Original article

MOLECULAR METABOLISM

Leptin receptor neurons in the dorsomedial hypothalamus are key regulators of energy expenditure and body weight, but not food intake

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ABSTRACT

Objective: Leptin responsive neurons play an important role in energy homeostasis, controlling specific autonomic, behavioral, and neuroendocrine functions. We have previously identified a population of leptin receptor (LepRb) expressing neurons within the dorsomedial hypothalamus/dorsal hypothalamic area (DMH/DHA) which are related to neuronal circuits that control brown adipose tissue (BAT) thermogenesis. Intra-DMH leptin injections also activate sympathetic outflow to BAT, but whether such effects are mediated directly via DMH/DHA LepRb neurons and whether this is physiologically relevant for whole body energy expenditure and body weight regulation has yet to be determined.
Methods: We used pharmacosynthetic receptors (DREADDs) to selectively activate DMH/DHA LepRb neurons. We further deleted LepRb with virally driven cre-recombinase from DMH/DHA neurons and determined the physiological importance of DMH/DHA LepRb neurons in whole body energy homeostasis.
Results: Neuronal activation of DMH/DHA LepRb neurons with DREADDs promoted BAT thermogenesis and locomotor activity, which robustly induced energy expenditure ($p < 0.001$) and decreases body weight ($p < 0.001$). Similarly, intra-DMH/DHA leptin injections normalized hypothermia and attenuated body weight gain in leptin-deficient *ob/ob* mice. Conversely, ablation of LepRb from DMH/DHA neurons remarkably drives weight gain ($p < 0.001$) by reducing energy expenditure ($p < 0.001$) and locomotor activity ($p < 0.001$). The observed changes in body weight were largely independent of food intake.
Conclusion: Taken together, our data highlight that DMH/DHA LepRb neurons are sufficient and necessary to regulate energy expenditure and body weight.

Keywords Leptin; Thermoregulation; Energy expenditure; Dorsomedial hypothalamus; DREADD

1. INTRODUCTION

Leptin regulates energy homeostasis by activating thermoregulatory responses, particularly brown adipose tissue (BAT) thermogenesis (also referred to as nonshivering thermogenesis). The importance of leptin-stimulated BAT thermogenesis has been well documented in rodent models [1–3]. For example, leptin-deficient mice are hypothermic and cold sensitive due to defects in BAT function [4–6]. Leptin also induces weight loss independent of food intake, via mechanisms that require functional BAT thermogenesis [7], suggesting that food-independent body weight control by leptin is mediated via BAT thermogenesis. Leptin treatment in obese humans is largely ineffective for reducing body weight [8], due to the common development of leptin resistance [9], but leptin's effect on energy expenditure has been demonstrated in weight-reduced and leptin-deficient humans where it prevents the drop in energy expenditure that is commonly associated with dieting [10,11]. Thus, the neuronal circuits that mediate leptin-stimulated BAT thermogenesis and energy expenditure hold promise as potential obesity therapy and warrant further investigation.

Despite the initial controversy surrounding the importance of BAT thermogenesis in adult humans and the control of body weight, thermogenic active BAT in adult humans is now well acknowledged and BAT size has been found to correlate negatively with body mass index [12,13]. BAT thermogenesis is governed by central mechanisms via the sympathetic nervous system [14,15], but the neuronal circuits that integrate specific peripheral signals into thermoregulatory responses are not entirely understood. Neuroanatomical and pharmacological approaches have identified several brain sites, including the dorsomedial hypothalamus/dorsal hypothalamic area (DMH/DHA), as key players in the control of BAT thermogenesis in rodents [14,16,17],

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but their role in body weight control has not been studied. We recently reported that BAT-related neurons in the DMH/DHA express leptin receptors (LepRb) and are activated by cold-exposure. Similar to cold- and pyrogen stimulated thermogenic pathways, DMH/DHA LepRb neurons innervate premotor neurons in the raphe pallidus (RPa) that control BAT thermogenesis [18]. Intra-DMH leptin activates sympathetic BAT inputs, but if this effect is indeed mediated directly by DMH/DHA LepRb neurons and if they contribute to whole body energy homeostasis remains unknown [19]. In this study we tested if DMH/DHA LepRb neurons are necessary and sufficient to promote BAT thermogenesis, energy expenditure, and body weight maintenance in mice.

2. METHODS

2.1. Mice

$LepRb^{GFP}$, $LepRb^{cre}$ (kindly provided by Dr. Martin Myers, Jr, University of Michigan, Ann Arbor, Michigan) and $LepRb^{fl/fl}$ mice (kindly provided by Dr. Streamson Chua, Jr, Albert Einstein College of Medicine, New York, New York) were bred in house from homozygous breeding pairs of $LepRb^{cre/cre}$, $Gt(ROSA)26Sor^{tm2Sho/tm2Sho}$, $LepRb^{cre/cre}$ mice, or $LepRb^{fl/fl}$ mice, respectively, and have been described in detail elsewhere [20–22]. Leptin-deficient *ob/ob* mice and their wildtype littermates (*+/?*) were purchased from the Jackson Laboratories (Bar Harbor, ME). All experiments were gender balanced, when both male and female mice were employed, and all animals were group housed at 22 °C–24 °C, maintained on a 12-h light/12-h dark cycle, and given *ad libitum* access to standard mouse chow and water unless stated otherwise. All animal experiments were approved by the institutional animal care and use committee.

2.2. Peripheral leptin treatment

Leptin was obtained from Dr. Parlow (National Hormone and Peptide Program, http://www.humc.edu/hormones). To identify LepRb neurons via leptin-induced pSTAT3, mice were injected intraperitoneally (i.p.) with leptin (5 mg/kg body weight) and perfused after one hour of incubation. For studies investigating leptin-induced cFos (as a surrogate of neuronal activity) in LepRb neurons, $LepRb^{GFP}$ mice were injected with vehicle (saline) or leptin (5 mg/kg, i.p.) and perfused three hours later.

2.3. Stereotaxic injection of adeno-associated viruses (AAVs) in the DMH/DHA

We used *designer-receptors-exclusively activated-by-designer drugs* (DREADD) technology to allow neuron-specific activation of DMH/DHA LepRb neurons. For DMH/DHA specific deletion of LepRb we injected a *cre*-expressing AAV or control virus into $LepRb^{fl/fl}$ mice. AAV-hSyn-DIO-hM3D(G_q)-mCherry (AAV-DREADD, kindly made available from Dr. Bryan Roth) and AAV-CMV-TR-EGFP (control virus) were obtained from the vector core of the University of North Carolina at Chapel Hill. AAV-CMV-HI-GFP-Cre-WPRE-SV40 virus (AAV-cre) was obtained from the vector core of the University of Pennsylvania. Three separate cohorts of $LepRb^{cre}$ mice (Group A–C) were used for AAV-DREADD injections targeted to the DMH/DHA as described earlier [23]. Two cohorts of $LepRb^{fl/fl}$ mice received control or AAV-cre virus injections targeted to the DMH/DHA. Mice were anesthetized with 5% isofluorane and their heads immobilized within the frame of a stereotaxic instrument (M1900 Stereotaxic alignment system; David Kopf Instruments, Tujunga, CA). Following sterilization of the surgical site, anesthesia was maintained at 1% isofluorane and an incision was made to expose the skull. Injections were aimed at −1.7 mm posterior, ±0.25 mm lateral and −4.6 mm ventral to Bregma according to the Paxinos Mouse Brain Atlas [24], and adjusted in $LepRb^{cre}$ mice to −1.1 mm posterior, ±0.25 mm lateral and −5 mm ventral to Bregma. Access holes were drilled and a bilateral guide cannula (Plastics One C253, Roanoke, VA) was inserted into the brain. A bilateral injector filled with virus (3–6 × 1012 viral molecules/ml) and attached to a 0.5 μl Hamilton syringe (Hamilton Company, Reno, NV) was threaded into the guide cannula and a volume of 200 nl per site was slowly infused into the DMH/DHA tissue at a rate of 20 nl per minute. Subsequently, the guide cannula and injector were removed, the skull access sealed with bone wax, and the incision closed with wound clips. Analgesics were applied once to the incision site (bupivacaine/lidocaine, 5 mg/kg) and subcutaneously during recovery every 12 h for two days (carprofen, 5–10 mg/kg). After the surgery mice were single housed for up to three weeks to allow for recovery and viral expression before further experimentation. Cannula placement and viral spread was verified histochemically at the end of all experiments.

2.4. Chronic intra-DMH/DHA cannulations for central leptin treatment

For DMH/DHA specific leptin or vehicle injections we chronically implanted bilateral cannulas (Plastics One C353, Roanoke, VA), as described in detail earlier [25], into the DMH/DHA. Briefly, procedures were identical to acute AAV injections, described above, but the bilateral guide cannula was secured in place with Loctite 454 (Fisher, Pittsburgh, PA) and dental cement before the incision was closed with wound clips. A dummy was inserted into the guide cannula to prevent contamination or blockage. Analgesics were applied as described above. Mice were allowed to recover for one week after surgery and adjusted to handling, twice daily saline injections, and body weight, food intake, and rectal temperature measurements over five days prior to leptin treatment to minimize stress. Body weight, food intake, and rectal temperature data were collected once per day prior to the second daily injection. After three consecutive days of leptin treatment, all mice were perfused and their brains were analyzed to confirm correct cannula placement.

2.5. Measurement of body temperature, energy expenditure, activity, body weight, and food intake in AAV-DREADD mice

Two cohorts of AAV-DREADD mice (group A, $n = 4$ and group B, $n = 4$) were injected once with the designer drug clozapine N-oxide (CNO, 0.3 mg/kg body weight; Sigma–Aldrich, St. Louis, MO) and rectal temperature was measured every 15 min for 90 min. In addition, two AAV-DREADD mice (from group B) were equipped with subcutaneous temperature transmitters (G2 E-Mitter; Respironics, Murrysville, PA). Temperature was continuously recorded in these two animals over three days of twice daily i.p. vehicle (saline) injections followed by three days of twice daily CNO (1.5 mg/kg body weight). Another separate cohort of DREADD mice (group C, $n = 8$) were shaved from head to tail along their backs to allow for temperature monitoring with an infrared/thermal camera (SC5000; FLIR Inc., Wilsonville, OR). Thermal photographs were taken at 10 min intervals following a single injection of vehicle or CNO (i.p., 1.5 mg/kg body weight). Two regions of interest (ROI) were applied to thermal photographs and average temperatures were calculated using Thermovison ExaminIR Beta software (FLIR Inc., Wilsonville, OR). The BAT ROI covered the shaved area from the back of the skull to the bottom of the shoulder blades, while the Body ROI spanned the remaining shaved area caudal the BAT ROI. Following temperature measurements, mice in groups A and B were adapted to oxymax chambers [Comprehensive Lab Animal Monitoring system (CLAMS); Columbus Instruments, Columbus, OH] and i.p. vehicle injections to minimize stress-induced energy expenditure. Then mice

