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Neuronal SH2B1 is essential for controlling energy and glucose homeostasis

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SH2B1 (previously named SH2-B), a cytoplasmic adaptor protein, binds via its Src homology 2 (SH2) domain to a variety of protein tyrosine kinases, including JAK2 and the insulin receptor. SH2B1-deficient mice are obese and diabetic. Here we demonstrated that multiple isoforms of SH2B1 (α, β, γ, and/or δ) were expressed in numerous tissues, including the brain, hypothalamus, liver, muscle, adipose tissue, heart, and pancreas. Rat SH2B1β was specifically expressed in neural tissue in SH2B1-transgenic (SH2B1Tg) mice. SH2B1Tg mice were crossed with SH2B1-knockout (SH2B1KO) mice to generate SH2B1TgKO mice expressing SH2B1 only in neural tissue but not in other tissues. Systemic deletion of the SH2B1 gene resulted in metabolic disorders in SH2B1KO mice, including hyperlipidemia, leptin resistance, hyperphagia, obesity, hyperglycemia, insulin resistance, and glucose intolerance. Neuron-specific restoration of SH2B1β not only corrected the metabolic disorders in SH2B1TgKO mice, but also improved JAK2-mediated leptin signaling and leptin regulation of orexigenic neuropeptide expression in the hypothalamus. Moreover, neuron-specific overexpression of SH2B1 dose-dependently protected against high-fat diet–induced leptin resistance and obesity. These observations suggest that neuronal SH2B1 regulates energy balance, body weight, peripheral insulin sensitivity, and glucose homeostasis at least in part by enhancing hypothalamic leptin sensitivity.

Introduction

Body weight is controlled by a balance between energy intake and expenditure. Excess energy derived from a positive energy imbalance is stored as triglyceride (TG) in adipose tissue, resulting in obesity. Body weight is maintained within a narrow range by a homeostatic control system in which the brain, particularly the hypothalamus, senses and integrates various neuronal, hormonal, and nutrient-related signals, thereby coordinating food intake and energy expenditure. Recent findings provide a framework for understanding this homeostatic regulation of body weight. Leptin, which serves as an essential adiposity signal, is produced primarily by white adipose tissue but not in other tissues. Systemic deletion of the SH2B1 gene results in morbid obesity and type 2 diabetes, demonstrating an essential role for these 2 pathways in mediating leptin regulation of energy metabolism and body weight (1–3). Genetic deficiency of either leptin or its receptor disrupts the communication between the peripheral energy stores and the central sensors/integrators, resulting in severe energy imbalance and morbid obesity (4–8). Leptin resistance plays a key role in the development of obesity, which is a primary risk factor for type 2 diabetes and various cardiovascular disorders.

Leptin binds to and activates its long form receptor (LEPRb) in the hypothalamus, initiating the activation of a variety of intracellular signaling pathways, including the STAT3 and PI3K pathways (8–12). Inhibition of either the STAT3 or PI3K pathways in the hypothalamus results in leptin resistance and obesity, demonstrating an essential role for these 2 pathways in mediating leptin regulation of energy metabolism and body weight (11–17). JAK2, a cytoplasmic tyrosine kinase, binds directly to LEPRb and is activated in response to leptin (9, 18). Activated JAK2 phosphorylates and activates downstream signaling molecules, including STAT3, insulin receptor substrate 1 (IRS1) and IRS2 (19). JAK2 is targeted by multiple negative regulators. PTP1B, a protein tyrosine phosphatase, binds to and dephosphorylates JAK2, thereby inhibiting JAK2 activation (20). Genetic deletion of PTP1B improves leptin sensitivity and protects against high-fat diet–induced (HFD-induced) obesity (21, 22). SOCS3 also inhibits JAK2 activation (23, 24). Both systemic SOCS3 haploinsufficiency and hypothalamus-specific deletion of SOCS3 improve leptin sensitivity (25, 26). Recent studies suggest that JAK2 may also be positively regulated by SH2-B, a JAK2-interacting protein (27).

SH2-B is one of 3 members of the SH2B family (SH2-B, APS, and Lnk), which have a conserved structure of a pleckstrin homology (PH) and Src homology 2 (SH2) domain (28–30). SH2-B, APS, and Lnk were recently renamed SH2B1, SH2B2, and SH2B3, respectively, by the HUGO Gene Nomenclature Committee. The SH2B1 (SH2-B) gene encodes, via alternative mRNA splicing, 4 isoforms (α, β, γ, and δ) that share an identical N-terminal region, including both PH and SH2 domains, but differ at their C termini following the SH2 domain (31). Since all forms contain conserved PH and SH2 domains, they are predicted to have similar functions. In cultured cells, SH2B1 binds via its SH2 domain to numerous protein tyrosine kinases, including both cytoplasmic tyrosine kinases (e.g., JAK1, JAK2, and JAK3) and receptor tyrosine kinases (e.g., the receptors for insulin, IGF-1, PDGF, FGF, nerve growth factor, brain-derived neurotrophic factor, and glial cell–derived neurotrophic factor) (29, 32–43). In animals, systemic deletion of SH2B1 results in morbid obesity and type 2 diabetes, demonstrating that SH2B1 is an essential player in the regulation of both body weight and glucose metabolism (44–46). In addition to obese and diabetic phenotypes, SH2B1-knockout mice also have defects in reproduction (47).

SH2B1 is abundantly expressed in both the central nervous system and peripheral tissues, including the brain, liver, muscle, and adipose tissue. However, relative contributions of SH2B1 in

Nonstandard abbreviations used: AgRP, agouti-related protein; GH, growth hormone; GTT, glucose tolerance test; HFD, high-fat diet; IRS1, insulin receptor substrate 1; ITT, insulin tolerance test; LEPRb, long form of the leptin receptor; MEF, mouse embryonic fibroblast; NPY, neuropeptide Y; NSE, neuron-specific enolase; PH, pleckstrin homology; POMC, proopiomelanocortin; SH2, Src homology 2; TG, triglyceride; TgKO, transgenic/knockout compound mutation.

Conflict of interest: The authors have declared that no conflict of interest exists.

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individual tissues to energy and/or glucose homeostasis remain unclear. This study investigated the potential role of central versus peripheral SH2B1 in regulating energy and glucose metabolism and provides convincing evidence that central rather than peripheral SH2B1 controls body weight, peripheral insulin sensitivity, and glucose metabolism at least partially by cell-autonomously enhancing leptin sensitivity in the hypothalamus.

Results

Neuron-specific restoration of SH2B1 rescues obesity and hyperlipidemia in SH2B1\textsuperscript{KO} mice. (A) Tissue extracts (2 mg protein in hypothalamic and 3 mg protein in other tissue extracts) were prepared from WT mice, immunoprecipitated with \(\alpha\)-SH2B1, and immunoblotted with \(\alpha\)-SH2B1. Lanes 1–8 represent tissue extracts from spleen, pancreas, heart, hypothalamus, muscle, liver, white adipose tissue, and brain, respectively. (B) Schematic representation of the Myc-tagged SH2B1\(\beta\) transgene. (C) Brain extracts were prepared from an SH2B1\textsuperscript{tg} mouse and a WT littermate, immunoprecipitated with \(\alpha\)-SH2B1, and immunoblotted with \(\alpha\)-Myc. (D) Brain extracts were prepared from WT, SH2B1\textsuperscript{KO}, and SH2B1\textsuperscript{tgko-437} mice and immunoblotted with \(\alpha\)-SH2B1. Each lane represents a sample from 1 mouse. (E) Tissue extracts were prepared from the hypothalamus (Hypo), brain, skeletal muscle, liver, pancreas (Pan), white adipose tissue (WAT), brown adipose tissue (BAT), heart, and lung from an SH2B1\textsuperscript{tgko-437} female (16 weeks old); immunoprecipitated with \(\alpha\)-SH2B1; and immunoblotted with \(\alpha\)-Myc. (F) Growth curves for WT, SH2B1\textsuperscript{tg}, SH2B1\textsuperscript{KO}, and SH2B1\textsuperscript{tgko-437} mice. Number in parentheses indicates the number of mice per group. (G) Levels of plasma FFAs and TGs in males (17 weeks old) fasted overnight. (H) Liver weight and TG levels in males (21–22 weeks old) fasted overnight. *\(P < 0.05\).
WT mice (Figure 1C). All 8 \(SH2B1^β\) lines expressed similar levels of recombinant \(SH2B1β\) (data not shown).

Two independent \(SH2B1^β\) lines (\(SH2B1^β\)-TgKO and \(SH2B1^β\)-TgKO) were crossed with heterozygous \(SH2B1\)-knockout mice to generate compound mutant mice (\(SH2B1^β\)-TgKO and \(SH2B1^β\)-TgKO) that were heterozygous for the \(SH2B1β\)-transgenic allele and homozygous for the \(SH2B1\) allele. The expression of recombinant \(SH2B1β\) was similar in \(SH2B1^β\)-TgKO and \(SH2B1^β\)-TgKO mice and restricted to neural tissues in both \(SH2B1^β\)-TgKO and \(SH2B1^β\)-TgKO mice (data not shown). To compare the expression levels of the \(SH2B1\) transgene and endogenous \(SH2B1\) gene, brain extracts were immunoblotted with \(α\)-SH2B1. Multiple forms of endogenous \(SH2B1\) were detected in WT but not homozygous \(SH2B1\)-knockout (\(SH2B1^ββ\)) mice (Figure 1D). Recombinant \(SH2B1β\) was the only form detected in \(SH2B1^β\)-TgKO mice and was expressed at levels similar to those of endogenous \(SH2B1\) in WT mice (Figure 1D). However, these data cannot exclude the possibility that the NSE promoter/GH enhancer may drive an overexpression of recombinant \(SH2B1β\) in a subpopulation of neurons that either express extremely low levels of \(SH2B1\) or do not express \(SH2B1\) at all. To confirm specific expression of the \(SH2B1β\) transgene in neural tissue, multiple tissue extracts were prepared from \(SH2B1^β\)-TgKO mice, immunoprecipitated with \(α\)-SH2B1, and immunoblotted with \(α\)-Myc. Recombinant \(SH2B1β\) was detected in both the hypothalamus and whole brain, but not in muscle, liver, pancreas, white adipose tissue, brown adipose tissue, heart, and lung (Figure 1E).

To determine the role of neuronal \(SH2B1\) in regulating growth and body weight, \(SH2B1^KO\), \(SH2B1^β\), \(SH2B1^β\), and WT mice were fed standard chow, and body weight was monitored. Systemic deletion of \(SH2B1\) resulted in a marked increase in body weight in both male and female \(SH2B1^β\) mice, and neuron-specific restoration of \(SH2B1\) (to endogenous levels) fully rescued the obese phenotype in \(SH2B1^β\)-TgKO mice (Figure 1F). Neuron-specific restoration of \(SH2B1\) also markedly reduced body weight in another independent line (\(SH2B1^β\), 32.3 ± 1.6 g, \(n = 12\); \(SH2B1^β\)-TgKO, 25.5 ± 1.1 g, \(n = 5\); 10 weeks). Neuron-specific restoration of recombinant \(SH2B1β\) alone was sufficient to rescue the obese phenotype observed in \(SH2B1^β\)-TgKO mice, suggesting that neuronal \(SH2B1\) is required for maintaining normal body weight and that multiple isoforms of \(SH2B1\) in the brain have similar functions in regulating body weight.

Obesity is commonly associated with hyperlipidemia. Systemic deletion of \(SH2B1\) markedly increased both plasma FFA and triglyceride (TG) levels in \(SH2B1^β\) mice; neuron-specific restoration of \(SH2B1\) completely corrected hyperlipidemia in \(SH2B1^β\)-TgKO mice (Figure 1G). \(SH2B1^β\) mice had hepatic steatosis as revealed by an enlarged liver mass and significantly increased hepatic lipid content (Figure 1H). Neuron-specific restoration of \(SH2B1\) largely reversed hepatic steatosis in \(SH2B1^β\)-TgKO mice (Figure 1H).

**Figure 2**
Neuronal and adipose \(SH2B1\) have opposite effects on adiposity. (A) Weight of epididymal (Epi) and inguinal fat depots (Ing) from \(SH2B1^KO\), \(SH2B1^β\)-TgKO, and WT males at age 23–24 weeks. (B) Whole body fat content in \(SH2B1^KO\), \(SH2B1^β\)-TgKO, and WT mice. (C) Representative H&E staining of epididymal fat depots from \(SH2B1^KO\), \(SH2B1^β\)-TgKO, and WT males at age 23 weeks (upper panels) or from \(SH2B1^β\)-TgKO and WT males at age 10 weeks (lower panels). (D) 3T3-L1 preadipocytes were differentiated into adipocytes for 0, 3, 6, or 10 days. Cell extracts were immunoprecipitated with \(α\)-SH2B1 and immunoblotted with \(α\)-SH2B1 (upper panel). Cell extracts were also immunoblotted with anti–β-actin antibodies (lower panel). (E) WT and \(SH2B1^β\)-Mef primary cultures were subjected to adipocyte differentiation for 10 days. Differentiated cells were stained with oil red O. *\(P < 0.05\).
Neuron-specific restoration of SH2B1 reverses energy imbalance in SH2B1KO mice. Systemic deletion of SH2B1 resulted in hyperphagia, markedly increasing food intake in SH2B1KO mice (Figure 3A). SH2B1KO mice also had significantly elevated energy expenditure, as revealed by significantly increased O2 consumption and CO2 production (Figure 3B). A previous report showed that energy intake still exceeds energy expenditure in this setting, resulting in obesity in SH2B1KO mice (45). Neuron-specific restoration of SH2B1 largely corrected hyperphagia and markedly reduced energy expenditure in SH2B1TKO mice (Figure 3B). These results suggest that SH2B1 in the brain controls body weight and adiposity by inhibiting both energy intake and expenditure.

Neuron-specific restoration of SH2B1 corrects leptin resistance and hypothalamic neuropeptide expression in SH2B1TKO mice. Systemic deletion of SH2B1 dramatically increased plasma leptin levels (hyperleptinemia) in both fasted and fed SH2B1KO mice, a hallmark of leptin resistance (Figure 4A). Neuron-specific restoration of SH2B1 expression completely reversed hyperleptinemia in SH2B1TKO mice (Figure 4A). Acute exogenous leptin treatment markedly reduced body weight and food intake in WT mice, as predicted (Figure 4B and data not shown). Systemic deletion of SH2B1 abolished these physiological responses to leptin in SH2B1KO mice, including leptin-induced reduction in body weight (Figure 4B). Neuron-specific restoration of SH2B1 fully rescued the ability of leptin to reduce body weight in SH2B1TKO mice (Figure 4B). These data indicate that neuron-specific restoration of SH2B1 (to endogenous levels) is sufficient to rescue leptin resistance in SH2B1KO mice.

To examine leptin signaling in the hypothalamus, mice were fasted overnight and injected intraperitoneally with leptin (1 mg/kg body weight). Hypothalamic extracts were immunoblotted with anti–phospho-STAT3 antibodies that specifically recognize phosphorylated and active STAT3. Systemic deletion of SH2B1 significantly impaired leptin-stimulated phosphorylation of STAT3 (Figure 4C). Neuron-specific restoration of SH2B1 increased leptin-stimulated phosphorylation of hypothalamic STAT3 in SH2B1TKO mice to levels similar to those in age-matched WT controls, suggesting that neuronal SH2B1 may cell-autonomously enhance leptin signaling in the hypothalamus (Figure 4C).

Leptin inhibits the expression of orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) and stimulates the expression of anorexigenic POMC in the arcuate nucleus of the hypothalamus (1, 3, 50). NPY and AgRP promote positive energy imbalance, whereas α-melanocyte-stimulating hormone (α-MSH), a proteolytic product of POMC, promotes negative energy imbalance (2, 3, 51). The abundance of hypothalamic NPY, AgRP, and POMC mRNA was measured using quantitative real-time PCR assays and normalized to the expression of β-actin. Systemic deletion of SH2B1 markedly increased NPY and AgRP but not POMC expression in SH2B1KO mice (Figure 4D). Neuron-specific restoration of SH2B1 dramatically reduced NPY expression in SH2B1TKO mice to levels similar to those in WT controls, suggesting that neuronal SH2B1 may cell-autonomously enhance leptin signaling in the hypothalamus (Figure 4C).

Neuron-specific overexpression of SH2B1 protects against HFD-induced leptin resistance and obesity. To determine whether a modest increase in neuronal SH2B1 expression protects against leptin resistance...
and obesity, SH2B1<sup>+/−</sup> and WT littermates were fed an HFD. Body weight and fat content were similar in SH2B1<sup>+/−</sup> and WT littermates (Figure 5, A and B). However, blood leptin levels were significantly reduced, by 58%, in SH2B1<sup>+/−</sup> mice, suggesting an increase in leptin sensitivity in SH2B1<sup>+/−</sup> mice (Figure 5C). These results suggest that while a modest increase in neuronal SH2B1 expression mildly increases leptin sensitivity, this is insufficient to protect against HFD-induced obesity in SH2B1<sup>+/−</sup> mice.

To increase neuronal SH2B1 expression, SH2B1<sup>+/−</sup> mice were bred to generate homozygous SH2B1-transgenic mice (SH2B1<sup>Tg<sub>407</sub>/437</sup>). Two independent SH2B1<sup>Tg<sub>407</sub>/437</sup> lines (SH2B1<sup>Tg<sub>407</sub>/437</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup>) were obtained. To examine the SH2B1 transgene expression, brain extracts were immunoprecipitated with α-SH2B1 and immunoblotted with α-Myc to detect Myc-tagged recombinant SH2B1<sup>α</sup>. The expression of the SH2B1<sup>α</sup> transgene was significantly higher in SH2B1<sup>Tg<sub>407</sub>/437</sup> than in SH2B1<sup>Tg<sub>407</sub>KO</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup> mice (heterozygous for the SH2B1<sup>α</sup> transgene) (Figure 6A). Neuron-specific overexpression of SH2B1 did not have an obviously deleterious effect on the overall health of SH2B1<sup>Tg<sub>407</sub>/437</sup> mice.

To determine the dosage effect of neuronal SH2B1 on leptin sensitivity and adiposity, body weight and blood leptin levels were measured. Body weight markedly decreased in both SH2B1<sup>Tg<sub>407</sub>/437</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup> compared with WT control mice fed normal chow (Figure 6B, left panel). Fasting plasma leptin levels were significantly lower in SH2B1<sup>Tg<sub>407</sub>/437</sup> than in WT control mice (Figure 6D).

Mice were fed an HFD at 7 weeks of age. HFD markedly increased both body weight and fat content in WT mice, whereas both SH2B1<sup>Tg<sub>407</sub>/437</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup> mice were resistant to HFD-induced obesity (Figure 6, B, right panel, and C). HFD induced severe hyperleptinemia (a hallmark of leptin resistance) in WT mice, increasing fasting blood leptin levels by 26-fold; in contrast, plasma leptin levels were only mildly elevated in HFD-fed SH2B1<sup>Tg<sub>407</sub>/437</sup> mice. Neuronal overexpression of SH2B1 reduced blood leptin levels by 98% in SH2B1<sup>Tg<sub>407</sub>/437</sup> and 92% in SH2B1<sup>Tg<sub>407</sub>/437</sup> compared with WT control mice (Figure 6D, right panel). Since the 2 lines of SH2B1<sup>Tg<sub>407</sub>/437</sup> mice were similarly protected against HFD-induced leptin resistance and obesity, overexpression of neuronal SH2B1, rather than other mutations derived from the random insertion of the SH2B1 transgene, enhances leptin sensitivity in SH2B1<sup>Tg<sub>407</sub>/437</sup> mice.

To avoid the complete disruption of a gene by the transgenic insertion, SH2B1<sup>Tg<sub>407</sub>/437</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup> mice were crossed to generate SH2B1<sup>Tg<sub>407</sub>/437</sup> mice heterozygous for both SH2B1<sup>Tg<sub>407</sub>/437</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup> alleles. The expression levels of recombinant SH2B1<sup>α</sup> mice were similar in SH2B1<sup>Tg<sub>407</sub>/437</sup> and SH2B1<sup>Tg<sub>407</sub>/437</sup> or SH2B1<sup>Tg<sub>407</sub>/437</sup> mice but higher in SH2B1<sup>Tg<sub>407</sub>/437</sup> than in heterozygous SH2B1<sup>α</sup> mice (Figure 6A). Importantly, SH2B1<sup>Tg<sub>407</sub>/437</sup> mice were protected against HFD-induced obesity to a similar extent as were SH2B1<sup>Tg<sub>407</sub>/437</sup> mice and SH2B1<sup>Tg<sub>407</sub>/437</sup> mice (Figure 6, B, right panel, and C). Blood leptin levels were also reduced by 97% in SH2B1<sup>Tg<sub>407</sub>/437</sup> mice compared with WT mice (Figure 6D, right panel).

**Figure 4**
Neuron-specific restoration of SH2B1 reverses leptin resistance in SH2B1<sup>KO</sup> mice. (A) Plasma leptin levels in fasted (10 weeks old) and fed ad libitum (13 weeks old) mice. (B) Male mice (9 weeks old) were housed individually and injected intraperitoneally with leptin (2 mg/kg body weight) or PBS (control) twice a day (6:00 pm and 12:00 am). Body weight was monitored both before and after the injection. Changes in body weight were calculated as a percentage of the initial values prior to the injection. (C) Female (right panel): 6 weeks old and male (middle panel): 12 weeks old; right panel: 9 weeks old) mice were fasted for 24 hours and injected intraperitoneally with leptin (1 mg/kg of body weight) or PBS as control. Hypothalamic extracts were prepared 45 minutes after injection and immunoblotted with α-p-STAT3 or α-STAT3. Each lane represents a combination of 2 hypothalami. (D) Hypothalamic RNA was prepared from males (22 weeks old, fasted overnight), NPY, AgRP, and POMC mRNA levels were measured using quantitative real-time PCR and normalized to the expression of β-actin. *P < 0.05.
Neuron-specific restoration of SH2B1 reverses peripheral insulin resistance and glucose intolerance in SH2B1KO mice. SH2B1 binds directly to the insulin receptor, thereby enhancing the activation of the insulin receptor and multiple downstream pathways in cultured cells (44, 52). Systemic deletion of SH2B1 results in severe insulin resistance and type 2 diabetes (44–46). However, it is unclear whether SH2B1 enhances insulin sensitivity directly by promoting insulin signaling in the liver, skeletal muscle, and/or adipose tissue or indirectly by reducing adiposity through its action in the brain.

Insulin sensitivity and glucose metabolism were compared in SH2B1KO mice (which completely lack SH2B1 in all tissues) and SH2B1TgKO mice (which only express SH2B1 in neural tissue). SH2B1KO mice developed severe hyperglycemia and hyperinsulinemia, hallmarks of insulin resistance (Figure 7A). Fasting plasma insulin levels increased by more than 26-fold in SH2B1KO compared with age-matched WT controls. Neuron-specific restoration of SH2B1 corrected both hyperglycemia and hyperinsulinemia in SH2B1TgKO mice (Figure 7A).

To further examine peripheral insulin sensitivity, glucose and insulin tolerance tests (GTTs and ITTs) were performed. In GTTs, mice were fasted overnight and injected intraperitoneally with D-glucose (2 g/kg body weight), and blood glucose levels were measured at various time points after glucose injection. Compared with WT controls, SH2B1KO mice were severely intolerant to exogenous
Neuronal SH2B1 regulates energy and glucose metabolism at least in part by enhancing leptin sensitivity in the brain, particularly in the hypothalamus. Systemic deletion of SH2B1 resulted in severe leptin resistance as demonstrated by marked hyperleptinemia, significantly reduced physiological responses to leptin (e.g., leptin-induced anorexia and inhibition of hypothalamic NPY and AgRP expression), and impaired leptin signaling in the hypothalamus. Leptin resistance precedes the onset of obesity in SH2B1KO mice (45). Leptin regulates energy metabolism and body weight mainly by activating LEPReb in the brain. Neuron-specific restoration of SH2B1 fully rescued not only leptin resistance but also obesity in SH2B1KO mice, which do not express SH2B1 in peripheral tissues (e.g., muscle, adipose tissue, and liver). A modest increase in neuronal SH2B1 expression reduced blood leptin levels by 58% in SH2B1KO mice, which were heterozygous for the SH2B1 transgene, compared with WT littermates fed an HFD. Further increases in neuronal SH2B1 expression reduced blood leptin levels to a much higher extent in SH2B1KO mice (by 98%), SH2B1KO (by 92%), and SH2B1KO (by 97%) mice. After normalization to total fat mass, blood leptin levels were still significantly reduced in SH2B1KO (by 93%), SH2B1KO (by 93%), and SH2B1KO (by 93%) mice. More importantly, SH2B1 in peripheral tissues (e.g., muscle, adipose tissue, and liver) and SH2B1 in neuronal tissues (e.g., the brain) similarly regulate energy and glucose metabolism.

Discussion

The SH2B family contains 3 members (SH2B1, SH2B2, and SH2B3) that contain a conserved PH and SH2 domain. SH2B1 is believed to mediate cell signaling in response to multiple hormones, growth factors, and cytokines, including GH, leptin, insulin, IGF-1, PDGF, FGF, nerve growth factor, brain-derived neurotrophic factor, and glial cell–derived neurotrophic factor (29, 32–42). Systemic deletion of SH2B1 resulted in energy imbalance, morbid obesity, and severe glucose intolerance, suggesting that SH2B1 has a unique function in the regulation of body weight and glucose metabolism, which cannot be compensated for by SH2B2 and SH2B3. SH2B1 is ubiquitously expressed in both neuronal and non-neuronal tissues, including the brain, liver, skeletal muscle, and adipose tissue. All these tissues are involved in the regulation of adiposity and glucose metabolism.

Interestingly, neuron-specific restoration of recombinant SH2B1 in SH2B1KO mice corrected the hyperphagia, obesity, hyperglycemia, and glucose intolerance observed in SH2B1KO mice. Moreover, neuron-specific overexpression of SH2B1 dose-dependently protected against HFD-induced obesity. These observations suggest that endogenous SH2B1 in the brain plays a key role in controlling body weight and glucose homeostasis. Multiple forms of SH2B1 were expressed in the brain; however, neuron-specific restoration of recombinant SH2B1 alone was sufficient to reverse obese and glucose-intolerant phenotypes observed in SH2B1KO mice, suggesting that SH2B1β and other isoforms of SH2B1 in the brain similarly regulate energy and glucose metabolism.

Neuronal SH2B1 regulates energy and glucose metabolism at least in part by enhancing leptin sensitivity in the brain, particularly in the hypothalamus. Systemic deletion of SH2B1 resulted in severe leptin resistance as demonstrated by marked hyperleptinemia, significantly reduced physiological responses to leptin (e.g., leptin-induced anorexia and inhibition of hypothalamic NPY and AgRP expression), and impaired leptin signaling in the hypothalamus. Leptin resistance precedes the onset of obesity in SH2B1KO mice (45). Leptin regulates energy metabolism and body weight mainly by activating LEPReb in the brain. Neuron-specific restoration of SH2B1 fully rescued not only leptin resistance but also obesity in SH2B1KO mice, which do not express SH2B1 in peripheral tissues (e.g., muscle, adipose tissue, and liver). A modest increase in neuronal SH2B1 expression reduced blood leptin levels by 58% in SH2B1KO mice, which were heterozygous for the SH2B1 transgene, compared with WT littermates fed an HFD. Further increases in neuronal SH2B1 expression reduced blood leptin levels to a much higher extent in SH2B1KO mice (by 98%), SH2B1KO (by 92%), and SH2B1KO (by 97%) mice. After normalization to total fat mass, blood leptin levels were still significantly reduced in SH2B1KO (by 93%), SH2B1KO (by 93%), and SH2B1KO (by 93%) mice. More importantly, SH2B1 in peripheral tissues (e.g., muscle, adipose tissue, and liver) and SH2B1 in neuronal tissues (e.g., the brain) similarly regulate energy and glucose metabolism.

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The SH2B family contains 3 members (SH2B1, SH2B2, and SH2B3) that contain a conserved PH and SH2 domain. SH2B1 is believed to mediate cell signaling in response to multiple hormones, growth factors, and cytokines, including GH, leptin, insulin, IGF-1, PDGF, FGF, nerve growth factor, brain-derived neurotrophic factor, and glial cell–derived neurotrophic factor (29, 32–42). Systemic deletion of SH2B1 resulted in energy imbalance, morbid obesity, and severe glucose intolerance, suggesting
Neuronal SH2B1 increases leptin sensitivity at least in part by directly enhancing leptin signaling in LEPRb-expressing neurons in a cell-autonomous manner. SH2B1 directly binds via its SH2 domain to phosphorylated Tyr813 in JAK2, enhancing JAK2 activation in cultured cells (27, 29, 55–57). Deletion of SH2B1 impairs leptin-stimulated activation of hypothyalamic JAK2 in SH2B1KO mice (45). Systemic deletion of SH2B1 markedly reduced leptin-stimulated phosphorylation of hypothyalamic STAT3 (a main substrate of JAK2), which was fully rescued by neuron-specific restoration of SH2B1 in SH2B1TgKO mice. Moreover, SH2B1 binds simultaneously to both JAK2 and IRS2, thereby promoting leptin-stimulated tyrosine phosphorylation of IRS2, presumably by both enhancing JAK2 activation and recruiting IRS2 to JAK2 (58). Disruption of SH2B1 blocks leptin-stimulated tyrosine phosphorylation of hypothyalamic IRS2 (45). IRS2 is an upstream activator of the PI3K pathway, which is required for leptin regulation of energy metabolism (16, 17, 59). Interestingly, PTP1B inhibits leptin signaling by inhibiting JAK2 activation and JAK2-mediated pathways, which is reversed by SH2B1 (27, 45, 56). Therefore, leptin sensitivity appears to be regulated by a balance between positive (e.g., SH2B1) and negative regulators (e.g., PTP1B and SOCS3) in LEPRb-expressing cells. JAK2 is likely to integrate signals from both intracellular positive (e.g., SH2B1) and negative modulators (e.g., PTP1B and SOCS3) in LEPRb-expressing neurons.

Leptin reduces body weight by both decreasing energy intake and increasing energy expenditure. Surprisingly, SH2B1KO mice have a marked increase in both energy intake and expenditure in the presence of severe systemic leptin resistance. Neuron-specific restoration of SH2B1 reversed both leptin resistance and energy imbalance in SH2B1TgKO mice. These results suggest that leptin regulates energy intake and expenditure by 2 distinct pathways in the brain. Consistent with this idea, melanocortin-4 receptor–expressing (MC4R-expressing) neurons in the paraventricular hypothalamus and/or the amygdala, which are directly innervated by hypothalamic LEPRb-neurons, control energy intake, whereas the MC4R-expressing neurons in other areas control energy expenditure (60). Energy intake and expenditure can be controlled by 2 distinct subpopulations of LEPRb-expressing neurons. SH2B1 may cell-autonomously enhance leptin signaling to a much higher degree in LEPRb-neurons controlling energy intake than in LEPRb-neurons controlling energy expenditure. Therefore, systemic deletion of SH2B1 may impair leptin sensitivity more severely in the LEPRb-neurons controlling energy intake than in the LEPRb-neurons controlling energy expenditure. Compensatory hyperleptinemia may not overcome severe leptin resistance in the LEPRb-neurons controlling energy intake, resulting in hyperphagia in SH2B1KO mice. Conversely, hyperleptinemia may be sufficient to overcome mild leptin resistance in the LEPRb-neurons controlling energy expenditure, resulting in increased energy expenditure in SH2B1KO mice. However, neuronal SH2B1 may also regulate energy intake and expenditure by additional leptin-independent mechanisms.

Adipose SH2B1 may also be involved in the regulation of adiposity. SH2B1 expression was upregulated during adipocyte differentiation of 3T3-L1 cells. Deletion of SH2B1 impaired the ability of MEFs to differentiate into adipocytes in vitro. Moreover, SH2B1TgKO mice, which do not express SH2B1 in adipose tissue, had a significant reduction in both fat content and the size of individual white adipocytes. These results suggest that adipose SH2B1 may cell-autonomously regulate adipocyte growth, differentiation, and/or function. SH2B1 binds to the insulin receptor, enhancing insulin signaling (44, 52). Insulin promotes adipogenesis; therefore, adipose SH2B1 may regulate adipogenesis by enhancing insulin signaling. However, disruption of both neuronal and adipose SH2B1 resulted in adipocyte hypertrophy and massive obesity in SH2B1KO mice, suggesting that neuronal SH2B1 play a dominant role in controlling adipocyte differentiation and/or growth in vivo.

SH2B1 was abundantly expressed in peripheral insulin target tissues, including the liver, muscle, and adipose tissues. In cultured cells, SH2B1 binds via its SH2 domain to the insulin receptor, enhancing insulin signaling (32, 33, 35, 40, 44, 52). Systemic deletion of SH2B1 resulted in marked hyperglycemia, hyperinsulinemia, and glucose and insulin intolerance in SH2B1KO mice, as expected. Surprisingly, neuron-specific restoration of SH2B1 fully rescued insulin resistance and glucose intolerance in SH2B1TgKO mice, even though these mice still lack SH2B1 in the liver, muscle, and adipose tissue. These results suggest that central rather than peripheral SH2B1 is essential for regulating systemic insulin sensitivity and glucose homeostasis in mice fed normal chow. However, it is unclear whether peripheral SH2B1 deficiency in the liver, muscle, and adipose tissue exacerbates insulin resistance and glucose intolerance induced by an HFD and/or other cellular stress. The mechanisms of neuronal SH2B1 regulation of systemic insulin sensitivity and glucose metabolism remain largely unknown. First, neuronal SH2B1 negatively regulates adiposity, thus enhancing peripheral insulin sensitivity. Second, central leptin action increases peripheral insulin sensitivity by an adiposity-independent mechanism (61, 62). The PI3K pathway mediates leptin-induced and adiposity-independent enhancement of peripheral insulin sensitivity (62). SH2B1 promotes leptin-stimulated activation of the PI3K pathway (45, 58); therefore, neuronal SH2B1 may promote peripheral insulin sensitivity via a leptin-dependent and adiposity-independent mechanism. Third, central insulin action improves hepatic insulin sensitivity (63–66). Neuronal SH2B1 may promote peripheral insulin sensitivity and glucose metabolism by cell-autonomously enhancing insulin signaling in the hypothalamus.

In summary, systemic deletion of SH2B1 resulted in severe leptin resistance, insulin resistance, morbid obesity, and glucose intolerance in SH2B1KO mice, all of which were largely reversed by neuron-specific restoration of SH2B1 in SH2B1TgKO mice. Neuron-specific overexpression of SH2B1 protected against HFD-induced leptin resistance and obesity in a dose-dependent manner. Therefore, neuronal SH2B1 may serve as a potential target for therapeutic treatment of both obesity and type 2 diabetes.

Methods

Animal experiments. SH2B1-knockout mice (129Sv/C57BL/6 genetic background) were generated by homologous recombination as described previously (44). An SH2B1 transgene construct was prepared by inserting Myc-tagged full-length rat SH2B1 cDNA 5′-prime of an NSE promoter/GH enhancer sequence. The SH2B1 transgene construct was microinjected into F2 mouse oocytes (C57BL/6 × SJL) and surgically transferred to recipients in the University of Michigan Transgenic Animal Model Core to generate heterozygous SH2B1-transgenic (SH2B1Tg) animals. Genotyping was performed by PCR-based assays. Two independent SH2B1Tg lines (407 and 437) were inbred to generate homogygous SH2B1-transgenic animals (SH2B1KO). In parallel experiments, these 2 lines were crossed with SH2B1-knockout mice to generate SH2B1-transgenic and -knockout compound mutants (SH2B1TgKO). Mice were housed on a 12-hour light/12-hour dark cycle in the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan, with free access to water and standard mouse chow (21% kcal from fat) or an HFD (45% kcal
from fat). Animal experiments were conducted following protocols approved by the University Committee on the Use and Care of Animals (UCUCA).

Blood samples were collected from the tail vein and assayed for plasma insulin and leptin using rat insulin or mouse leptin ELISA kits (Crystal Chem Inc.), respectively. FFAs and TGs were measured using Wako NEFAC and Free Glycerol Reagent (Sigma-Aldrich), respectively.

For GTTs, mice were fasted overnight (approximately 16 hours), and t-glucose (2 g/kg of body weight) was injected intraperitoneally. Blood glucose was monitored at 0, 15, 30, 60, and 120 minutes after glucose injection. For ITTs, mice were fasted for 6 hours, and human insulin (1 IU/kg of body weight) was injected intraperitoneally. Blood glucose was monitored at 0, 15, 30, and 60 minutes after insulin injection.

For histological analysis, adipose tissues were isolated and fixed in Bouins’ solution (Sigma-Aldrich). Paraffin sections were prepared and stained with H&E. Images were visualized using a BX51 microscope (Olympus) and captured using a DP70 Digital Camera (Olympus).

To examine leptin inhibition of food intake and weight gain, mice were housed individually with free access to food and water. Mice were injected with leptin (2 mg/kg body weight) or PBS (as control) twice daily (6:00 pm and 12:00 am) for 2 days. Food intake and body weight were monitored both before and after leptin injection.

To measure tissue TG, tissues were homogenized in chloroform/methanol (2:1) and incubated at room temperature for 4 hours. Tissue extracts were air dried, resuspended in KOH (3 M), incubated at 70°C for 1 hour, neutralized with MgCl2, and subjected to a TG assay as described above.

Measurements of energy expenditure. Metabolic rates were measured by indirect calorimetry (Windows Oxymax Equal Flow system; Columbus Instruments).

Mice were housed individually in air-tight respiratory cages through which room air was passed at a flow rate of 0.5 L/min. Exhaust air was sampled at 27-minute intervals for a period of 1 minute; O2 and CO2 content of the exhaust air was determined by comparison with the O2 and CO2 content of standardized sample air. Mice were acclimatized to the cages for 48 hours before measurements were taken. Lean body mass and fat content were determined using the DEXA method (Dexa Sabre Bone Densitometry; Norland). VO2, VCO2, and heat production were normalized to lean body mass.

Quantitative real-time PCR analysis. Mice were fasted for 8 hours (from 9:00 am to 5:00 pm) and sacrificed by decapitation. The hypothalamus was isolated immediately, and total hypothalamic RNA was prepared using TRIzol reagent (Invitrogen). The first-strand cDNAs were synthesized using oligo-dT(12-18) and M-MLV reverse transcriptase (Promega).

NPY, POMC, AgRP, and β-actin mRNA levels were measured using the Brilliant SYBR Green QPCR Kit and Mx3000P Real-Time PCR System (Stratagene). The expression of NPY, AgRP, and POMC was normalized to β-actin mRNA levels. The expression of β-actin primers for real-time RT-PCR were: NPY sense 5′-TCAGACCTCTTAATAGAAGAAAGCA-3′, NPY antisense 5′-GAGACTCAAGTAACTCAAAGA-3′, AgRP sense 5′-GGCTTCAGAAAGACCGTGC-3′, AgRP antisense 5′-GACTCCGTGCAAGCTTACACA-3′; POMC sense 5′-CTGCTTCAGACCTCATGATG-3′, POMC antisense CAGCGAGGCTGATTTGGC; β-actin sense 5′-AACCTTGCTGGAACCATC-3′, β-actin antisense 5′-AAGGAAGCTGGAAGAAGAC-3′.

Adipocyte differentiation. Confluent 3T3-L1 preadipocytes were grown for 2 additional days in DMEM–high glucose supplemented with 8% calf serum at 5% CO2 and 37°C. Cells were then cultured for 3 days in a differentiation medium (DMEM–high glucose supplemented with 10% FCS, 0.1 μM insulin, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine) and 3 days in DMEM–high glucose supplemented with 10% FCS, 0.1 μM insulin. Differentiated adipocytes were maintained in DMEM–high glucose supplemented with 8% fetal calf serum. MEF primary cultures were differentiated similarly, except that 0.1 μM rosiglitazone was added into the differentiation medium.

Adipocytes were washed with PBS, fixed in 10% formalin for 5 minutes, stained in oil red O working solution for 2 hours, and washed extensively with water. Oil red O working solution was prepared by diluting oil red O stock solution (0.5% in isopropanol) with water to a 6:4 ratio. Adipocytes were visualized using a BX51 microscope, and images were captured using a DP70 Digital Camera.

Immunoprecipitation and immunoblotting. Mice were fasted for 24 hours and injected intraperitoneally with leptin (1 mg/kg body weight) or PBS (as control). Forty-five minutes later, mice were sacrificed by decapitation, and the hypothalamus was isolated and homogenized in lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 10 mM Na3PO4, 100 mM NaF, 250 mM sucrose, 1 mM Na3VO4, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.2 mM benzamidine, 2 mM DTT, 1% Nonidet P-40). The same amount of protein in hypothalamic extracts was immunoblotted with anti–phospho-STAT3 (pTyr705) antibody (Santa Cruz Biotechnology Inc.). The same blots were reprobed with anti-STAT3 antibody (Santa Cruz Biotechnology Inc.) to estimate total STAT3 protein.

Statistics. The data are presented as mean ± SEM. Two-tailed Student’s t tests were used for comparisons between 2 groups. P < 0.05 was considered statistically significant.

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