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PTP1B Regulates Leptin Signal Transduction In Vivo

Janice M. Zabolotny,[1,6] Kendra K. Bence-Hanulec,[2,6] Alain Stricker-Krongrad,[4] Fawaz Haj,[2] Yongping Wang,[2] Yasuhiko Minokoshi,[1] Young-Bum Kim,[1] Joel K. Elmquist,[1,3] Louis A. Tartaglia,[4] Barbara B. Kahn,[1,5,6] and Benjamin G. Neel[2,6]

[1]Division of Endocrinology and Metabolism and
[2]Cancer Biology Program
Division of Hematology and Oncology
Department of Medicine
[3]Department of Neurology and Program in Neuroscience
Beth Israel Deaconess Medical Center and
Harvard Medical School
Boston, Massachusetts 02215
[4]Millennium Pharmaceuticals
Cambridge, Massachusetts 02138

Summary

Mice lacking the protein-tyrosine phosphatase PTP1B are hypersensitive to insulin and resistant to obesity. However, the molecular basis for resistance to obesity has been unclear. Here we show that PTP1B regulates leptin signaling. In transfection studies, PTP1B dephosphorylates the leptin receptor-associated kinase, Jak2. PTP1B is expressed in hypothalamic regions harboring leptin-responsive neurons. Compared to wild-type littermates, *PTP1B*$^{-/-}$ mice have decreased leptin/body fat ratios, leptin hypersensitivity, and enhanced leptin-induced hypothalamic Stat3 tyrosyl phosphorylation. Gold thioglucose treatment, which ablates leptin-responsive hypothalamic neurons, partially overcomes resistance to obesity in *PTP1B*$^{-/-}$ mice. Our data indicate that PTP1B regulates leptin signaling in vivo, likely by targeting Jak2. PTP1B may be a novel target to treat leptin resistance in obesity.

Introduction

Regulation of body mass is essential for mammalian survival (Spiegelman and Flier, 2001), and disorders of body mass control (e.g., obesity) contribute to the pathogenesis of many important human diseases, including type II diabetes (Kopelman, 2000). The adipocyte-derived hormone leptin plays a key role in body mass regulation (Ahima and Flier, 2000). Although leptin affects multiple tissues, in normal rodents (and probably normal humans), many of its effects on body mass are mediated by neurons in the mediobasal hypothalamus (Dawson et al., 1997; Elmquist et al., 1999; Fei et al., 1997). Leptin binding to receptors on these neurons suppresses food intake and increases energy expenditure (Ahima and Flier, 2000). Naturally occurring mutations of leptin or the leptin receptor (LepR) cause morbid obesity and its complications in rodents and humans (Ahima and Flier, 2000). Therefore, understanding the regulation of LepR signaling is of substantial biomedical importance.

LepR is a type I cytokine receptor that associates with the protein-tyrosine kinase Jak2 (Ahima and Flier, 2000; Tartaglia, 1997). Upon leptin binding, Jak2 becomes activated and phosphorylates itself and LepR, which then recruits signal relay molecules, such as Stat3 (Ahima and Flier, 2000; Tartaglia, 1997). Tyrosyl phosphorylated Stat3 dimerizes, translocates to the nucleus, and regulates the transcription of target genes, including those encoding orexigenic and anorexigenic neuropeptides (Ahima and Flier, 2000; Spiegelman and Flier, 2001). Activated LepR also stimulates the Erk MAP kinase pathway (Banks et al., 2000; Bjorbaek et al., 2001) and regulates ion channels (Cowley et al., 2001; Spanswick et al., 1997).

Little is known about how LepR signaling is terminated. Initial studies of LepR mutants suggested a negative regulatory role for the protein-tyrosine phosphatase Shp2 (Carpenter et al., 1998; Li and Friedman, 1999). However, Socs-3, which binds to the same site on LepR as Shp2, may actually mediate this inhibitory signal (Bjorbaek et al., 2000). Negative regulation by Socs-3 involves LepR-evoked transcription and new protein synthesis (Bjorbaek et al., 2000; Krebs and Hilton, 2000). Whether (and how) acute regulation of LepR signaling occurs has remained unclear.

Recently, *PTP1B*$^{-/-}$ mice were generated (Elchebly et al., 1999; Klaman et al., 2000). Consistent with earlier suggestions that PTP1B regulates insulin receptor (IR) signaling (Goldstein et al., 1998), these mice are hypersensitive to insulin and exhibit enhanced and/or prolonged IR tyrosyl phosphorylation in liver and muscle (Elchebly et al., 1999; Klaman et al., 2000). Together with structural and kinetic studies (Salmeen et al., 2000), these findings establish PTP1B as a physiological IR phosphatase. *PTP1B*$^{-/-}$ mice also display resistance to high-fat diet-induced obesity (Elchebly et al., 1999; Klaman et al., 2000). This is due, at least in part, to increased energy expenditure in *PTP1B*$^{-/-}$ mice, although their food intake may also not be increased appropriately for their lowered body fat (Klaman et al., 2000). How PTP1B regulates body mass is unknown.

Results

PTP1B Regulates Leptin Signaling In Vitro

A recent study suggested that PTP1B might dephosphorylate Stat5 (Aoki and Matsuda, 2000), whereas structural and kinetic analyses indicated that Janus kinases (e.g., Jak2) should be favorable PTP1B substrates (Salmeen et al., 2000). To test the hypothesis that PTP1B might regulate LepR signaling, we first carried out transient transfection studies. Transfection of a LepR expression construct into Cos-7 cells conferred leptin-evoked tyrosyl phosphorylation of Jak2 and Stat3 (Figure 1), as reported (Bjorbaek et al., 1997; Carpenter et al., 1998; Li and Friedman, 1999). Coexpression of PTP1B

[5]Correspondence: bkahn@caregroup.harvard.edu
[6]These authors contributed equally to this work.

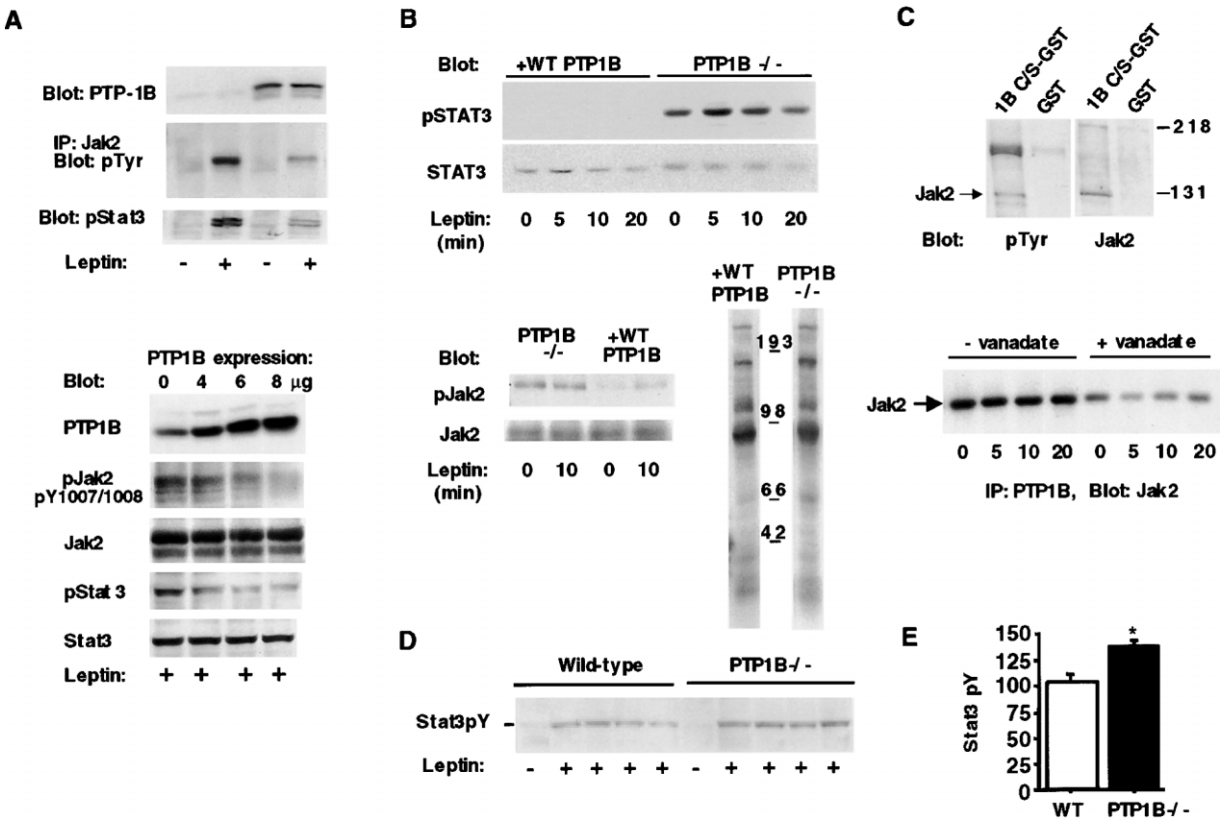

Figure 1. PTP1B Regulates Leptin Signaling

(A) PTP1B regulates LepR signaling in Cos-7 cells. Upper panel: Cos-7 cells transfected with expression constructs for LepR alone (left two lanes) or together with PTP1B (right two lanes) were stimulated with leptin or left unstimulated. Anti-PTP1B and anti-pStat3 immunoblots of total lysates and anti-pTyr immunoblots of anti-Jak2 immunoprecipitates are shown. Lower panel: Cos-7 cells transfected with a LepR expression construct and increasing amounts of PTP1B expression vector were stimulated with leptin. Lysates resolved by SDS-PAGE were immunoblotted with anti-PTP1B, anti-phospho-Jak2 (Y1007/Y1008), anti-Jak2, anti-phospho-Stat3, or anti-Stat3 antibodies.

(B) PTP1B regulates Jak2 phosphorylation in *PTP1B*$^{-/-}$ fibroblasts. Lysates from LepR-expressing *PTP1B*$^{-/-}$ fibroblasts or LepR-expressing *PTP1B*$^{-/-}$ fibroblasts reconstituted with PTP1B were stimulated with leptin (+) or left unstimulated (−), and immunoblotted with anti-pStat3 (upper panel), anti-pJak2 (lower left panel), or anti-phosphotyrosine (lower right panel). In some experiments, there was a further increase in Jak2 and Stat3 tyrosyl phosphorylation following leptin stimulation, but this was not observed consistently.

(C) Upper panel: 293 cells transiently expressing LepR were stimulated with leptin, and incubated with GST-PTP1BC215S or GST. Bound proteins were resolved by SDS-PAGE and immunoblotted with anti-pTyr or anti-Jak2 antibodies, as indicated. The 180 kDa pY species bound to the GST-PTP1BC215S is probably the EGFR (see Flint et al., 1997). Lower panel: *PTP1B*$^{-/-}$ fibroblasts expressing PTP1BD181A were stimulated with leptin, lysed in the absence (left four lanes) or presence (right four lanes) of sodium orthovanadate, immunoprecipitated with anti-PTP1B, and immunoblotted with anti-Jak2.

(D and E) Enhanced Stat3 activation in *PTP1B*$^{-/-}$ hypothalami. Hypothalamic extracts from male mice injected with saline (−) or leptin (+) were resolved by SDS-PAGE and immunoblotted with anti-pStat3 antibodies. A representative immunoblot of 4-month-old mice is shown (D); similar data were obtained in 8- to 10-week-old mice. Results from multiple experiments were quantified by densitometry and expressed as percent of the WT value (E). WT (n = 19), *PTP1B*$^{-/-}$ (n = 18) mice. *p = 0.006.

suppressed leptin-induced Jak2 and Stat3 phosphorylation (Figure 1A, left panel) and induction of a Stat-dependent transcriptional reporter (data not shown). Immunoblots with phospho-specific antibodies confirmed that PTP1B suppresses phosphorylation of the Jak2 activation site tyrosines (Y1007/Y1008) and Stat3 in a dose-dependent manner (Figure 1A, lower panel). These two Jak2 phosphotyrosines are those predicted to be preferred PTP1B targets (Salmeen et al., 2000). Our results suggest that PTP1B acts on Jak2, although additional actions on Stat3 or LepR are not excluded. Similar results were obtained using 293 cells (data not shown).

We also analyzed fibroblast lines derived from *PTP1B*$^{-/-}$ mice and engineered to stably express LepR and either an empty expression vector, or wild-type (WT) or mutant forms of PTP1B. *PTP1B*$^{-/-}$ cells reconstituted with WT PTP1B showed little or no phospho-Stat3 reactivity either basally (i.e., serum-starved) or in response to leptin stimulation. In contrast, Jak2 and Stat3 tyrosine phosphorylation were detectable constitutively in *PTP1B*$^{-/-}$ cells (Figure 1B, upper and lower left panels). Constitutive Jak2 and Stat3 phosphorylation in these cells also is consistent with PTP1B regulation of Jak2 phosphorylation. Notably, total cellular tyrosine phosphorylation in fibroblasts lacking PTP1B was similar to WT (Figure 1B, lower right panel), arguing against a global perturbation of tyrosine kinase pathways in the absence of PTP1B.

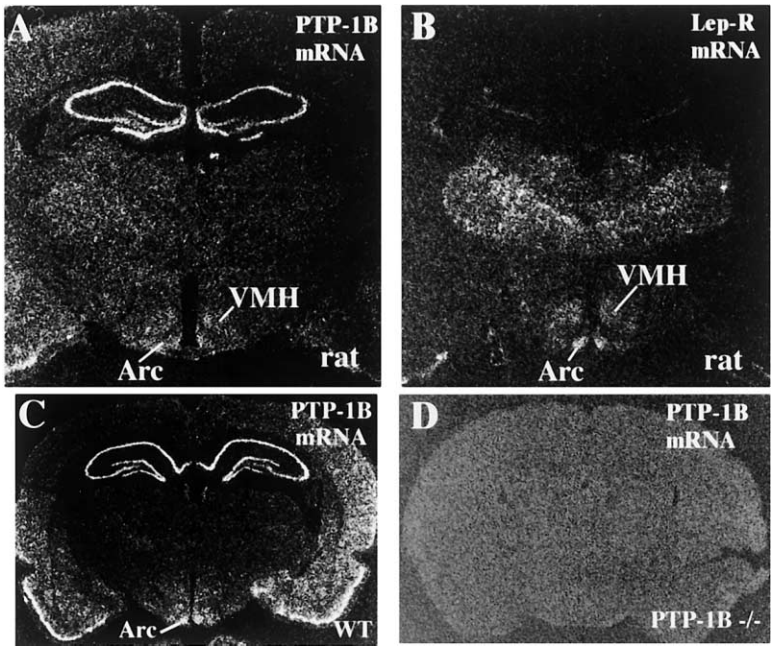

Figure 2. In Situ Hybridization

(A and B) PTP1B (A) and LepR (B) antisense riboprobes hybridized to rat brain.

(C) PTP1B antisense riboprobe hybridized to WT mouse brain. Note in (A) and (C) the strong hybridization to CA3 of the hippocampus, as well as hybridization elsewhere, including the arcuate (Arc), ventromedial hypothalamic (VMH), and dorsomedial hypothalamic (DMH) nuclei.

(D) PTP1B antisense probe hybridized to *PTP1B*$^{-/-}$ mouse brain.

Jak2 Is a Substrate of PTP1B

Further evidence was provided by experiments with two PTP1B "substrate-trapping" mutants, C215S and D181A, which have markedly diminished catalytic activity but retain substrate binding ability (Flint et al., 1997). A GST-PTP1BC215S fusion protein bound Jak2 from leptin-stimulated 293 cells transiently expressing LepR (Figure 1C, upper panel). Likewise, Jak2 coimmunoprecipitated with PTP1BD181A reexpressed in *PTP1B*$^{-/-}$ fibroblasts. Importantly, this interaction was reduced markedly in the presence of the competitive inhibitor sodium orthovanadate (Figure 1C, lower panel). We did not detect association of Stat3 with PTP1B in the same immunoprecipitation (not shown). Thus, D181A/Jak2 interaction occurs via the active site of PTP1B, consistent with Jak2 being a PTP1B substrate in vivo. Furthermore, transient coexpression of the D181A mutant and LepR in 293 cells resulted in enhanced leptin-evoked Jak2 tyrosine phosphorylation (data not shown).

PTP1B Is Expressed in Hypothalamus

We next asked whether PTP1B is expressed in regions of the brain important for body mass control. In situ hybridizations to both rat and WT mouse brains revealed significant hybridization in the hippocampus, as reported (Guan and Dixon, 1990), and throughout the brain including the hypothalamus, where hybridization was detected in the arcuate, ventromedial hypothalamic, and dorsomedial hypothalamic nuclei (Figures 2A and 2C). Notably, the pattern of PTP1B expression in the hypothalamus was similar to that of LepR (Figure 2B). No signal was observed using the antisense probe and brains from *PTP1B*$^{-/-}$ mice (Figure 2D) or the corresponding sense probe and WT brains (data not shown). Immunoblots confirmed widespread expression of PTP1B protein in murine brain including the hypothalamus (see Supplemental Figure S1 at http://www.developmentalcell.com/cgi/content/full/2/4/489/DC1). Thus, PTP1B is expressed within hypothalamic regions in which LepR functions to regulate body mass.

PTP1B$^{-/-}$ Mice Are Hypersensitive to Leptin

Comparisons of body fat and leptin levels in *PTP1B*$^{-/-}$ and WT mice raised the possibility that *PTP1B*$^{-/-}$ mice might be hypersensitive to leptin. On high-fat diets, *PTP1B*$^{-/-}$ mice gain much less weight than WT (Elchebly et al., 1999; Klaman et al., 2000) and have markedly diminished body fat (Klaman et al., 2000). On a low-fat (4.5% by weight) diet, *PTP1B*$^{-/-}$ and WT mice gain weight at similar rates (Elchebly et al., 1999 and data not shown). However, even under these conditions, *PTP1B*$^{-/-}$ mice had decreased fat pad mass and total carcass lipid (Table 1). Normally, serum leptin levels are proportional to body fat mass (Ahima and Flier, 2000) and, consistent with their decreased fat mass, *PTP1B*$^{-/-}$ mice had lower leptin levels. However, the leptin/body fat ratio also was significantly decreased in *PTP1B*$^{-/-}$ mice on low- (Table 1) and high-fat (data not shown; see Klaman et al., 2000) diets. This suggests increased leptin sensitivity, since *PTP1B*$^{-/-}$ mice maintain their leanness at lower leptin levels than WT.

To directly assess leptin sensitivity, we compared the response of WT, *PTP1B*$^{+/-}$, and *PTP1B*$^{-/-}$ mice to exogenous leptin. Body fat levels influence leptin sensitivity (Ahima and Flier, 2000). Therefore, for these studies, we used lean mice with similar levels of body fat as measured by dual energy X-ray absorptiometry (see Figure 3 legend). Mice (male, 6–8 per group) from each genotype were injected intraperitoneally (i.p.) with phosphate-buffered saline (PBS) vehicle or leptin (1–20 mg/kg/day in two divided doses; Figures 3A and 3B). In WT mice, only the highest leptin dose caused significant declines in body mass and food intake (left panels). In contrast, *PTP1B*$^{-/-}$ mice responded to all doses of leptin tested (right panels), whereas *PTP1B*$^{+/-}$ mice exhibited intermediate leptin sensitivity (middle panels). Thus, in a

Table 1. Body Composition Analysis

Genotype	BW (g)	Carcass Lipid (g)	Perigonadal Fat Pad (mg)	Leptin (ng/ml)	Leptin/Fat Pad (ng/ml-mg^{-1})
Wild-type	25.79 ± 0.74 (12)	8.84 ± 0.55 (6)	375.7 ± 29.8 (12)	4.20 ± 0.73 (6)	10.74 ± 1.07 (6)
PTP1B$^{-/-}$	25.21 ± 0.56 (12)	5.62 ± 0.73* (5)	237.1 ± 22.9* (12)	2.17 ± 0.28* (7)	8.56 ± 0.60* (7)

Body weight (BW), carcass lipid, perigonadal fat pad weight, and fed leptin levels of 8- to 11-week-old male mice fed a 4.5% (by weight) fat diet are shown. Number of animals is indicated in parentheses. Data are expressed as means ±SEM. *$p < 0.05$ by Student's t test.

gene dosage-dependent manner, PTP1B regulates total body leptin sensitivity of pathways regulating body mass.

Increased leptin sensitivity correlated with enhanced hypothalamic LepR signaling. Following peripheral leptin injection, Stat3 activation was ~35% higher ($p < 0.01$) in the hypothalami of *PTP1B*$^{-/-}$ mice (n = 18, 135.8 ± 7.3 arbitrary units) compared with WT (n = 19, 100 ± 8.2), as determined by densitometry of immunoblots from four separate experiments (Figures 1D and 1E). We were unable to detect LepR or Jak2 tyrosyl phosphorylation or Erk activation in hypothalami from leptin-stimulated WT or *PTP1B*$^{-/-}$ mice (data not shown), most likely reflecting inadequate sensitivity of available reagents. Enhanced Stat3 tyrosyl phosphorylation in *PTP1B*$^{-/-}$ hypothalami could reflect action of PTP1B on Stat3 or on an upstream component(s) of the LepR pathway, such as Jak2 and/or LepR. Based on our transient transfection studies (Figure 1) and the structural/kinetic studies of Salmeen et al. (2000), we believe PTP1B action is most likely at the level of Jak2. Importantly, LepR mRNA levels, measured by real-time PCR, were the same in WT and *PTP1B*$^{-/-}$ mice (data not shown). Thus, our results suggest that PTP1B acutely regulates hypothalamic LepR signaling.

Intact Hypothalamic Function Is Necessary but Not Sufficient for Leanness of *PTP1B*$^{-/-}$ Mice

Loss of PTP1B regulation of LepR signaling could contribute to the decreased fat mass and resistance to obesity of *PTP1B*$^{-/-}$ mice. To assess whether altered hypothalamic signaling contributes to the *PTP1B*$^{-/-}$ body mass phenotype, we administered gold thioglucose (GTG) to WT and *PTP1B*$^{-/-}$ mice. GTG destroys LepR-expressing neurons in the mediobasal hypothalamus (Bergen et al., 1998; Elmquist et al., 1998; Fei et al.,

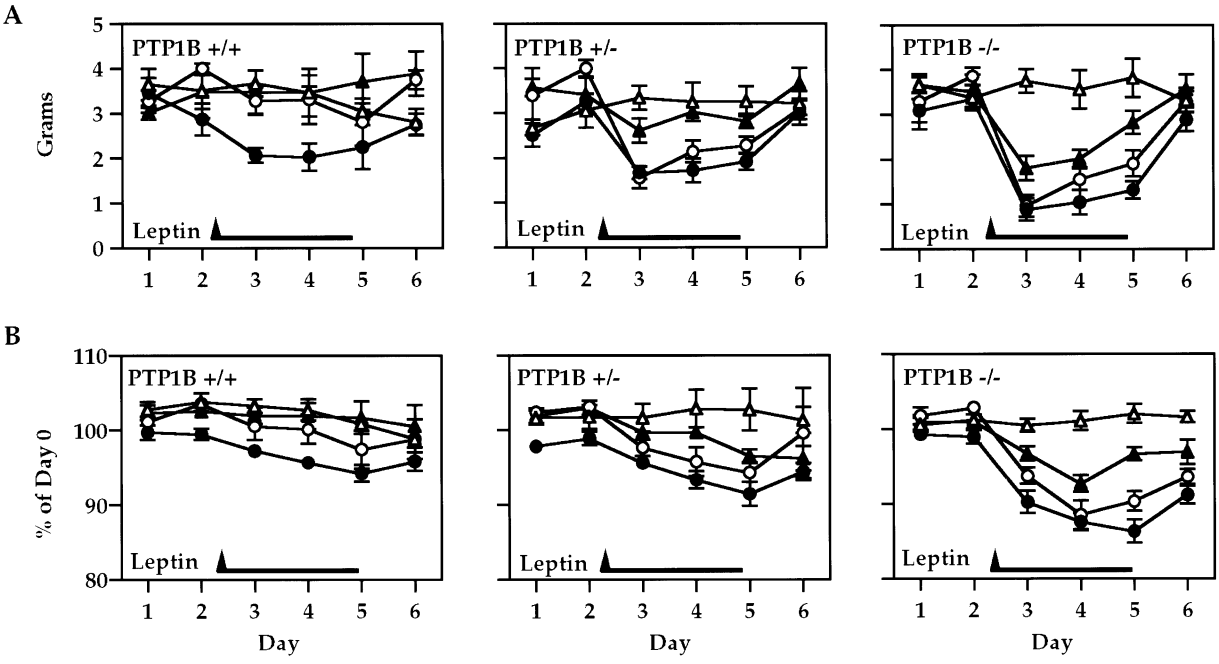

Figure 3. *PTP1B*$^{-/-}$ Mice Are Hypersensitive to Leptin

(A) Food intake in leptin-treated wild-type (WT), *PTP1B*$^{+/-}$ (Hets), and *PTP1B*$^{-/-}$ (KO) mice. Since there is a large variation in fat content in outbred mice, we were able to select 8- to 11-week-old mice that were matched for similar body fat content as measured by dual energy X-ray absorptiometry. Male animals (6–8/group) were injected with vehicle (PBS) (open triangle) or 1 (filled triangles), 10 (open circles), or 20 (filled circles) mg/kg/day leptin i.p. (in two divided doses) during the 3 consecutive days indicated. Values represent mean daily food intake (in grams) ±SEM.

(B) Effect of leptin on body weight. Shown are body weights expressed as % of weights at day 0 of mice in (A). Similar results were obtained with female mice (data not shown). *$p < 0.05$.

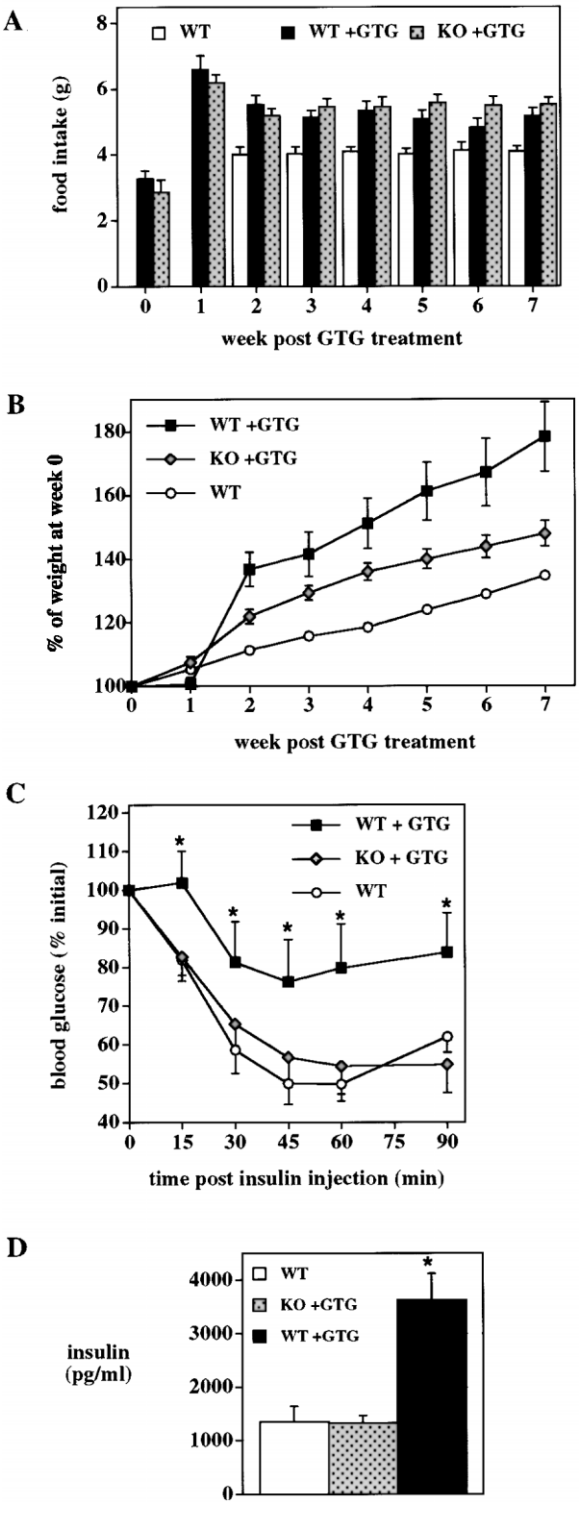

Figure 4. Effect of GTG on WT and *PTP1B*$^{-/-}$ (KO) Mice

WT (n = 11) and *PTP1B*$^{-/-}$ (n = 13) male mice were treated with GTG (0.4 mg/g body weight) at 5 weeks of age. Untreated WT mice (n = 5–12) served as controls. Data are means ±SEM.

(A) Food intake of WT + GTG and KO + GTG groups is increased significantly compared to untreated WT mice by 1 week post-GTG treatment ($p < 0.05$).

(B) Weight gains expressed as % of weight at week 0 of WT + GTG, KO + GTG, and WT are statistically different ($p < 0.05$) at all points from 2 to 7 weeks posttreatment.

(C and D) Insulin tolerance tests (C) and fed serum insulin levels (D) were measured at 7 weeks post-GTG treatment. *WT + GTG is different from WT control and from KO + GTG; $p < 0.05$.

1997) and causes hyperphagia and obesity (Debons et al., 1977). If hypothalamic leptin hypersensitivity is an important cause of the resistance to obesity in *PTP1B*$^{-/-}$ mice, then *PTP1B*$^{-/-}$ and WT mice should respond similarly to GTG treatment. As expected, GTG injection markedly disrupted the normal architecture of the mediobasal hypothalami, including the arcuate nuclei, of both WT and *PTP1B*$^{-/-}$ mice (Supplemental Figure S2). GTG-treated WT and *PTP1B*$^{-/-}$ mice exhibited significantly and comparably increased food intake, indicating that hypothalamic neurons had been ablated to similar extents (Figure 4A). Compared to untreated controls, GTG-treated WT and *PTP1B*$^{-/-}$ mice also exhibited increased body mass. However, the increase in body mass of GTG-treated *PTP1B*$^{-/-}$ mice was only about half that of GTG-treated WT mice, despite their comparably increased food intake (Figure 4B). These data further support our previous finding that *PTP1B*$^{-/-}$ mice have enhanced energy expenditure (Klaman et al., 2000). Moreover, as GTG-treated *PTP1B*$^{-/-}$ mice are no longer fully resistant to obesity, intact hypothalamic signaling, most likely initiated by leptin-responsive neurons in GTG-sensitive areas, appears to be required for the full manifestation of the *PTP1B*$^{-/-}$ body mass phenotype. Importantly, however, since GTG-treated *PTP1B*$^{-/-}$ mice gain less weight than WT despite comparable food intake and brain lesions, PTP1B also must regulate body mass pathways distal to those ablated by GTG, either in the brain or the periphery.

Glucose homeostasis also differed in GTG-treated WT and *PTP1B*$^{-/-}$ mice. Blood glucose (at noon) was significantly lower in GTG-treated *PTP1B*$^{-/-}$ mice (135 ± 6.7 mg/dl versus 164 ± 6.8 mg/dl; $p < 0.05$). Insulin tolerance tests (ITTs; Figure 4C) confirmed that GTG-treated WT mice became more insulin resistant than untreated controls. In contrast, GTG-treated *PTP1B*$^{-/-}$ mice remained as insulin sensitive as control untreated WT mice (again, in spite of the increased body mass of *PTP1B*$^{-/-}$ mice). Likewise, serum insulin levels were elevated in GTG-treated WT mice, but not in GTG-treated *PTP1B*$^{-/-}$ mice (Figure 4D). Thus, the two most striking phenotypes caused by PTP1B deficiency, insulin hypersensitivity and resistance to diet-induced obesity, appear to involve different signaling pathways and/or sites of action.

Discussion

Our results, together with previous studies (Elchebly et al., 1999; Klaman et al., 2000), indicate that PTP1B is a key regulator of at least two critical metabolic pathways. In muscle and liver (and possibly other tissues), PTP1B negatively regulates insulin signaling by directly dephosphorylating the IR (Elchebly et al., 1999). Loss of this regulation in *PTP1B*$^{-/-}$ mice results in increased insulin sensitivity (Elchebly et al., 1999; Klaman et al., 2000). The results herein suggest that PTP1B also is a

physiologically significant negative regulator of hypothalamic LepR signaling, where it most likely acts on Jak2. Resistance of *PTP1B*$^{-/-}$ mice to high-fat diet-induced obesity may be explained, in part, by loss of this regulatory function.

PTP1B deficiency only partially "rescues" the effects of GTG ablation on body mass, so leptin hypersensitivity in GTG-sensitive neurons accounts for only part of the *PTP1B*$^{-/-}$ body mass phenotype. Therefore, PTP1B must have important targets in tissues distal to those ablated by GTG. These could include leptin-responsive neurons elsewhere in the brain (Elias et al., 2000; Elmquist et al., 1998) or leptin-responsive cells in the periphery, such as muscle, fat, and liver (Ahima and Flier, 2000; Minokoshi et al., 2002). PTP1B also may regulate central IR signaling, and loss of this regulation could contribute to resistance to obesity in *PTP1B*$^{-/-}$ mice. Although the role of IR signaling in the brain remains unclear, the phenotypes of brain-specific *IR*$^{-/-}$ (Bruning et al., 2000) and *IRS-1*$^{-/-}$ (Burks et al., 2000) mice and data with insulinomimetic small molecule treatment (Air et al., 2002) are consistent with insulin action in the brain regulating body mass. Alternatively, PTP1B may regulate another pathway(s) besides the IR and LepR that controls body mass; candidates include other cytokine receptors that signal via Jak2. Tissue-specific PTP1B deletion should help to clarify these issues. It will also be important to determine why PTP1B appears to regulate Jak2 in some cell types (e.g., hypothalamic neurons), whereas other PTPs are required for Jak2 regulation in other cells (Irie-Sasaki et al., 2001; You et al., 1999), even though PTP1B is expressed ubiquitously.

Our studies, along with those of Cheng et al. in this issue of *Developmental Cell*, indicate that PTP1B deficiency can attenuate weight gain in mice lacking normal hypothalamic leptin signaling. Since nearly all forms of human obesity involve leptin resistance (Spiegelman and Flier, 2001), these findings, along with the apparently benign effects of PTP1B deficiency in the mouse, support the identification of PTP1B as a potential target for prevention and therapy of obesity.

Experimental Procedures

Cells and Transfections

293 and Cos-7 cells were maintained in DMEM plus 10% FCS and antibiotics. Transfections were performed with Superfect using expression constructs for LepR (under CMV control) and PTP1B (under SV40 promoter/enhancer control). Details are available from K.K.B.-H. upon request. Twenty-four to 36 hr posttransfection, cells were placed in serum-free DMEM for 14 hr, prior to stimulation with leptin (20–50 nM). The *PTP1B*$^{-/-}$ fibroblast cell line was generated by immortalizing primary embryo fibroblasts from *PTP1B*$^{-/-}$ mice with large T antigen. *PTP1B*$^{-/-}$ cells were infected with retroviruses expressing WT or D181A PTP1B or the parental virus (LaMontagne et al., 1998), and pools of infected cells were selected. WT, C215S, and D181A PTP1B constructs were derived from the human PTP1B cDNA (Chernoff et al., 1990) using standard techniques. LepR was introduced into these lines by transfection of a CMV-based expression construct (pCMVZeo). Selected cells expressed comparable levels of LepR (data not shown). Further details regarding these lines will be published elsewhere, and are available from Haj et al. (2002).

Immunoprecipitations and Immunoblotting

For substrate-trapping experiments, cell lysates were prepared in 1% NP-40 buffer with a protease inhibitor cocktail with or without sodium orthovanadate (Harlow and Lane, 1988). In other experiments, cells were lysed in RIPA buffer (Harlow and Lane, 1988). Immunoprecipitations (typically from 500 μg clarified lysate) and immunoblotting were carried out as described (Klaman et al., 2000) with anti-phosphotyrosine (PY99; Santa Cruz Biotechnology), anti-PTP1B (FG6; Oncogene Sciences), anti-pStat3 and polyclonal anti-Jak2 (for immunoblotting; Upstate Biotechnology), anti-Jak2 (for immunoprecipitation; Dwayne Barber, Ontario Cancer Institute), anti-pJak2 (Y1007/1008; Biosource), and anti-leptin receptor (Martin Myers, Joslin Diabetes Center) antibodies.

Mice

WT, *PTP1B*$^{+/-}$, and *PTP1B*$^{-/-}$ mice (Klaman et al., 2000) were housed at 22°C with a 12 hr light/dark cycle and fed a low-fat (4.5% by weight) diet (Purina Autoclavable Rodent Diet 5010) ad libitum.

In Situ Hybridizations

Serial sections (30 μm) from brains of 5- to 8-week-old mice or adult rats perfused with 10% neutral buffered formalin were subjected to in situ hybridization as described previously (Elias et al., 2000) using ^{35}S-labeled sense and antisense riboprobes corresponding to nucleotides 614–964 of murine PTP1B cDNA (GenBank accession number GI 1292901).

Leptin Signaling in *PTP1B*$^{-/-}$ Mice

Murine leptin (Eli Lilly) was administered i.p. (1.67 μg/g body weight) to mice fasted for 15–18 hr. Mice were sacrificed 45 min later and the hypothalamus was rapidly dissected and frozen in liquid N_2. The same fraction of hypothalamic lysate (in RIPA buffer) from each mouse was subjected to SDS-PAGE and anti-pStat3 immunoblotting.

Gold Thioglucose Treatment

Male mice (5 weeks old) were injected i.p. (0.4 mg/g body weight) with aurothioglucose (Solganal; Schering). Twenty-four hr food intake and body weights were measured weekly. Lesions were confirmed by histological analysis. Mice were perfused with 10% neutral buffered formalin, brains were cut at 30 μm on a freezing microtome, mounted on Superfrost Plus slides (Fischer), and stained with thionine.

Metabolic Measurements

Blood glucose was measured using a One Touch II glucometer (Lifescan). ITTs were performed as described (Klaman et al., 2000) on mice fasted for 5 hr using 0.5 mU/g body weight of insulin (Novolin R, Novo Nordisk). Serum insulin and leptin and body composition analysis of carcass hydrolysates was carried out as described (Klaman et al., 2000).

Statistical Analyses

Data are presented as means ±SEM. Statistical analyses were performed using StatView or SuperANOVA software (Abacus Concepts). Statistical significance was tested with unpaired Student's t tests or factorial analysis of variance followed by Newman-Keuls multiple range test. Differences were considered significant if $p < 0.05$.

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