice) are resistant to diet-induced obesity (DIO) but develop severe steatohepatitis [15]. The reason for the resistance to DIO in *Pemt*$^{-/-}$ mice is not simply due to either reduced PC synthesis or VLDL secretion, as the liver specific CTα knockout mice (*LCTα*$^{-/-}$ mice) are not protected from obesity [15].

Inter-organ communication through the autonomic nervous system is an essential regulator of metabolism [16]. The vagus nerve is an important collection of nerve fibres, including sensor (afferent) and motor (efferent) nerves [17]. Selective vagotomy has uncovered a role for the vagus nerve in regulating satiety, hormone secretion (insulin) and response to hormones (ghrelin) [17,18]. Adenoviral expression of PPARγ2 in livers of wild-type mice increases energy expenditure, decreases adiposity and improves insulin sensitivity, all of which were prevented by HV [19]. Similarly, the vagus nerve was shown to be involved in linking lower hepatic PPARα expression to insulin resistance [20]. Consequently, long-term stimulation of the hepatic vagus nerve in rats result in reduced body weight gain [21].

We performed HV on both *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice to investigate whether signals through the hepatic vagus nerve contribute to the protection from obesity in *Pemt*$^{-/-}$ mice. Our data show that neuronal signals through hepatic vagus nerve are critical for the resistance to DIO in *Pemt*$^{-/-}$ mice. Surprisingly, HV also completely abolished HFD-induced NASH in these mice. To further examine the contribution of afferent fibres of the hepatic branch of vagus nerve alone, we disrupted the afferent nerve with capsaicin. Capsaicin did not reverse the protection against DIO, nor improve the HFD-induced NASH in *Pemt*$^{-/-}$ mice. The current study highlights the fundamental role of the hepatic vagus nerve in the development of adiposity and NASH in *Pemt*$^{-/-}$ mice.

Materials and methods

Materials

Primers for real-time quantitative polymerase chain reaction (PCR) were purchased from the Institute of Biomolecular Design at the University of Alberta and are listed in Supplementary Table 1.

Animal handling, diets and surgery

All procedures were approved by the University of Alberta's Institutional Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care. Male C57BL/6 (backcrossed >7 generations) *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice were housed with free access to water and standard chow diet (LabDiet, #5001) before surgery.

The common hepatic branch of the vagus nerve (both afferent and efferent nerves) of 8-week-old *Pemt*$^{+/+}$ and *Pemt*$^{-/-}$ mice was cut and control mice were subjected to sham operation, as described [22]. For the capsaicin experiment, the hepatic vagal branch was freed from the surrounding tissues by paraffin paper and then wrapped for 30 min with gauze soaked with vehicle (Tween 80: olive oil = 1:9) alone or 10 mg/ml capsaicin dissolved in the vehicle solution as described [20]. One week after the surgery, mice were fed the HFD (Bio-Serv, #F3282) for 10 weeks. Mice were fasted for 12 h before sacrifice. Tissues were collected and stored at −80 °C until analysis or preserved in 10% phosphate-buffered formalin for histology.

Table 1. Plasma and liver parameters in HV- and sham-operated mice.

Operation	Sham		HV	
PEMT genotype	*Pemt*$^{+/+}$	*Pemt*$^{-/-}$	*Pemt*$^{+/+}$	*Pemt*$^{-/-}$
Liver (% b.w.)	4.2 ± 0.3	10.1 ± 0.3*	3.3 ± 0.2	4.7 ± 0.7$^{\#}$
Plasma C (mg/L)	15.7 ± 0.6	6.7 ± 1.0*	11.1 ± 0.9	12.2 ± 2.5$^{\#}$
Plasma CE (mg/L)	23.7 ± 3.8	2.3 ± 0.3*	16.7 ± 3.1	15.9 ± 4.3$^{\#}$
Plasma TG (mg/L)	18.4 ± 2.2	10.8 ± 2.5*	18.8 ± 2.5	12.7 ± 2.1
Hepatic C (μg/mg)	7.09 ± 0.74	7.73 ± 0.68	6.52 ± 0.55	7.13 ± 0.49
Hepatic CE (μg/mg)	1.73 ± 0.31	5.26 ± 1.24*	1.58 ± 0.19	2.58 ± 0.25$^{\#}$
Hepatic TG (μg/mg)	86.4 ± 34.4	326.0 ± 85.7*	88.7 ± 14.6	112.1 ± 21.0$^{\#}$
Hepatic PC (nmol/mg)	87 ± 3	73 ± 1*	86 ± 4	86 ± 1$^{\#}$
Hepatic PE (nmol/mg)	64 ± 8	55 ± 1	40 ± 1	47 ± 1

Pemt$^{-/-}$ and *Pemt*$^{+/+}$ mice were subjected to hepatic vagotomy (HV) and sham operation. After surgery, mice were fed a HFD for 10 weeks. Data are presented as mean values ± SEM. (n = 5–7). $^{*}p$ <0.05, *vs. Pemt*$^{+/+}$ mice on the same surgery; $^{\#}p$ <0.05, sham-operated mice *vs.* HV-operated mice of the same genotype.

In vivo metabolic analysis

Metabolic measurements (O_2 consumption, CO_2 production and heat production) were obtained using a Comprehensive Lab Animal Monitoring System (Columbus Instruments, OH). Mice were housed individually for 3 days before being placed in metabolic cages. Following an initial 24-h acclimatization period, measurements were taken every 13 min for 24 h. Mice were fasted for 12 h before a glucose tolerance test. Briefly, mice were administered glucose (2 g/kg body weight) by intraperitoneal injection. Blood glucose was measured by a glucometer prior to injection and at indicated times afterwards.

Analytical procedures

The mass of cholesterol-and cholesteryl ester (CE) was measured by gas-liquid chromatography [23]. The mass of triacylglycerol (TG) was either measured by gas-liquid chromatography or commercially available kits according to the manufacturer's protocols from Roche Diagnostics. Plasma alanine aminotransferase was measured using commercially available kits (Biotron Diagnostics). Hepatic cytokines and chemokines were quantified using ELISA kits from eBioscience or Preprotech. Protein concentrations were determined by the Bradford assay (Bio-Rad). Hepatic PC and PE were quantified as previously described [24].

Histology

A portion of the liver and visceral white adipose tissue was subjected to hematoxylin and eosin staining. Liver slides were scored for steatosis, hepatocellular ballooning, and portal inflammation and lobular inflammation, using a modified NAFLD activity score system [25].

Electron microscopy

The common truncal vagus nerve and the hepatic branch of vagus nerve were collected from non-treated mice and from mice treated with vehicle or capsaicin 7 days after surgery, and then processed as before [15]. Electron micrographs were taken with a Hitachi H7000 transmission electron microscope.

Real-time quantitative PCR

Total RNA was isolated from snap-frozen liver tissue using TRIzol reagent (Invitrogen). Total RNA was treated with DNase I (Invitrogen) to degrade genomic DNA, then reverse-transcribed using an oligo(dT)12–18 primer and Superscript II