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MOLECULAR METABOLISM

Improved metabolic phenotype of hypothalamic PTP1B-deficiency is dependent upon the leptin receptor

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ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is a known regulator of central metabolic signaling, and mice with whole brain-, leptin receptor (LepRb) expressing cell-, or proopiomelanocortin neuron-specific PTP1B-deficiency are lean, leptin hypersensitive, and display improved glucose homeostasis. However, whether the metabolic effects of central PTP1B-deficiency are due to action within the hypothalamus remains unclear. Moreover, whether or not these effects are exclusively due to enhanced leptin signaling is unknown. Here we report that mice with hypothalamic PTP1B-deficiency (Nkx2.1-PTP1B$^{-/-}$) display decreased body weight and adiposity on high-fat diet with no associated improvements in glucose tolerance. Consistent with previous reports, we find that hypothalamic deletion of the LepRb in mice (Nkx2.1-LepRb$^{-/-}$) results in extreme hyperphagia and obesity. Interestingly, deletion of hypothalamic PTP1B and LepRb (Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$) does not rescue the hyperphagia or obesity of Nkx2.1-LepRb$^{-/-}$ mice, suggesting that hypothalamic PTP1B contributes to the central control of energy balance through a leptin receptor-dependent pathway.

KEYWORDS Phosphatase; Leptin; Obesity; Hypothalamus

1. INTRODUCTION

Obesity continues to be a major public health crisis in the United States and worldwide [1–3]. Given the numerous metabolic comorbidities and an overall increased all-cause mortality associated with obesity [4,5], understanding the underlying biological systems that regulate body weight and adiposity is of great importance. Though obesity is commonly thought of as a disease affecting the periphery (i.e. increased body fat), the central nervous system (CNS) plays a key role in regulating appetite, metabolism, and body weight. Neurons within the hypothalamus integrate neuroendocrine signals from the periphery, gauging short term and long term energy status. The adipocyte-secreted hormone leptin is one such signal whose effects on the central control of energy homeostasis have been studied in depth. Circulating leptin acts on leptin receptors (LepRbs) expressed within the hypothalamus and extrahypothalamic sites (hindbrain nucleus tractus solitarius, parabrachial nucleus) [6,7] to suppress food intake and increase energy expenditure, ultimately promoting negative energy balance. Leptin- (*ob/ob*) and LepRb-deficient (*db/db*) mice are hyperphagic and develop extreme obesity [8–10]. Moreover, deletion of LepRb within the hypothalamus in mice, driven by the ventral forebrain specific Nkx2.1-Cre, recapitulates much of the *db/db* phenotype [11]. LepRb-deficiency within the hindbrain NTS in mice also results in hyperphagia and increased weight gain [12].

At the molecular level, when LepRb is activated, several tyrosine phosphorylation events occur. Initially, leptin binding to LepRb results in a conformational change of the receptor and activation of the associated tyrosine kinase Janus kinase 2 (JAK2). JAK2 autophosphorylates and subsequently phosphorylates tyrosine residues along the intracellular tail of LepRb, which can further recruit downstream signaling molecules necessary for eliciting leptin's physiological effects [13,14]. Protein tyrosine phosphatase 1B (PTP1B) shows enriched expression correlating with areas of LepRb expression [15], and is a known negative regulator of leptin signaling via direct dephosphorylation of JAK2 [15–17]. In mice, PTP1B is encoded by the *Ptpn1* gene, and whole body, whole brain-, LepRb-expressing cell-, or POMC neuron-specific PTP1B-deficiency results in decreased body weight and adiposity on HFD [18–22]. In contrast, deletion of PTP1B in peripheral tissues does not affect body weight [23–26]. Since CNS PTP1B-deficient models to date have used holistic (whole brain) or neuron specific approaches (POMC- or LepRb-targeted), the anatomic specificity of PTP1B's metabolic effects remains unclear. Like the LepRb, POMC is expressed both in the hypothalamus and hindbrain, and there is evidence of enhanced hypothalamic and hindbrain leptin signaling in POMC-PTP1B$^{-/-}$ mice [22,27], suggesting a metabolic role for PTP1B in both regions. Thus, the extent to which the metabolic effects of PTP1B deficiency are due to action within the hypothalamus or in extrahypothalamic sites remains unknown. Here, to determine the metabolic contribution of

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Abbreviations: BAT, Brown adipose tissue; CNTF, Ciliary neurotrophic factor; Cre, Cre recombinase; *db/db*, Leptin receptor-deficient mice; GTT, Glucose tolerance test; HFD, High-fat diet; HPA, hypothalamus–pituitary–adrenal; IL-6, Interleukin-6; ITT, Insulin tolerance test; JAK2, Janus kinase 2; LepRb, Leptin receptor long form; Nkx2.1, NK2 homeobox 1 protein or thyroid transcription factor-1; *ob/ob*, leptin-deficient mice; PI3K, Phosphatidylinositol 3-kinase; POMC, Proopiomelanocortin; Prdm16, PR domain containing 16; PTP1B, Protein tyrosine phosphatase 1B; PTPs, Protein tyrosine phosphatases; SHP2, Src homology 2 domain-containing protein tyrosine phosphatase; STAT3, Signal transducer and activator of transcription 3; UCP1, Uncoupling protein 1; WAT, White adipose tissue

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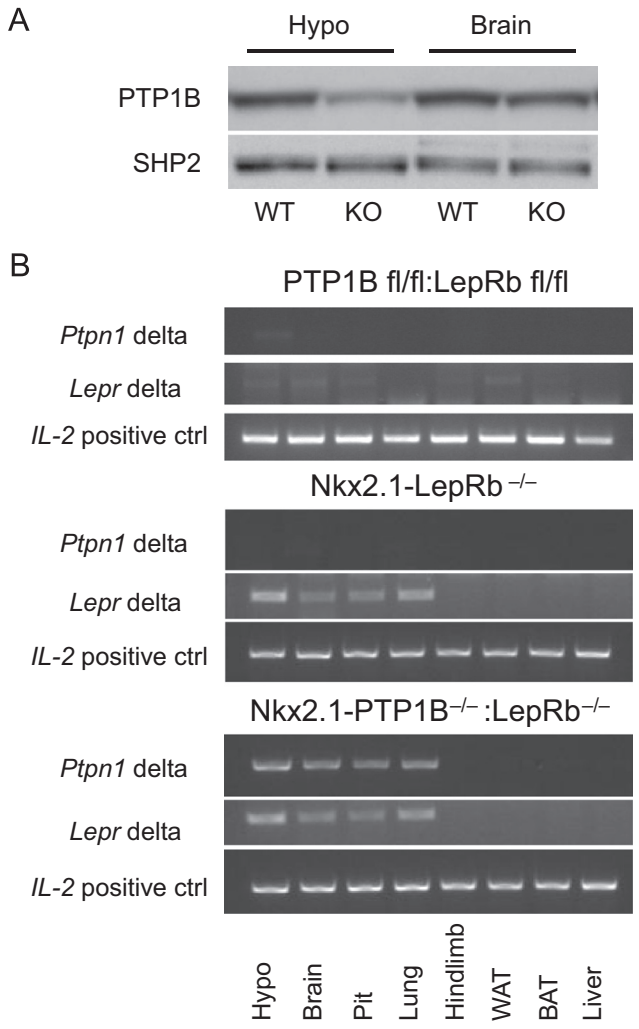

Figure 1: Detection of PTP1B deletion in Nkx2.1-PTP1B deficient mouse models. (A) PTP1B protein levels in the hypothalamus and brain of Nkx2.1-PTP1B$^{-/-}$ (KO) mice compared with PTP1B fl/fl controls (WT). SHP2 protein levels are shown as a loading control. (B) Detection of deletion of PTP1B or LepRb floxed alleles in PTP1B fl/fl:LepRb fl/fl, Nkx2.1-LepRb$^{-/-}$, and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice. DNA was isolated from different tissues [hypothalamus (Hypo), extrahypothalamic brain, pituitary (Pit), lung, hindlimb, perigonadal white adipose tissue (WAT), brown adipose (BAT), and liver], and deletion of floxed allele was detected by PCR.

hypothalamic PTP1B, we generated a genetic PTP1B deficient mouse model using the Nkx2.1-Cre line, which leads to widespread recombination within the ventral forebrain.

The improved metabolic phenotype of central PTP1B-deficient models is largely attributed to enhanced leptin sensitivity. Interestingly, however, compound *ob/ob*:PTP1B$^{-/-}$ mice show attenuated weight gain in comparison to *ob/ob* mice [17], suggesting that there may be leptin-independent metabolic effects of PTP1B deficiency. Furthermore, *db/db*:PTP1B$^{-/-}$ mice display decreased plasma triglycerides and serum free fatty acids when compared to *db/db* :PTP1B$^{+/-}$ [28], and *ob/ob* mice treated with PTP1B antisense oligonucleotides possess decreased epididymal fat compared to saline-treated controls [29]. Thus, we were interested in examining whether or not the metabolic effects of PTP1B deficiency are exclusively leptin receptor dependent. For these studies, we crossed the Nkx2.1-Cre line with *Ptpn1*$^{loxP/loxP}$:*Lepr*$^{loxP/loxP}$ mice in order to generate compound hypothalamic Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice. Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice were compared to Nkx2.1-LepRb$^{-/-}$ mice as well as wildtype controls to determine whether PTP1B's metabolic effects within the hypothalamus are dependent upon functional leptin receptor signaling.

2. MATERIALS AND METHODS

2.1. Animal care

All animal care protocols and procedures were approved by the University of Pennsylvania Institutional Care and Use Committee. We maintained mice on a 12-h light/12-h dark cycle in a temperature controlled barrier facility, with free access to water and food: standard chow autoclavable Lab Diet 5010 (calories provided by protein [28.7%], fat [12.7%], and carbohydrate [58.5%]) or custom HFD Teklad TD93075 (calories provided by protein [21.2%], fat [54.8%], and carbohydrate [24%]). Age-matched littermates were used for all experiments.

2.2. Generation of Nkx2.1-PTP1B$^{-/-}$, Nkx2.1-LepRb$^{-/-}$, and Nkx2.1-PTP1B$^{-/-}$:LepRb$^{-/-}$ mice

All mice were on a C57BL/6 background. *Ptpn1*$^{loxP/loxP}$ mice were generated previously [20] on a mixed 129Sv/J × C57BL/6 background but were backcrossed at least 10 generations onto C57BL/6 background prior to mating with other lines. *Lepr*$^{loxP/loxP}$ mice on a C57BL/6 background were obtained from S. Chua (Albert Einstein College of Medicine) and S. Obici (University of Cincinnati, Ohio). Nkx2.1-Cre transgenic mice were obtained from The Jackson Laboratory (Stock #008661, Bar Harbor, ME). Genotyping primer sequences were as follows: PTP1B fl forward 5′-TGCTCACTCACCCTGCTACAA, reverse 5′-GAAATGGCTCACTCCTACTGG. Lepr fl forward 5′-AACGGTTTTA-CAGTCTCCA, reverse 5′-AAGGCCCATTTAGTCAAC. Nkx2.1-Cre forward 5′-CCACAGGCACCCCACAAAAATG, reverse 5′-GCCTGGCGATCCCTGAACAT.

2.3. Isolating DNA from tissues for detection of recombination of the floxed alleles

Tissues were digested at 55 °C overnight in proteinase K digestion buffer (100 mM Tris–HCl pH 8.5, 5 mM EDTA, 0.2% SDS, 200 mM NaCl, 300 μg/ml proteinase K). Saturated NaCl (~6 M) was added to the digestion, and samples were vortexed vigorously for 1 min. Samples were centrifuged for 20 min at 13,700*g*, and supernatants were transferred to a fresh tube. DNA was precipitated by adding 1 ml 100% ethanol, and pellets were washed once with 70% ethanol and were resuspended in 100 μl of sterile PCR water for analysis. PCR primers for detection of recombined alleles: *Ptpn1*Δ/Δ forward 5′-GTGGTGCCTGCAAGAGAACTGAC, reverse 5′-GAAATGGCTCACTCCTACTGG. *Lepr*Δ/Δ forward 5′-GTCTGATTTGATAGA-TGGTCTT, reverse 5′-ACAGGCTTGAGAACATGAACAC. *IL-2* internal control forward 5′-CTAGGCCACAGAATTGAAAGATCT, reverse 5′-GTAGGTGGAAAT-TCTAGCATCATCC.

2.4. Immunoblotting

Mouse tissues were dissected and immediately frozen in liquid nitrogen. Whole cell lysates were prepared in modified RIPA buffer containing fresh protease inhibitors, and PTP1B and SHP2 immunoblotting was performed as described previously [15,22]. PTP1B immunoblots were normalized to SHP2 (Santa Cruz Biotechnology Inc., sc-280) to control for loading.

2.5. Body composition and food intake

At weaning, mice were placed on diets of either standard laboratory chow or HFD. Body weights were assessed weekly and food intake was measured daily at indicated age. Body length was measured as nose-rump length at indicated age. Epididymal fat pads were dissected and weighed at indicated age. Total fat and lean mass was measured in conscious mice using NMR (Echo Medical Systems) at indicated age in the Penn IDOM Mouse Phenotyping, Physiology and Metabolism Core.

2.6. Energy expenditure measurements

Rectal temperature was measured with a thermistor during the light cycle in animals at 14–17 weeks of age (MicroTherma 2T; ThermoWorks). Feed

