Original Article

MOLECULAR METABOLISM

Loss of dorsomedial hypothalamic GLP-1 signaling reduces BAT thermogenesis and increases adiposity

Shin J. Lee [1,*], Graciela Sanchez-Watts [2], Jean-Philippe Krieger [1], Angelica Pignalosa [1], Puck N. Norell [1], Alyssa Cortella [2], Klaus G. Pettersen [1], Dubravka Vrdoljak [1], Matthew R. Hayes [3], Scott Kanoski [2], Wolfgang Langhans [1,4], Alan G. Watts [2,4]

ABSTRACT

Objective: Glucagon-like peptide-1 (GLP-1) neurons in the hindbrain densely innervate the dorsomedial hypothalamus (DMH), a nucleus strongly implicated in body weight regulation and the sympathetic control of brown adipose tissue (BAT) thermogenesis. Therefore, DMH GLP-1 receptors (GLP-1R) are well placed to regulate energy balance by controlling sympathetic outflow and BAT function.

Methods: We investigate this possibility in adult male rats by using direct administration of GLP-1 (0.5 ug) into the DMH, knocking down DMH GLP-1R mRNA with viral-mediated RNA interference, and by examining the neurochemical phenotype of GLP-1R expressing cells in the DMH using in situ hybridization.

Results: GLP-1 administered into the DMH increased BAT thermogenesis and hepatic triglyceride (TG) mobilization. On the other hand, *Glp1r* knockdown (KD) in the DMH increased body weight gain and adiposity, with a concomitant reduction in energy expenditure (EE), BAT temperature, and uncoupling protein 1 (UCP1) expression. Moreover, DMH *Glp1r* KD induced hepatic steatosis, increased plasma TG, and elevated liver specific de-novo lipogenesis, effects that collectively contributed to insulin resistance. Interestingly, DMH *Glp1r* KD increased neuropeptide Y (NPY) mRNA expression in the DMH. GLP-1R mRNA in the DMH, however, was found in GABAergic not NPY neurons, consistent with a GLP-1R-dependent inhibition of NPY neurons that is mediated by local GABAergic neurons. Finally, DMH *Glp1r* KD attenuated the anorexigenic effects of the GLP-1R agonist exendin-4, highlighting an important role of DMH GLP-1R signaling in GLP-1-based therapies.

Conclusions: Collectively, our data show that DMH GLP-1R signaling plays a key role for BAT thermogenesis and adiposity.

Keywords Neuropeptide; Hypothalamus; Sympathetic nerve; Adipose tissue; Obesity

1. INTRODUCTION

The intestine and brain both produce glucagon-like peptide-1 (GLP-1), which plays an important role in the control of food intake and glycemia [1,2]. GLP-1 producing neurons are primarily located in the nucleus tractus solitarii (NTS) in the hindbrain, where they integrate neural, hormonal, and viscerosensory information arising from meal ingestion [3]. GLP-1 neurons send projections to numerous brain areas involved in the neuroendocrine and autonomic regulation of energy homeostasis [4–6]. Recent studies have highlighted the role of central GLP-1R in mediating the anorexigenic effects of GLP-1R agonists (e.g., exenatide and liraglutide), which are pharmacological treatment options for Type II diabetes and, more recently, obesity [7–11]. Intracerebroventricular injection of GLP-1 or GLP-1R agonists decreases food intake and increases energy expenditure (EE) [12–14], resulting in weight loss.

The recent emergence of GLP-1's role in the control of the sympathetic nervous system (SNS) highlights novel mechanisms through which GLP-1 contributes to energy balance [15]. Stimulation of central GLP-1R increases brown adipose tissue (BAT) thermogenesis and causes weight loss in a way that is largely independent of its anorexigenic effects [14,16–19]. Central GLP-1 infusion also decreases de-novo lipogenesis in the liver and hepatic as well as adipose tissue triglyceride content [20,21]. It is unknown, however, whether central GLP-1R-mediated effects on sympathetic regulation of adipose tissue and liver are associated with the pathogenesis of obesity and insulin resistance. A recent study showed that GLP-1R expression is reduced in postmortem hypothalamic nuclei of obese diabetic humans [22], suggesting that reduced hypothalamic GLP-1R signaling may contribute to the development of the metabolic syndrome.

The dorsomedial nucleus of the hypothalamus (DMH), a key part of the sympathetic control network [23], expresses GLP-1R and is densely

[1]Physiology and Behavior Laboratory, ETH Zürich, 8603 Schwerzenbach, Switzerland [2]Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089, USA [3]Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

[4] Co-senior authors.

*Corresponding author. Physiology and Behavior Laboratory, ETH Zürich, 8603 Schwerzenbach, Switzerland. E-mail: shin-lee@ethz.ch (S.J. Lee).

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innervated by GLP-1 fibers [4,5,24,25], suggesting a possible role of DMH GLP-1R signaling in SNS regulation. DMH neurons send monosynaptic projections to the rostral raphe pallidus (rRPa), which contains sympathetic premotor neurons regulating BAT activity [26–29]. Disinhibiting DMH glutamatergic neurons stimulates BAT sympathetic nerve activity (SNA) and thermogenesis [30,31]. Moreover, the DMH mediates increased blood pressure and heart rate during psychogenic stress [32] and obesity [33]. DMH leptin receptor (LEPR) expressing neurons have been synaptically and functionally linked to BAT thermogenesis [34–36]. Blocking or ablating DMH LepR reduced EE and the thermogenic effects of leptin [34,35]. Similar to LepR in the DMH [37], GLP-1R are expressed in the anterior and ventral part of the DMH [5,38]. However, the neurochemical phenotype and function of GLP-1R expressing neurons are unknown. Given the changes in GLP-1R expression observed in the hypothalamus of obese diabetic humans [22], a decrease in DMH GLP-1R signaling may also play an important role in the pathogenesis of obesity via a SNS-dependent mechanism. Neuropeptide Y (NPY) expressing neurons in the DMH have long been implicated in controlling ingestive behavior and body weight (BW) [39–43]. Knockdown of DMH *NPY* expression decreases food intake, reduces fat mass, and ameliorates diet-induced obesity (DIO) and insulin resistance [44–46]. Interestingly, DMH *NPY* knockdown also increases BAT thermogenesis and white adipose tissue (WAT) browning, suggesting that DMH NPY neurons contribute to the development of obesity, in part by decreasing sympathetic outflow [47]. However, the mechanisms through DMH NPY neurons reduce sympathetic outflow are poorly understood. Arcuate nucleus (ARC) NPY neurons are inhibited by local GLP-1-sensitive GABAergic neurons [9], raising the possibility that a similar mechanism may contribute to DMH NPY neuronal regulation.

Here we investigate the role of DMH GLP-1R signaling in the sympathetic regulation of BAT in male rats by assessing 1) the short-term effects of DMH GLP-1 injections on BAT thermogenesis, 2) the effects of long-term DMH *Glp1r* knockdown (KD) on BW, EE, BAT thermogenesis, and glucose metabolism, and 3) the neurochemical identity of DMH GLP-1R expressing neurons. We found that DMH GLP-1R stimulation increased BAT thermogenesis and hepatic fuel mobilization, whereas DMH *Glp1r* KD increased adiposity, decreased EE, impaired BAT function, and hepatic steatosis; effects which collectively contributed to increased insulin resistance and blunted the anorexigenic response to the long-lasting GLP-1R agonist, exendin-4 (Ex-4). In addition, DMH *Glp1r* KD locally increased *NPY* expression, implicating NPY as a downstream substrate for DMH neuronal regulation of BAT thermogenesis. Collectively, our results show that DMH GLP-1R signaling is a physiologically important component of the central GLP-1 system that controls BAT thermogenesis in a way that helps maintain energy balance.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague Dawley (SD) rats were purchased from Charles River Laboratories (Sulzfeld, Germany) or Harlan (Envigo US) and housed individually in a climate-controlled room (temperature: 23 ± 2 °C, humidity: 55 ± 5%). Rats were maintained on a 12 h/12 h light/dark cycle with ad libitum access to standard chow diet (No 3436, Provimi Kliba AG, Kaiseraugust, Switzerland) and tap water, except as noted. All procedures were approved by the Cantonal Veterinary Office of Zurich, or the University of Southern California Institutional Animal Care and Use Committee.

2.2. Stereotaxic surgery

SD rats (320–340 g; pre-surgical BW) were anesthetized by intraperitoneal injection of 2 mg/kg Xylazine (Rompun 2%, Provet AG, Lyssach, Switzerland) and 10 mg/kg BW Ketamin (Ketalar 50 mg/ml, Pfizer AG, Zurich, Switzerland), and positioned in the stereotaxic apparatus. *Acute DMH GLP-1R activation study*: Bilateral guided cannulas (Bilaney, Dusseldorf, Germany) were positioned immediately above the DMH (3.3 mm caudal to the bregma, 1 mm from center to center, 8 mm below pedestal). Sham injections started after 7 days of recovery (injectors fit to 8 mm guided cannula with 1.5 mm projections). On the experiment day, GLP-1 (0.5 ug in 0.5 ul volume, n = 8) or saline (0.5 ul, n = 8) was injected bilaterally 4 h after dark onset. The placement of the cannula was verified during cryosectioning the brains.

DMH Glp1r knockdown: AAV-GFP or AAV GLP-1R shRNA constructs were previously described in detail [48,49]. 200 nl of AAV-GFP or AAV GLP-1R shRNA (5.22 e12 GC/ml) was bilaterally injected into the DMH (3.3 mm caudal, 0.5 mm lateral to the bregma, and 8.4 mm ventral to dura) using a glass capillary micropipette connected with polyethylene (PE)-tubing to a microinjector (Picospritzer III, Parker Hannifin, Hollis, USA). All injected rats (n = 7 for AAV-GFP and n = 10 for AAV GLP-1R) were also implanted with intraperitoneal catheters for subsequent drug injections. Food intake and BW were measured daily. Behavioral and metabolic effects were assessed beginning approximately 3 weeks after surgery. Only the animals with bilateral GFP expression within the DMH were included in the final analyses.

2.3. Indirect calorimetry

Respiratory exchange ratio (RER) and energy expenditure (EE) measurements were conducted in an open circuit calorimetry Phenomaster system (TSE) after 3 days of habituation. TSE Indirect calorimetry estimates EE (kcal/h/kg) from the animals's O2 consumption (mL/kg/h) and CO2 production (mL/kg/h) using the Weir equation [50,51]. RER: VCO2/VO2. EE: (3.941 × VO2 + 1.106 × VCO2)/1000. For the *Glp1r* KD study, RER and EE were measured at 8 weeks after surgery. For the acute study, RER and EE were measured before and after intra-DMH GLP-1 (Bachem, 0.5 μg in 0.5 μl saline, n = 8) or vehicle (n = 8) administration for comparison. Data are presented as the average RER and EE values in dark, light, and total diurnal cycle for 3 consecutive days.

2.4. BAT and core temperature measurements

Infra-red (IR) pictures were taken with an E60 camera (FLIR), mounted vertically over the shaved interscapular area (distance 30 cm). Rats were lightly restrained in a stretched position under the camera and 3 snapshots/time point were taken for further analysis. For the acute study, baseline IR pictures were taken prior to intra-DMH vehicle/GLP-1 injection (0.5 μg in 0.5 μl saline) and a second series of IR pictures was taken 4 h after the injection. Body core temperature was measured with a rectal thermometer (WD-35427-20, Oakton instruments) before and 4 h after the injection. For the *Glp1r* KD study, all rats were subjected to BAT IR recording 5 h after dark onset at 4 weeks. For the β-3 receptor agonist study, rats had ad lib access to food for 2 h after dark onset. After 1.5 h of fasting, rats received 1 ug/kg IP injection of β-3 receptor agonist (Sigma–Aldrich CL316243) or PBS. IR pictures were taken 2 h after the injection. Final analyses of the IR pictures were done by defining a standard rectangular area (60 × 20 px) over the interscapular region and averaging the mean area temperatures of two pictures (FLIR Tool software for PC).

