Brief communication

The role of autonomic efferents and uncoupling protein 1 in the glucose-lowering effect of leptin therapy

Heather C. Denroche [1], Michelle M. Kwon [1], Maria M. Glavas [1], Eva Tudurí [1], Marion Philippe [1], Whitney L. Quong [1], Timothy J. Kieffer [1,2,*]

ABSTRACT

Objective: Leptin reverses hyperglycemia in rodent models of type 1 diabetes (T1D). Direct application of leptin to the brain can lower blood glucose in diabetic rodents, and can activate autonomic efferents and non-shivering thermogenesis in brown adipose tissue (BAT). We investigated whether leptin reverses hyperglycemia through a mechanism that requires autonomic innervation, or uncoupling protein 1 (UCP1)-mediated thermogenesis.

Methods: To examine the role of parasympathetic and sympathetic efferents in the glucose-lowering action of leptin, mice with a subdiaphragmatic vagotomy or 6-hydroxydopamine induced chemical sympathectomy were injected with streptozotocin (STZ) to induce hyperglycemia, and subsequently leptin treated. To test whether the glucose-lowering action of leptin requires activation of UCP1-mediated thermogenesis in BAT, we administered leptin in STZ-diabetic *Ucp1* knockout (*Ucp1*$^{-/-}$) mice and wildtype controls.

Results: Leptin ameliorated STZ-induced hyperglycemia in both intact and vagotomised mice. Similarly, mice with a partial chemical sympathectomy did not have an attenuated response to leptin-mediated glucose lowering relative to sham controls, and showed intact leptin-induced *Ucp1* expression in BAT. Although leptin activated BAT thermogenesis in STZ-diabetic mice, the anti-diabetic effect of leptin was not blunted in *Ucp1*$^{-/-}$ mice.

Conclusions: These results suggest that leptin lowers blood glucose in insulin-deficient diabetes through a manner that does not require parasympathetic or sympathetic innervation, and thus imply that leptin lowers blood glucose through an alternative CNS-mediated mechanism or redundant target tissues. Furthermore, we conclude that the glucose lowering action of leptin is independent of UCP1-dependent thermogenesis.

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Keywords Type 1 diabetes; Glucose; Vagotomy; Sympathectomy; Brown adipose tissue; Streptozotocin

1. INTRODUCTION

The hormone leptin plays a critical role in the control of glucose metabolism [1]. Leptin can reverse hyperglycemia in rodent models of type 1 diabetes (T1D), independent of food intake, and without raising circulating insulin levels [2–8]. Central leptin administration or gene therapy can reverse hyperglycemia in rodent models of T1D in a similar manner to peripheral administration [9–16], suggesting a critical role of the central nervous system (CNS) in mediating leptin action. CNS leptin action can modulate peripheral tissues via both sympathetic and parasympathetic branches of the autonomic nervous system (ANS). Leptin-induced glucose uptake in brown adipose tissue (BAT) and skeletal muscle, and activation of hepatic 5′AMP-activated protein kinase in non-diabetic rodents have been shown to occur in a sympathetic-dependent manner [17–22]. In addition, disruption of the parasympathetic branch of the ANS with a surgical vagotomy diminishes improvements in glucose homeostasis following central leptin administration in rodent models of type 2 diabetes [23,24]. Thus, autonomic efferents play a key role in mediating leptin-induced changes to glucose metabolism in peripheral tissues and may contribute to leptin-mediated reversal of hyperglycemia.

Activated BAT thermogenesis by sympathetic stimulation causes robust glucose uptake and energy dissipation as heat [25,26], and holds therapeutic potential for obesity and metabolic disorders [27,28]. BAT thermogenesis requires mitochondrial uncoupling protein 1 (UCP1) [25,29]. Interestingly, central leptin can stimulate UCP1-dependent thermogenesis and glucose utilization in BAT in a noradrenergic-dependent manner [30]. Furthermore, central leptin

[1]Laboratory of Molecular and Cellular Medicine, Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada [2]Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada

*Corresponding author. Department of Cellular and Physiological Sciences, University of British Columbia, 2350 Health Sciences Mall, Vancouver V6T 1Z3, Canada. Tel.: +1 (604) 822 2156; fax: +1 (604) 822 2316. E-mail: tim.kieffer@ubc.ca (T.J. Kieffer).

Abbreviations: 6OHDA, 6-hydroxydopamine; ANS, autonomic nervous system; BAT, brown adipose tissue; CNS, central nervous system; CCK, cholecystokinin; iBAT, interscapular BAT; STZ, streptozotocin; T1D, type 1 diabetes; TH, tyrosine hydroxylase; UCP1, uncoupling protein 1

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therapy has been found to robustly induce *Ucp1* expression and BAT glucose uptake in rodent models of T1D [9,11,16,31]. Therefore, the activation of UCP1-dependent BAT thermogenesis may play a role in leptin-induced glucose lowering.

We aimed to elucidate the role of the ANS in the anti-diabetic effect of leptin therapy. Although leptin has been shown to improve cardiovascular function in rodent models of T1D by increasing sympathetic tone [14,15], studies using pharmacological or genetic means to partially attenuate sympathetic innervation or signaling have not found any evidence that the anti-diabetic action of leptin in rodent models of T1D requires sympathetic innervation [14,16,31]. Furthermore, the role of parasympathetic efferents in leptin-mediated glucose lowering has not been reported. Thus, we assessed whether leptin could reverse streptozotocin (STZ)-induced hyperglycemia in mice following 6-hydroxydopamine (6OHDA)-induced sympathectomy or surgical vagotomy. Subsequently, we examined the role of leptin-induced BAT thermogenesis as a possible downstream mechanism. We report that neither subdiaphragmatic vagotomy, nor partial chemical sympathectomy attenuates the glucose-lowering action of leptin. In addition, we found that although leptin induces *Ucp1* expression and thermogenesis in BAT of STZ-diabetic mice, diabetic *Ucp1*$^{-/-}$ mice are not refractory to leptin therapy.

2. MATERIALS AND METHODS

2.1. Animals

Male C57Bl/6J mice for vagotomy studies, and *Ucp1*$^{+/-}$ mice [32] (Jackson Laboratories, Bar Harbor, ME), and male C57Bl/6J mice for sympathectomy studies (Centre for Disease Modeling, Vancouver, Canada) were housed with a 12/12-hour light—dark cycle with *ad libitum* access to food (2918, Harlan Laboratories, Madison, WI) and water. All animal procedures were approved by the UBC Animal Care Committee and carried out in accordance with the Canadian Council on Animal Care guidelines. *Ucp1*$^{+/-}$ mice were interbred to generate *Ucp1*$^{+/+}$, *Ucp1*$^{+/-}$ and *Ucp1*$^{-/-}$ mice, and genotyped using the *Ucp1*$^{+}$ primer (GATTTGCCTCTGAATGCCCGC), *Ucp1*$^{-}$ primer (CCTA CCCGCTTCCATTGCTCA) and, *Ucp1* common primer (GCACG GGGTGGTGTACTATCC). Experimental *Ucp1*$^{-/-}$ and *Ucp1*$^{+/+}$ controls were housed in cages maintained at thermoneutrality (29.0—30.5 °C) from 3 weeks of age to experimental endpoint.

2.2. Chemical sympathectomy

Male C57Bl/6J mice, aged 12 weeks, were injected intraperitoneally (i.p.) with 250 mg/kg 6-hydroxydopamine hydrobromide (6OHDA, Sigma—Aldrich, St. Louis, MO), prepared in sterile saline containing 0.05% L-ascorbic acid (Sigma—Aldrich, St. Louis, MO) [33], 3 weeks before STZ administration.

2.3. Vagotomy

C57Bl/6J mice received subdiaphragmatic vagotomies at 6 weeks of age, performed by Jackson Laboratories (Bar Harbor, ME) as previously described [34]. A section of both the dorsal and ventral vagal trunks, adjacent to the esophagus, were excised. In sham operated mice the vagus was exposed but not excised.

2.4. STZ administration

STZ (Sigma—Aldrich, St. Louis, MO) prepared in acetate buffer, pH 4.5, was injected i.p. at a dose of 170 mg/kg in *Ucp1*$^{-/-}$ mice and controls. For all other studies, mice received 180 mg/kg STZ. Diabetes was defined as two consecutive measures of fasting blood glucose $\geq$16.5 mmol/L.

2.5. Leptin administration via mini-osmotic pump

Alzet osmotic pumps (DURECT Corporation, Cupertino, CA) were implanted subcutaneously as previously described [2,3], delivering 10 μg/day recombinant mouse leptin (National Hormone & Peptide Program, Torrance, CA, USA), or 20 μg/day recombinant mouse leptin (Peprotech, Rocky Hill, NJ), or vehicle. The doses of leptin were adjusted between studies to account for different glucose lowering efficacies observed between the peptide sources. For the vagotomy study, mice received leptin supplied by the National Hormone & Peptide Program (Torrance, CA) prepared in PBS or PBS as vehicle. All other studies employed leptin from Peprotech (Rocky Hill, NJ) dissolved in water or water as vehicle.

2.6. Metabolic assessments

Blood glucose, plasma leptin, and insulin were measured from blood collected through the saphenous vein in conscious mice following a 4-hour fast as previously described [2,34].

2.7. Cholecystokinin (CCK)-induced satiety

CCK octapeptide (26—33) (American Peptide, Sunnyvale, CA) was prepared as previously described [34]. Following an overnight fast, mice were injected with CCK or vehicle i.p., placed individually in cages with pre-weighed food, and allowed to feed for 1 h. Food intake was measured as the difference in food weight prior to and 1-hour post-injection.

2.8. Gastric distension

Stomachs were harvested 32 days post-pump implant following a 4-hour fast. Gastric contents were removed, and stomach weights were measured.

2.9. BAT measurements

BAT was harvested 19 days post-pump implant, fixed overnight in 4% paraformaldehyde at 4 °C, incubated in 25% sucrose in PBS for 72 h, and subsequently frozen in an isopentane bath. BAT was sectioned by Wax-it Histology Services Inc. (Vancouver, Canada). Sections were immunostained for tyrosine hydroxylase (TH) (rabbit anti-TH, Millipore, Cat# AB152, 1:1000 dilution), subsequently incubated with secondary antibody, mounted and scanned as previously described [35]. Total TH positive area was expressed relative to total cell count (based on DAPI fluorescence) and total section area. RNA was isolated from BAT on day 19 and DNase treated using RNeasy Lipid Tissue Mini Kit (Qiagen, Mississauga, Canada) and RNase free DNase kit (Qiagen). RNA was converted to cDNA and RT-qPCR was performed as previously described [36] using forward primer (GGCCCTTGTAAACAACAAAATAC) and reverse primer (GGCAACAAGAGCTGACAGTAAAT). Beta-actin was selected as a reference gene by geNorm analysis as previously described [36]. Relative transcript abundance was determined by the Pfaffl method [37].

2.10. Interscapular BAT (iBAT) thermogenesis

Temperature transponders (Implantable Programmable Temperature Transponder IPTT-300; Bio Medic Data Systems Inc, Seaford, USA) were implanted interscapularly at the same time as osmotic pumps. Non-fasted temperatures were recorded using a hand held Pocket Scanner (DAS-5007; Bio Medice Data Systems Inc).

2.11. Statistical analyses

Data are presented as mean ± SEM. Data and statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla,

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