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 SH2B112/12 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 SH2B112/12 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 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 SH2B112/12 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.

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; POMC, proopiomelanocortin; SH2, Src homology 2; TG, triglyceride; TgKO, transgenic/knockout compound mutation.

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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<sup>KO</sup> mice. Tissue extracts (2 mg protein in hypothalamic and 3 mg protein in other tissue extracts) were prepared from WT mice, immunoprecipitated with α-SH2B1, and immunoblotted with α-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 SH2B1β transgene. (C) Brain extracts were prepared from an SH2B1<sup>Tg</sup> mouse and a WT littermate, immunoprecipitated with α-SH2B1, and immunoblotted with α-Myc. (D) Brain extracts were prepared from WT, SH2B1<sup>Tg</sup>, and SH2B1<sup>TgKO-437</sup> mice and immunoblotted with α-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<sup>TgKO-437</sup> female (16 weeks old); immunoprecipitated with α-SH2B1; and immunoblotted with α-Myc. (F) Growth curves for WT, SH2B1<sup>Tg</sup>, SH2B1<sup>KO</sup>, and SH2B1<sup>TgKO-437</sup> 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 mice (21–22 weeks old) fasted overnight. *P < 0.05.
WT mice (Figure 1C). All 8 SH2B1KO lines expressed similar levels of recombinant SH2B1β (data not shown).

Two independent SH2B1β lines (SH2B1βKO and SH2B1βKO-407) were crossed with heterozygous SH2B1 knockout mice to generate compound mutant mice (SH2B1βKO-437 and SH2B2βKO-407) that were heterozygous for the SH2B1β-transgenic allele and homozygous for the SH2B1α allele. The expression of recombinant SH2B1β was similar in SH2B1βKO and SH2B1βKO-407 mice and restricted to neural tissues in both SH2B1βKO and SH2B1βKO-407 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 (SH2B1βKO) mice (Figure 1D). Recombinant SH2B1β was the only form detected in SH2B1βKO 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 SH2B1βKO 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).

Obesity is commonly associated with hyperlipidemia. Systemic deletion of SH2B1 resulted in a marked increase in body weight in both male and female SH2B1αKO mice, and neuron-specific restoration of SH2B1 (to endogenous levels) fully rescued the obese phenotype in SH2B1βKO mice (Figure 1F). Neuron-specific restoration of SH2B1 also markedly reduced body weight in another independent line (SH2B1KO, 32.3 ± 1.6 g, n = 12; SH2B1βKO-407, 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 SH2B1KO 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.

Neuronal and adipose SH2B1 have opposite effects on adiposity. (A) Weight of epidymal (Epi) and inguinal fat depots (Ing) from SH2B1αKO, SH2B1βKO-437, and WT males at age 23–24 weeks. (B) Whole body fat content in SH2B1αKO, SH2B1βKO-437, and WT mice. (C) Representative H&E staining of epididymal fat depots from SH2B1αKO, SH2B1βKO-437, and WT males at age 23 weeks (upper panels) or from SH2B1βKO-437 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 SH2B1αKO MEF primary cultures were subjected to adipocyte differentiation for 10 days. Differentiated cells were stained with oil red O. *P < 0.05.
ly 2.5-fold in female $SH2B1^{KO}$ mice (Figure 2B). Neuron-specific restoration of $SH2B1$ (to endogenous levels) completely reversed the elevation in fat mass in $SH2B1^{TgKO}$ mice (Figure 2, A and B). Histological examination of epididymal fat depots revealed that systemic deletion of $SH2B1$ markedly increased the size of individual adipocytes in $SH2B1^{KO}$ mice; neuron-specific restoration of $SH2B1$ completely reversed adipocyte hypertrophy in $SH2B1^{TgKO}$ mice (Figure 2C, upper panels). These data demonstrated that neuronal $SH2B1$ negatively controls adiposity in animals.

Compared with those in age-matched WT control mice, both total fat mass and the size of individual white adipocytes were significantly reduced in $SH2B1^{TgKO}$ mice, which lacked $SH2B1$ in adipose tissue (Figure 2, A–C). These results suggest that adipocyte $SH2B1$ may be involved in adipocyte growth and/or differentiation. To obtain additional evidence, we examined $SH2B1$ expression during 3T3-L1 adipocyte differentiation. Cell extracts were immunoprecipitated with $\alpha$-SH2B1 and immunoblotted with $\alpha$-SH2B1. 3T3-L1 adipocytes expressed low levels of endogenous $SH2B1$. $SH2B1$ expression increased progressively during adipocyte differentiation, reaching maximal levels within 6 days after the induction of differentiation (Figure 2D). The effect of $SH2B1$ deficiency on adipocyte differentiation was examined using mouse embryonic fibroblasts (MEFs). Both WT and $SH2B1^{TgKO}$ MEF primary cultures were subjected to an in vitro adipocyte differentiation treatment and then stained with oil red O to identify differentiated adipocytes. Deletion of $SH2B1$ impaired the ability of MEFs to differentiate into adipocytes (Figure 2E). These results suggest that adipocyte $SH2B1$ is involved in adipogenesis in a cell-autonomous fashion. However, neuronal $SH2B1$ may play a dominant role over adipocyte $SH2B1$ in controlling adiposity in vivo; therefore, deletion of $SH2B1$ in both the brain and adipose tissue results in adipocyte hypertrophy and obesity in $SH2B1^{KO}$ mice.

**Neuron-specific restoration of $SH2B1$ reverses energy imbalance in $SH2B1^{KO}$ mice.** Systemic deletion of $SH2B1$ resulted in hyperphagia, markedly increasing food intake in $SH2B1^{KO}$ mice (Figure 3A). $SH2B1^{KO}$ mice also had significantly elevated energy expenditure, as revealed by significantly increased $O_2$ consumption and $CO_2$ production (Figure 3B). A previous report showed that energy intake still exceeds energy expenditure in this setting, resulting in obesity in $SH2B1^{KO}$ mice (45). Neuron-specific restoration of $SH2B1$ largely corrected hyperphagia and markedly reduced energy expenditure in $SH2B1^{TgKO}$ mice (Figure 3, A and B). 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 $SH2B1^{KO}$ mice.** Systemic deletion of $SH2B1$ dramatically increased plasma leptin levels (hyperleptinemia) in both fasted and fed $SH2B1^{KO}$ mice, a hallmark of leptin resistance (Figure 4A). Neuron-specific restoration of $SH2B1$ expression completely reversed hyperleptinemia in $SH2B1^{TgKO}$ 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 $SH2B1^{KO}$ 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 $SH2B1^{TgKO}$ mice (Figure 4B). These data indicate that neuron-specific restoration of $SH2B1$ (to endogenous levels) is sufficient to rescue leptin resistance in $SH2B1^{KO}$ 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 $SH2B1^{TgKO}$ 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 $\alpha$-melanocyte-stimulating hormone ($\alpha$-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 $\beta$-actin. Systemic deletion of $SH2B1$ markedly increased NPY and AgRP but not POMC expression in $SH2B1^{KO}$ mice (Figure 4D). Neuron-specific restoration of $SH2B1$ dramatically reduced NPY expression in $SH2B1^{TgKO}$ mice to levels similar to those in WT controls (Figure 4D). AgRP expression was also significantly reduced in $SH2B1^{TgKO}$ mice, but to a lesser extent (Figure 4D). Neuronal $SH2B1$ may inhibit the expression of orexigenic NPY and AgRP presumably by enhancing leptin signaling in the hypothalamus.

**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\textsuperscript{f/f} and WT littermates were fed an HFD. Body weight and fat content were similar in SH2B1\textsuperscript{f/f} and WT littermates (Figure 5, A and B). However, blood leptin levels were significantly reduced, by 58%, in SH2B1\textsuperscript{f/f} mice, suggesting an increase in leptin sensitivity in SH2B1\textsuperscript{f/f} 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\textsuperscript{f/f} mice.

To increase neuronal SH2B1 expression, SH2B1\textsuperscript{f/f} mice were bred to generate homozygous SH2B1-transgenic mice (SH2B1\textsuperscript{Tg/Tg}). Two independent SH2B1\textsuperscript{Tg/Tg} lines (SH2B1\textsuperscript{Tg/Tg}-407 and SH2B1\textsuperscript{Tg/Tg}-437) 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 SH2B1β. The expression of the SH2B1β transgene was significantly higher in SH2B1\textsuperscript{Tg/Tg} than in SH2B1\textsuperscript{f/f} and SH2B1\textsuperscript{Tg/KO} mice (heterozygous for the SH2B1β transgene) (Figure 6A). Neuron-specific overexpression of SH2B1 did not have an obviously deleterious effect on the overall health of SH2B1\textsuperscript{Tg/Tg} 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\textsuperscript{Tg/Tg} and SH2B1\textsuperscript{Tg/KO} compared with WT control mice fed normal chow (Figure 6B, left panel). Fasting plasma leptin levels were significantly lower in SH2B1\textsuperscript{Tg/Tg} 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\textsuperscript{Tg/Tg} and SH2B1\textsuperscript{Tg/KO} 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\textsuperscript{Tg/Tg} mice. Neuronal overexpression of SH2B1 reduced blood leptin levels by 98% in SH2B1\textsuperscript{Tg/Tg} mice compared with WT control mice (Figure 6D, right panel). Since the 2 lines of SH2B1\textsuperscript{Tg/Tg} 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\textsuperscript{Tg/Tg} mice.

To avoid the complete disruption of a gene by the transgenic insertion, SH2B1\textsuperscript{Tg/Tg} and SH2B1\textsuperscript{Tg/KO} mice were crossed to generate SH2B1\textsuperscript{Tg/Tg} mice heterozygous for both SH2B1\textsuperscript{Tg/Tg} and SH2B1\textsuperscript{Tg/KO} alleles. The expression levels of recombinant SH2B1β were similar in SH2B1\textsuperscript{Tg/Tg} and SH2B1\textsuperscript{Tg/KO} or SH2B1\textsuperscript{Tg/Tg} mice but higher in SH2B1\textsuperscript{Tg/Tg} than in heterozygous SH2B1\textsuperscript{Tg/KO} mice (Figure 6A). Importantly, SH2B1\textsuperscript{Tg/Tg} mice were protected against HFD-induced obesity to a similar extent as were SH2B1\textsuperscript{Tg/Tg} and SH2B1\textsuperscript{Tg/KO} mice (Figure 6, B, right panel, and C). Blood leptin levels were also reduced by 97% in SH2B1\textsuperscript{Tg/Tg} mice compared with WT mice (Figure 6D, right panel).
Neuron-specific restoration of SH2B1 reverses peripheral insulin resistance and glucose intolerance in SH2B1\textsuperscript{KO} 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 SH2B1\textsuperscript{KO} mice (which completely lack SH2B1 in all tissues) and SH2B1\textsuperscript{TgKO} mice (which only express SH2B1 in neural tissue). SH2B1\textsuperscript{KO} mice developed severe hyperglycemia and hyperinsulinemia, hallmarks of insulin resistance (Figure 7A). Fasting plasma insulin levels increased by more than 26-fold in SH2B1\textsuperscript{KO} compared with age-matched WT controls. Neuron-specific restoration of SH2B1 corrected both hyperglycemia and hyperinsulinemia in SH2B1\textsuperscript{TgKO} 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 \textdelta-glucose (2 g/kg body weight), and blood glucose levels were measured at various time points after glucose injection. Compared with WT controls, SH2B1\textsuperscript{KO} mice were severely intolerant to exogenous...
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 SH2BIKO 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 SH2BIKO mice, suggesting that SH2B1β is an important isoform in the brain that regulates 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 SH2BIKO mice (45). Leptin regulates energy metabolism and body weight mainly by activating LEPRb in the brain. Neuron-specific restoration of SH2B1 fully rescued not only leptin resistance but also obesity in SH2BIKO 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 SH2BIKO 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 SH2BIKO mice (by 98%), SH2BIKO (by 92%), and SH2BIKO (by 97%) mice. After normalization to total fat mass, blood leptin levels were still significantly reduced in SH2BIKO (by 93%), SH2BIKO (by 93%), and SH2BIKO (by 92%) mice. Therefore, neuronal SH2B1 improves leptin sensitivity in a dose-dependent manner. More importantly, SH2BIKO, SH2BIKO, and SH2BIKO mice were protected against HFD-induced obesity, supporting the idea that neuronal SH2B1 may negatively regulate adiposity at least in part by enhancing leptin sensitivity. However, these studies do not exclude the possibility that neuronal SH2B1 may regulate energy and glucose metabolism by additional leptin-independent mechanisms. SH2B1 directly enhances insulin signaling (44, 52). Insulin regulates energy metabolism and body weight by activating its receptor in the brain (53, 54). SH2B1 may also regulate energy and glucose metabolism by enhancing insulin sensitivity in the brain.

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
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 hypothalamic JAK2 in SH2B1KO mice (45). Systemic deletion of SH2B1 markedly reduced leptin-stimulated phosphorylation of hypothalamic STAT3 (a main substrate of JAK2), which was fully rescued by neuron-specific restoration of SH2B1 in SH2B1KO 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 hypothalamic 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 SH2B1KO 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, SH2B1KO 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 SH2B1KO 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 SH2B1KO 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 3′-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 homozygous 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

Mice were fasted for 24 hours and 10% FCS, 0.1 mM 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 mM 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 Na3P2O7, 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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