

Food intake reductions and increases in energetic responses by hindbrain leptin and melanotan II are enhanced in mice with POMC-specific PTP1B deficiency

Bart C. De Jonghe,^{1,4} Matthew R. Hayes,³ Derek J. Zimmer,¹ Scott E. Kanoski,² Harvey J. Grill,² and Kendra K. Bence¹

¹Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania;

²Department of Psychology, School of Arts and Sciences, University of Pennsylvania, Philadelphia, Pennsylvania;

³Translational Neuroscience Program, Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; and ⁴Department of Biobehavioral and Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, Pennsylvania

Submitted 6 January 2012; accepted in final form 2 July 2012

De Jonghe BC, Hayes MR, Zimmer DJ, Kanoski SE, Grill HJ, Bence KK. Food intake reductions and increases in energetic responses by hindbrain leptin and melanotan II are enhanced in mice with POMC-specific PTP1B deficiency. *Am J Physiol Endocrinol Metab* 303: E644–E651, 2012. First published July 3, 2012; doi:10.1152/ajpendo.00009.2012.—Leptin regulates energy balance through central circuits that control food intake and energy expenditure, including proopiomelanocortin (POMC) neurons. POMC neuron-specific deletion of protein tyrosine phosphatase 1B (PTP1B) (*Ptpn11^{loxP/loxP}* POMC-Cre), a negative regulator of CNS leptin signaling, results in resistance to diet-induced obesity and improved peripheral leptin sensitivity in mice, thus establishing PTP1B as an important component of POMC neuron regulation of energy balance. POMC neurons are expressed in the pituitary, the arcuate nucleus of the hypothalamus (ARH), and the nucleus of the solitary tract (NTS) in the hindbrain, and it is unknown how each population might contribute to the phenotype of POMC-*Ptp1b*^{-/-} mice. It is also unknown whether improved leptin sensitivity in POMC-*Ptp1b*^{-/-} mice involves altered melanocortin receptor signaling. Therefore, we examined the effects of hindbrain administration (4th ventricle) of leptin (1.5, 3, and 6 μ g) or the melanocortin 3/4R agonist melanotan II (0.1 and 0.2 nmol) in POMC-*Ptp1b*^{-/-} (KO) and control PTP1B^{fl/fl} (WT) mice on food intake, body weight, spontaneous physical activity (SPA), and core temperature (T_C). The results show that KO mice were hypersensitive to hindbrain leptin- and MTII-induced food intake and body weight suppression and SPA compared with WT mice. Greater increases in leptin- but not MTII-induced T_C were also observed in KO vs. WT animals. In addition, KO mice displayed elevated hindbrain and hypothalamic MC4R mRNA expression. These studies are the first to show that hindbrain administration of leptin or a melanocortin receptor agonist alters energy balance in mice likely via participation of hindbrain POMC neurons.

proopiomelanocortin; protein tyrosine phosphatase 1B; phosphatase; energy expenditure; melanotan II; melanocortin 4 receptor

THE INCIDENCE OF OBESITY, type 2 diabetes, and associated chronic diseases has reached epidemic proportions worldwide (10, 25). A key regulating signal of energy balance is the adipocyte-secreted hormone leptin, which acts centrally to reduce food intake and increase energy expenditure (see Refs.

5 and 21 for review) via activation of its receptor (LepR), which is highly expressed throughout the central nervous system (CNS) in nuclei contributing to energy balance control (8, 9, 20). Unfortunately, the efficacy of leptin therapy in obese humans has been unsuccessful due to the development of leptin resistance (i.e., a loss or reduction in signaling response at a behavioral, physiological, and cellular level) (19, 29, 37, 39). One mechanism leading to leptin resistance is an inhibition of the intracellular leptin signaling cascade (22). Protein tyrosine phosphatase 1B (PTP1B) is a nonreceptor protein tyrosine phosphatase that acts as a negative regulator of leptin receptor signaling by dephosphorylating the Janus-activated kinase 2 (JAK2) associated with the leptin receptor (6, 23, 41). Mice globally lacking the gene encoding PTP1B are leptin hypersensitive (41), consistent with a role for PTP1B as a negative regulator of leptin signaling in vivo.

Within the CNS, the physiological relevance of proopiomelanocortin (POMC) neurons in the control of energy balance has been highlighted by studies showing that deletion of LepR in POMC neurons (POMC-Cre: LepR^{fllox/fllox}) results in body weight and adiposity, hyperleptinemia, and altered hypothalamic neuropeptide expression (1, 31), whereas restoration of LepR expression only in POMC neurons in *db/db* mice normalizes blood glucose and decreases the extent of hyperphagia and obesity (17). These studies indicate that this small population of neurons is an important component of leptin's overall effects on energy balance. We showed recently that POMC neuron-specific deletion of PTP1B in mice leads to reduced body weight and adiposity due to increased energy expenditure, resistance to fatty liver induced by a high-fat diet, improved sensitivity to peripheral leptin administration, and enhanced thermogenic responses to cold temperatures via upregulation of the hypothalamic-pituitary-thyroid axis (2, 4). These findings implicate PTP1B as an important component of POMC regulation of energy balance.

Two distinct populations of neurons synthesize either POMC or agouti-related protein (AgRP) and mediate opposing catabolic and anabolic effects on energy balance (5). Circulating leptin acts upon these neurons as a catabolic hormone to decrease appetite and increase energy expenditure via simultaneous suppression of AgRP neurons and stimulation of POMC neurons. Although much of what is known about the role of POMC neurons in energy balance comes from studies

Address for reprint requests and other correspondence: K. K. Bence, Dept. of Animal Biology, School of Veterinary Medicine, Univ. of Pennsylvania, 3800 Spruce St., Philadelphia, PA 19104 (e-mail: kbence@vet.upenn.edu).

of POMC neurons within the arcuate nucleus of hypothalamus (ARH), POMC neurons are also highly expressed in the nucleus of the solitary tract (NTS) of the hindbrain (24), a nucleus that receives and integrates both vagal afferent satiation and blood-borne energy status signals and issues output commands essential to energy balance control (12, 26, 27, 35).

In rats, administration of leptin restricted to the hindbrain (i.e., 4th icv) decreases food intake and increases energy expenditure (15, 16, 34). In both rats and mice, hindbrain leptin receptor signaling in the NTS is required for normal control of energy balance regulation (16, 30). The effects of hindbrain leptin delivery are mediated in part through downstream hindbrain melanocortin receptor (MCR) signaling, since pharmacological blockade of hindbrain MC3R/MC4R abolishes leptin-induced reductions in both food intake and body weight as well as the induction of hyperthermia (33, 34, 44). Furthermore, 4th ventricle or intra-NTS delivery of the MC3R/MC4R agonist melanotan II (MTII) in the rat produces robust body weight loss, anorexia, and hyperthermia (11, 32). Intriguingly, however, the existing literature in terms of rat and mouse species congruency with respect to leptin receptor and POMC neuron functioning within the hindbrain remains largely unexplored (7, 18), and it is not known how exogenous hindbrain delivery of leptin or MCR agonists affects energy balance in mice.

Here, we examined the effects of hindbrain administration (4th ventricle) of leptin or MTII on energy balance in POMC-Ptp1b^{-/-} and control PTP1B^{+/+} mice to 1) test whether hindbrain delivery of these agents in a mouse model produces results comparable with those obtained in rats and 2) determine whether the peripheral leptin hypersensitivity observed in POMC-Ptp1b^{-/-} mice, relative to controls, involves enhanced responsiveness to leptin or melanocortin receptor signaling within the hindbrain.

METHODS

Animals

Two- to three-month-old male Ptp1b^{loxP/loxP} POMC-Cre mice (hereafter termed KO) and Ptp1b^{loxP/loxP} (hereafter termed WT) littermate controls were used for experiments. Mice were generated and genotyped by PCR, as described previously (2). Specificity of PTP1B deletion in KO mice has also been described (2). Mice were housed individually in small plastic bins in a temperature- and humidity-controlled room and maintained in a 12:12-h light-dark cycle (lights on at 2000). Animals were maintained ad libitum on pelleted chow (Lab Diet 5010) and water unless otherwise indicated. All protocols and procedures were approved by the Institutional Care and Use Committee at University of Pennsylvania.

Fourth icv Cannula and Telemetric Transponder Surgery

Under anesthesia (ketamine: 90 mg/kg im), miniature telemetric transponders (G2 VitalView; Mini Mitter/Respironics) were implanted within the abdominal cavity to record core temperature (T_c) or spontaneous physical activity (SPA). During the same surgical procedure, mice were implanted with fourth ventricular chronic indwelling cannulas (-6.0 mm bregma, flush with midline, at a depth of 2.5 mm, with injector extending 1.5 mm beyond guide). Cannulae were attached to the skull with dental acrylic and a jeweler's screw and closed with an obturator. Following the surgical recovery period (7 days), animals were tested for proper

cannula placement via injection of 50 μg of 5-thio-D-glucose (dose adapted for the mouse from rat studies; see Refs. 13 and 32). Animals that showed increased blood glucose >100% of baseline were used. For all experiments in mice receiving 4th ventricular injection of vehicles/drugs, the total number of injections per animal was between eight and 10.

Effects of Hindbrain Leptin or MTII Administration on Food Intake, Body Weight, SPA, and T_c

Experiment 1. Just prior to the dark cycle onset, all mice (*n* = 4–6/genotype) received 200 nl of 4th icv injections using a microsyringe pump (Hamilton) in a counterbalanced fashion of either leptin (1.5, 3, and 6 μg; National Hormone and Peptide Program) or vehicle (sodium bicarbonate) in a within-subjects design. After a 1-wk washout, MTII (0.1 and 0.2 nmol; Phoenix, Belmont, CA) or vehicle (artificial cerebrospinal fluid) was administered using the same design. SPA was recorded continuously every 5 min. Food intake measurements were taken 6 and 24 h postinjection. Body weight was measured every 24 h. Chow and water were available ad libitum. A 48-h interval occurred between drug treatments.

Experiment 2. To assess changes in T_c following fourth icv injection independent of diet-induced thermogenesis, mice (*n* = 4–6/genotype) were deprived of chow 1 h prior to fourth icv injections [200 nl; counterbalanced respective vehicle, leptin (6 μg) or MTII (0.2 nmol)] using a within-subjects design during the early light phase. T_c was monitored every 5 min, starting 1 h preinjection and continuing for 7 h (i.e., until 6 h postinjection). A 48-h interval separated injections. The same mice were used for both experiments 1 and 2.

MC4R mRNA Expression in the Hypothalamus and Caudal Dorsal Vagal Complex of WT and KO Mice

Experiment 3. In a separate group of ad libitum-fed adult female mice, WT (*n* = 9; body weight 25.9 ± 1.1 g) and KO (*n* = 9; body weight 24.5 ± 0.8 g) aged 8 mo were euthanized 2 h into the light cycle. Whole hypothalamus and dorsal vagal complex (DVC)-enriched hindbrain tissues were collected and flash-frozen in liquid nitrogen according to procedures adapted from Hayes et al. (15) for determination of MC4R mRNA expression levels. Briefly, total RNA was extracted from DVC-enriched hindbrain and hypothalamus tissues using TRIzol (Invitrogen) and the RNeasy kit (Qiagen). cDNA was synthesized from 1 μg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The relative mRNA level of MC4R was quantified by quantitative real-time PCR. Mouse β-actin (VIC-MGB, no. 4352341E; Applied Biosystems) was used as an internal control. PCR reactions were carried out using the TaqMan gene expression kit (MC4R: Mm00457483_s1) and PCR reagents from Applied Biosystems. Samples were analyzed using the Eppendorf Mastercycler ep realplex2. Relative mRNA expression was calculated using the comparative threshold cycle method, as described previously (3).

Statistics

All data are expressed as means ± SE. For statistical analyses, drug treatment (leptin or MTII) values were compared with respective vehicle injection results, which served as baseline. Percent suppression of body weight and food intake following drug treatment relative to baseline was calculated using the following formula: %suppression = 1 - (drug treatment/baseline) × 100. Appropriate one- or two-way ANOVA with repeated measures was performed to evaluate group differences, using drug dose (leptin vs. vehicle, MTII vs. vehicle) and genotype (KO and WT) as main effects. Fisher's least significant difference test was used for post hoc comparisons when applicable. Real-time PCR data were analyzed using one-way

ANOVA. Statistical differences between mean values were calculated using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

POMC-Ptp1b^{-/-} Mice Show Enhanced Reductions in Food Intake and Body Weight Following Hindbrain Leptin Administration Compared with WT Controls

Acute hindbrain ventricular administration of leptin significantly reduced food intake and body weight in both POMC-Ptp1b^{-/-} and wild-type control mice at all doses tested relative to vehicle; however, important differences were observed in magnitude and time course of leptin effects between genotypes. Consistent with previous findings (2, 4), no statistical differences were noted for baseline (vehicle injection) conditions, body weight (26.3 ± 1.2 vs. 25.1 ± 1.7 g for WT and KO, respectively), or daily food intake (5.07 ± 0.5 vs. 5.04 ± 1.0 g for WT and KO, respectively) between KO and WT mice. Figure 1A shows a significant main effect for leptin dose on 6-h food intake, where POMC-Ptp1b^{-/-} mice dose-dependently suppressed intake ($P < 0.05$), with a similar suppression of intake across all doses in WT mice ($P < 0.05$). A main effect of genotype was also observed ($P < 0.05$), with the degree of 6-h food intake suppression in KO mice exceeding that of WT in magnitude at the highest doses of leptin tested (3 and 6 μg, $P < 0.05$). A main effect for leptin treatment was also observed for food intake at 24 h postinjection, with significant intake suppression at all doses in both genotypes (all $P < 0.05$). WT mice showed a dose-dependent suppression at this time point ($P < 0.05$), whereas KO mice showed a maximal suppression at all doses (Fig. 1B). As depicted in Fig. 1C, hindbrain leptin administration significantly reduced body weight in both genotypes dose dependently (all $P < 0.05$); however, KO mice exhibited a larger magnitude of body weight loss at all doses tested.

POMC-Ptp1b^{-/-} Mice Show Enhanced Reductions in Food Intake and Body Weight Following Hindbrain MTII Administration Compared with WT Mice

Hindbrain delivery of MTII produced dose-dependent food intake suppression at 6 h in WT mice ($P < 0.05$), whereas KO mice showed similar suppressions at both doses administered (Fig. 2A). A genotype × MTII dose interaction for food intake suppression was found at 24 h ($P < 0.05$), with the 0.2-nmol dose resulting in significantly greater suppressions in KO compared with WT mice ($P < 0.05$) (Fig. 2B). A main effect for genotype was observed for reductions in 24-h body weight following 4th icv MTII, with greater body weight loss occurring in KO mice relative to WT mice at the 0.2-nmol dose ($P < 0.05$; Fig. 2C).

POMC-Ptp1b^{-/-} Mice Show Enhanced Increases in SPA Following Hindbrain Leptin or MTII Administration Compared with WT Mice

SPA induced by hindbrain leptin was increased significantly during the light cycle for KO mice at 3- and 6-μg doses ($P < 0.05$; Fig. 3A), whereas this was not observed for WT mice. The highest dose of MTII caused a small but significant

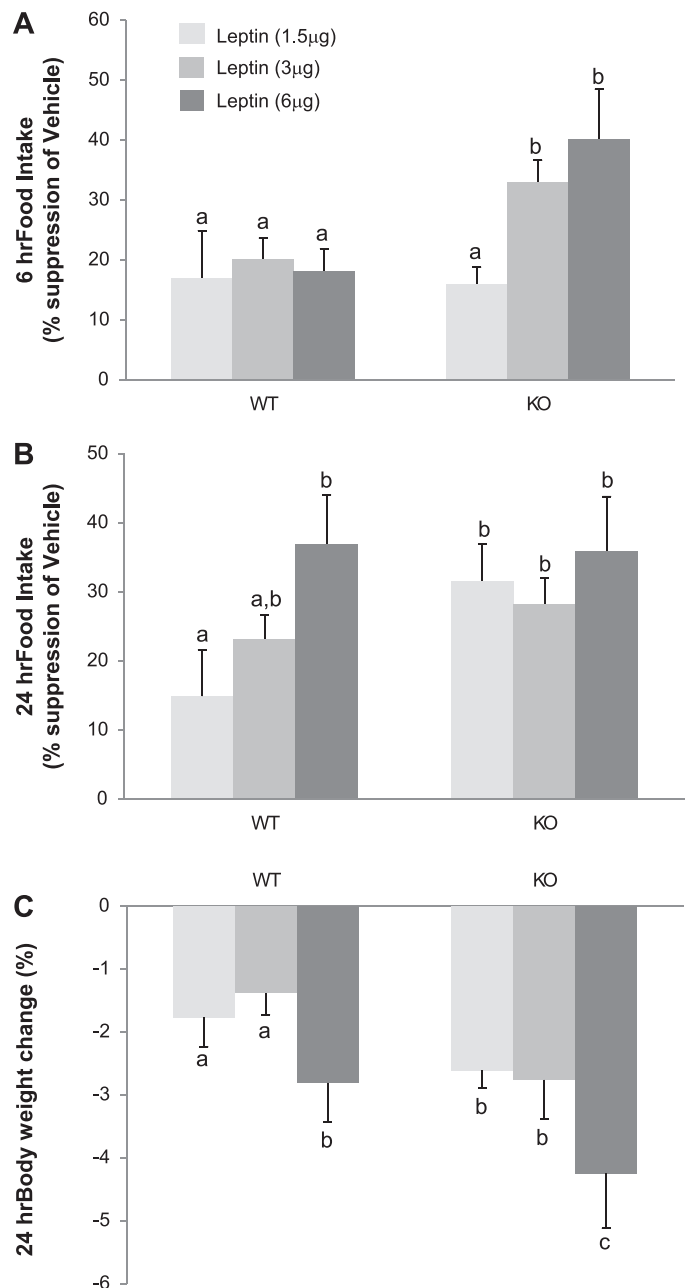


Fig. 1. A: food intake at 6 h following hindbrain leptin administration was dose-dependently suppressed in proopiomelanocortin-protein tyrosine phosphatase 1B (POMC-Ptp1b^{-/-}; KO) mice but not in control PTP1B^{fl/fl} (WT) mice. B: food intake at 24 h postinjection was decreased significantly at all doses in both genotypes, with WT mice showing a dose-dependent suppression. C: hindbrain leptin administration reduced body weight significantly to a greater extent in KO mice relative to controls. Bars with different letters indicate significant group differences in post hoc comparisons ($P < 0.05$). Bars with the same letters are not significantly different from each other.

increase during the light phase of KO animals ($P < 0.05$) but not WT animals (Fig. 3B).

POMC-Ptp1b^{-/-} Mice Show Enhanced Leptin- but not MTII-Induced Hyperthermia

Both leptin and MTII increased core temperature significantly in food-restricted WT and KO mice. A genotype × drug

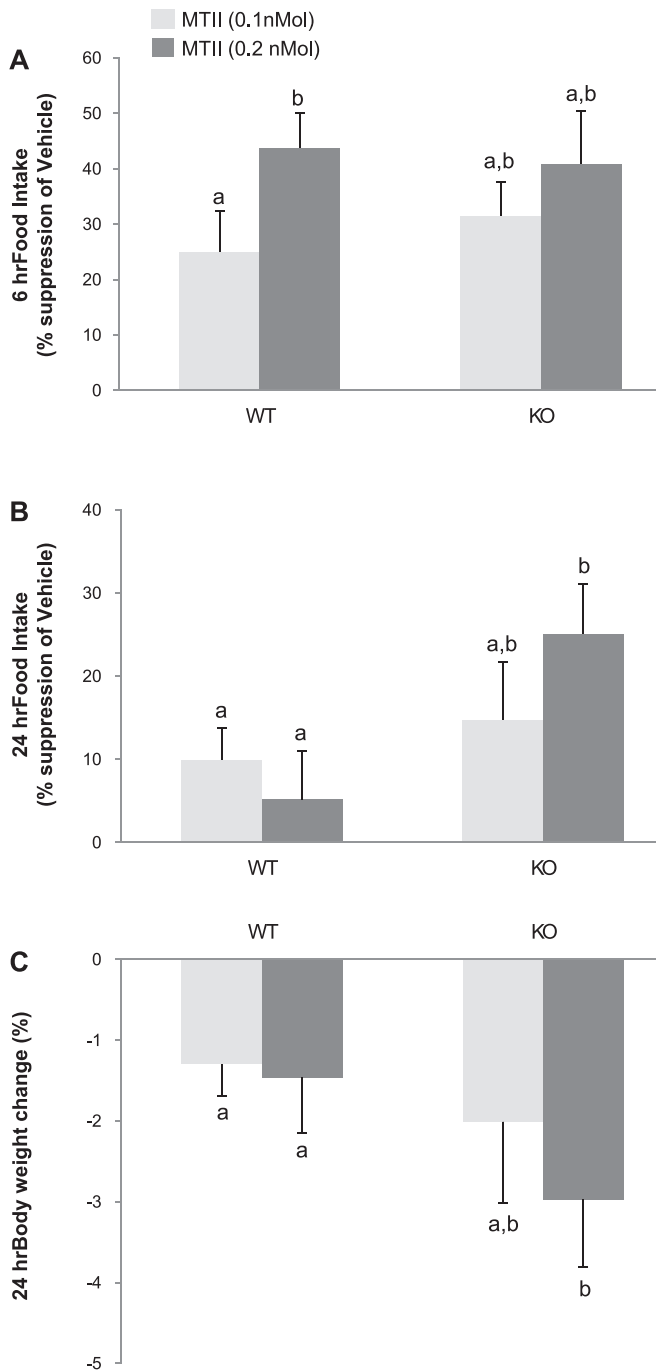


Fig. 2. A: hindbrain delivery of melanotan II (MTII) produced dose-dependent decreases in food intake and body weight in WT mice but a maximal effect in KO mice at 6 h. B: at 24 h, food intake suppression was greater in KO vs. WT mice at the highest dose. C: reductions in 24-h body weight were also achieved in both KO and WT mice, with greater body weight loss in KO mice at the highest dose. Bars with different letters indicate significant group differences in post hoc comparisons ($P < 0.05$). Bars with the same letters are not significantly different from each other.

interaction was achieved for T_C change, with 6 μ g of leptin-induced hyperthermia significantly greater in KO vs. WT mice relative to vehicle treatment ($P < 0.05$; Fig. 4A). A main effect for 0.2 nmol MTII was seen for T_C change compared with vehicle ($P < 0.05$), with similar increases in T_C between genotypes (Fig. 4B).

Increased MC4R mRNA Expression in POMC-Ptp1b^{-/-} Mice

Figure 5 shows that in nontreated naïve adult female mice, higher expression of MC4R mRNA is observed in POMC-Ptp1b^{-/-} mice compared with controls in both the hypothalamus as well as the caudal DVC-enriched hindbrain ($P < 0.05$).

DISCUSSION

The present findings illustrate for the first time that hindbrain administration of leptin or the melanocortin receptor agonist MTII reduces food intake and body weight and invokes thermogenic responses in a mouse model, similar to previous results found in the rat. We also demonstrate support for our hypothesis that enhanced sensitivity to energy balance effects of hindbrain leptin and MTII administration in POMC-Ptp1b^{-/-} mice suggests a role for hindbrain POMC leptin/melanocortineric signaling in the obesity-resistant phenotype of these animals. Finally, we show that MC4R mRNA expression is enhanced in POMC-Ptp1b^{-/-} mice compared with controls, suggesting alterations in melanocortineric signaling potentially downstream of POMC neuron activation.

Our recent findings demonstrate that POMC neuron-specific deletion of PTP1B in mice results in resistance to diet-induced obesity via enhanced energy expenditure and improved sensitivity to peripheral leptin administration as well as enhanced thermogenic responses to cold temperatures (2, 4). Presumably, these phenotypes occur as a result of enhanced POMC neuron sensitivity (via a lack of inhibitory PTP1B signaling) to leptineric signals, leading to greater stimulation of thermogenic pathways via MC4R-expressing neurons that control thermogenesis (36, 38, 40). Our previous report showed that ARH peptide levels of the endogenous MC3/4R receptor ligand α -melanocyte stimulating hormone (α -MSH) and α -MSH-containing projections to the paraventricular nucleus of the hypothalamus (PVH) are similar in POMC-Ptp1b^{-/-} and control mice (2). However, these findings do not rule out possible downstream effects such as altered MC4R activation in the PVH and/or hindbrain, which could lead to enhanced thermogenesis in POMC-Ptp1b^{-/-} animals via increased sympathetic outflow (4).

Hindbrain leptin receptor activation plays a critical role in the endogenous control of energy balance (11, 16, 30, 34). Previous experiments have shown that the effects of leptin administration are accomplished in part through downstream activation of hindbrain MC3/4Rs (34), suggesting that both hindbrain leptin and melanocortin signaling endemic to the hindbrain are capable of influencing energy balance in the rat.

Here, we show for the first time that, in mice, hindbrain-delivered leptin and MTII are capable of reducing food intake and body weight and increasing SPA as well as inducing hyperthermia. Interestingly, recent findings in the rat suggest a maximal suppression of food intake following hindbrain leptin administration at $\sim 40\%$ of vehicle intake in this species, similar in magnitude to what we have currently observed in mice (43). In addition, since direct hindbrain administration of leptin and MTII result in both enhancements in food intake reductions and energy expenditure increases in POMC-Ptp1b^{-/-} animals compared with WT mice, it is likely that augmented hindbrain integration of leptin/melanocortineric

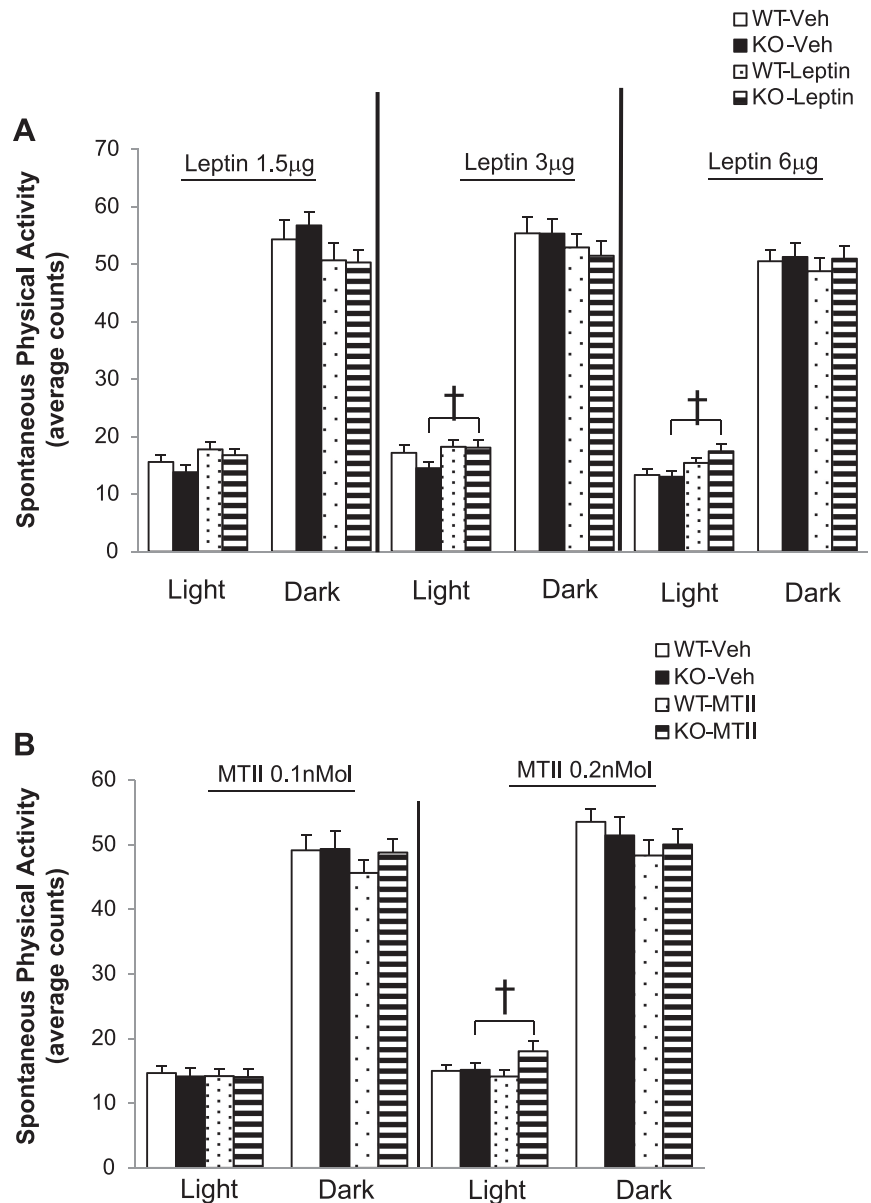


Fig. 3. *A*: spontaneous physical activity (SPA) induced by hindbrain leptin showed a small but significant increase during the light cycle in KO mice at the 3- and 6- μ g doses. *B*: only the highest dose of MTII caused a significant increase in SPA during the light phase in KO but not WT animals. †Significant differences between vehicle (Veh) and drug ($P < 0.05$).

signals plays a critical role in preventing the development of diet-induced obesity in these mice.

We also show that POMC-Ptp1b^{-/-} mice show greater MC4R mRNA expression in both hypothalamus and hindbrain tissues, which may suggest altered melanocortineric “tone” in these mice. To relate these data to our behavioral findings of enhanced 4th icv MTII-induced reductions in food intake in POMC-Ptp1b^{-/-} mice, it is possible that elimination of PTP1B from POMC neurons results in an elevation of downstream MC4R levels possibly through an increase in endogenous α -MSH signaling endemic to the hindbrain.

One population of hindbrain neurons that contributes to the neural control of energy balance is indeed POMC neurons either through direct or indirect activation of leptin receptors on these or nearby cells in the NTS. However, the function of hindbrain POMC neurons has not been studied extensively (especially in mice), and more data are necessary to characterize the physiological role of this population of highly special-

ized neurons. Specifically, the function of POMC neurons within the NTS may differ significantly from those in the ARH, although the literature contains only a handful of reports addressing this issue. For example, one report has shown that leptin does not induce p-STAT3 or Fos-like immunoreactivity (Fos-Li) in POMC NTS neurons, in contrast to robust activation in ARH POMC neurons (18). However, another report suggests the opposite, that p-STAT3 is induced in a significant population of POMC NTS neurons following leptin treatment (7). Notably, both NTS and ARH POMC mRNA have been shown to decrease following periods of food restriction (18). More recent data show that NTS-specific POMC gene transfer ameliorates diet-induced obesity and metabolic defects, whereas identical treatment in the ARH produces only transient improvements (42). These regional differences in treatment duration presumably involve compensatory AgRP activity, which is expressed in the ARH but not in the hindbrain, or altered GABAergic

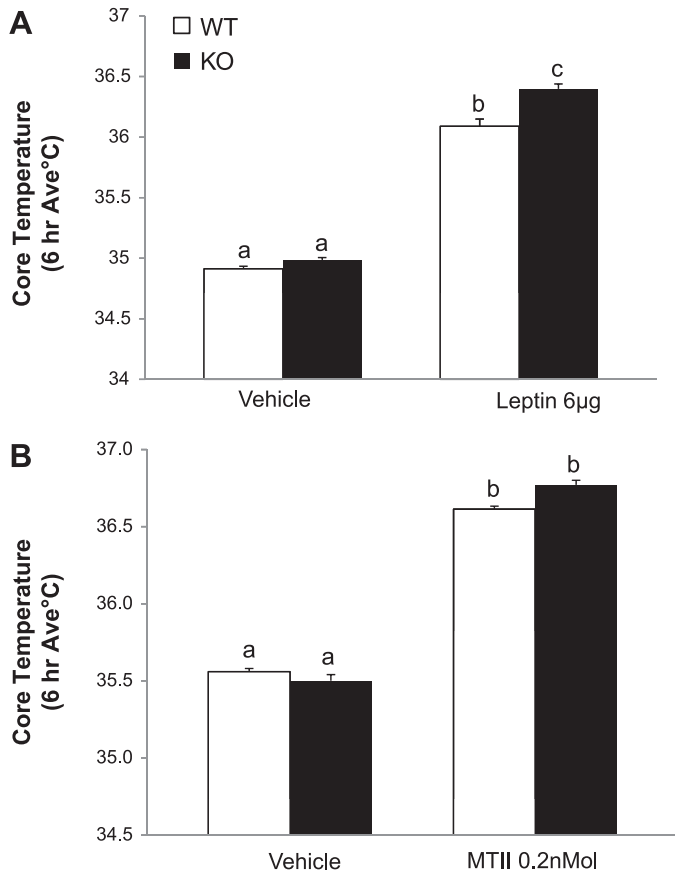


Fig. 4. Leptin-induced hyperthermia was significantly greater in KO vs. WT mice (A), whereas MTII-induced increases in core temperature were similar between genotypes (B). Bars with different letters indicate significant group differences in post hoc comparisons ($P < 0.05$). Bars with the same letters are not significantly different from each other.

tone. Overall, these findings illustrate the need to better characterize the functional role of the hindbrain POMC neurons.

Whether our results are mediated by enhanced efficiency of leptin receptor signaling by leptin acting directly on hindbrain POMC neurons is not clear; however, we can conclude that hindbrain POMC neurons are critical to leptinergic controls of energy balance in mice and that this pathway is PTP1B dependent. In addition, because POMC-Ptp1b^{-/-} mice are generated through the specific deletion of PTP1B in POMC neurons, it may be that the observed enhanced central sensitivity to exogenous leptin is due to enhanced sensitivity directly within POMC neurons. The kinetics of our observed leptin effect (i.e., greater food intake suppressions in KO vs. WT at an earlier time point) could be evidence for a direct effect of leptin on “leptin-hypersensitive” PTP1B-deficient hindbrain POMC neurons. However, it must be considered that the deletion of PTP1B in POMC neurons alters the sensitivity in nearby LepR or MC4R-expressing non-POMC neurons, leading to an enhancement of sensitivity to exogenous stimulation via an overall altered endogenous tone with the hindbrain. Along these lines, although leptin and MTII were hindbrain administered, thereby restricting the injection volume to hindbrain sites due to the caudal flow of cerebrospinal fluid (14, 32), it is likely that ascending/descending feedback cir-

cuits involving POMC- or PTP1B-containing neurons of the ARH also contribute to our effects. Indeed, it has been reported recently in the rat that hindbrain administration of leptin directly induces p-STAT3 not only in the hindbrain but also indirectly in the ventromedial hypothalamus due to a noncirculating effect of leptin, but rather likely involving reciprocal hypothalamic hindbrain projections (28). Thus, it is possible that greater leptin signaling transduction within the hindbrain of POMC-Ptp1b^{-/-} mice could in turn act to further enhance responsiveness of hypothalamic/forebrain POMC neurons in an overall increased central sensitivity to leptin and MTII, although this notion is speculative and remains to be determined.

In summary, our results show that POMC-Ptp1b^{-/-} mice are hypersensitive to ligand-driven increases in hindbrain LepR and MC4R signaling, as evidenced by food intake reduction, body weight suppression, and SPA increases compared with WT mice. Greater increases in leptin- but not MTII-induced core temperature were also observed in KO vs. WT animals. These studies are the first to show that hindbrain administration of leptin or a melanocortin receptor agonist alters energy in mice and further show that hindbrain POMC neurons regulate energy balance via a PTP1B-dependent mechanism.

Perspectives and Significance

Rats and mice are the most widely used animal models for the study of feeding behavior and body weight regulation. The present findings illustrate evidence for similar responsiveness to exogenous hindbrain application of leptin and a melanocortin receptor agonist between these two species. Importantly, these experiments involve the incorporation of a conditional KO model of selective POMC neuron PTP1B deficiency, a technique common to mouse models, with pharmacological (hindbrain icv) application of ligands, a technique used more commonly in rats than in mice. Via complementary genetic and behavioral pharmacology approaches, we establish an important congruency between rats and mice in the hindbrain control of energy balance as well as a role for PTP1B signaling within hindbrain POMC neurons to energy balance regulation.

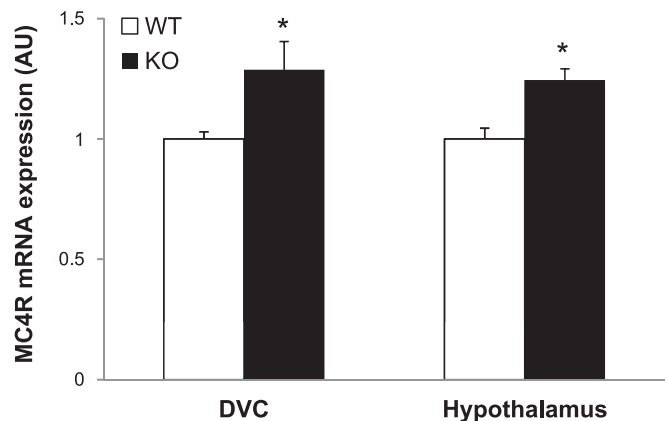


Fig. 5. Nontreated naïve adult female mice; higher expression of melanocortin 4 receptor (MC4R) mRNA is observed in POMC-Ptp1b^{-/-} mice compared with controls in both the hypothalamus and the dorsal vagal complex (DVC)-enriched tissue of the caudal hindbrain. *Significant differences between WT and KO ($P < 0.05$).

ACKNOWLEDGMENTS

We thank Anthony Balduzzi for technical assistance with experiments.

GRANTS

This work was supported by American Heart Association Grant 10POST3910000 (B. C. De Jonghe), National Institute of Diabetes and Digestive and Kidney Diseases Grants R01-DK-082417 (K. K. Bence), DK-21397 (H. J. Grill), and P30-DK-019525 (Penn DERC), The McCabe Fund, and the University of Pennsylvania Research Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.C.D., M.R.H., S.E.K., and K.K.B. did the conception and design of the research; B.C.D. and D.J.Z. performed the experiments; B.C.D. analyzed the data; B.C.D., M.R.H., and S.E.K. interpreted the results of the experiments; B.C.D. prepared the figures; B.C.D. drafted the manuscript; B.C.D., M.R.H., S.E.K., H.J.G., and K.K.B. edited and revised the manuscript; B.C.D., M.R.H., S.E.K., H.J.G., and K.K.B. approved the final version of the manuscript.

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