PI3K signalling in the ventromedial hypothalamic nucleus is required for normal energy homeostasis

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Summary

Phosphatidyl inositol 3-kinase (PI3K) signalling in the hypothalamus has been implicated in the regulation of energy homeostasis, but the critical brain sites where this intracellular signal integrates various metabolic cues to regulate food intake and energy expenditure are unknown. Here we show that mice with reduced PI3K activity in the ventromedial hypothalamic nucleus (VMH) are more sensitive to high fat diet-induced obesity due to reduced energy expenditure. In addition, inhibition of PI3K in the VMH impaired the ability to alter energy expenditure in response to acute high fat diet feeding and food deprivation. Furthermore, the acute anorexigenic effects induced by exogenous leptin were blunted in the mutant mice. Collectively, our results indicate that PI3K activity in VMH neurons plays a physiologically relevant role in the regulation of energy expenditure.

showed that lesions of the VMH produce obesity due to both increased food intake and decreased energy expenditure (see (King, 2006)). However, many of these results were questioned because the electrolytic insults and knife cuts used to lesion the VMH likely damaged the surrounding regions (such as the arcuate nucleus) as well as neuronal fibers passing through the VMH (Gold, 1973). Recent mouse genetic studies have begun to circumvent these issues by performing deletion of key genes specifically in VMH neurons. These studies were based on the finding that a transcription factor, steroidogenic factor-1 (SF1), is expressed exclusively in the VMH neurons within the brain (Ikeda et al., 1995). Deletion of SF1 in mice disrupts VMH structure (Dellovade et al., 2000) and leads to obesity (Majdic et al., 2002). These findings supported the model that VMH neurons are physiological regulators of body weight homeostasis.

Multiple metabolic signals have been demonstrated to regulate energy homeostasis via actions in the VMH. For example, leptin directly activates SF1 neurons in the VMH and selective deletion of leptin receptors from SF1 neurons produces obesity (Bingham et al., 2008; Dhillon et al., 2006). Estrogen acts on the estrogen receptor-α (ERα) expressed by VMH neurons to regulate energy expenditure as animals with ERα knocked down in the VMH develop obesity due to reduced energy expenditure (Musatov et al., 2007). Likewise, knock-down of brain-derived neurotrophic factor (BDNF) in the VMH and dorsomedial hypothalamic nucleus produces hyperphagic obesity (Unger et al., 2007).

Interestingly, all of the aforementioned hormonal and neural signals have been shown to activate the phosphatidyl inositol 3-kinase (PI3K) signalling pathway in neurons. For example, leptin activates the PI3K pathway in the hypothalamus (Niswender et al., 2001; Zhao et al., 2002), and pharmacological evidence indicates that leptin's effects on feeding (Zhao et al., 2002) and lipolysis (Buettner et al., 2008) require intact PI3K activity in the hypothalamus. Similarly, estrogen regulates expression of PI3K subunits in the hypothalamus including the VMH and the PI3K/Akt cascade mediates estrogen's actions in hypothalamic neurons (Malyala et al., 2008). Furthermore, BDNF activates the PI3K pathway in neurons to promote synaptic formation (Yoshii and Constantine-Paton, 2007). Therefore, PI3K signalling in VMH neurons may be a common pathway that integrates metabolic cues to provide a coordinated control of energy homeostasis. In the present study, we generated a mouse model with reduced PI3K signalling specifically in the VMH to assess the physiological relevance of PI3K in VMH neurons in the regulation of energy balance.

Results and Discussions

Generation of mice lacking PI3K in VMH neurons

PI3K consists of an 85kDa regulatory subunit (p85) and a 110kDa catalytic subunit (p110) (Cantley, 2002). As the primary insulin responsive isoform of PI3K, p110β is selectively activated by the insulin receptor substrate (IRS) signaling complex and is required for IRS-associated PI3K activity in the hypothalamus (Foukas et al., 2006; Knight et al., 2006). Thus, we crossed mice carrying loxP flanked p110β alleles ($p110\beta^{lox/lox}$ mice) (Zhao et al., 2006) with transgenic SF1-Cre mice which express Cre-recombinase driven by SF1 regulatory elements (Dhillon et al., 2006). These crosses produced mice lacking p110β in SF1 neurons ($p110\beta^{lox/lox}$/SF1-Cre mice) and mice bearing $p110\beta^{lox/lox}$ alleles alone. The latter group of littermates served as controls in all experiments.

We first validated the selective deletion of p110β in SF1 cells in $p110\beta^{lox/lox}$/SF1-Cre mice. We found that the p110β alleles were deleted from the genome in tissues that express SF1, including the hypothalamus, pituitary, adrenal gland and testis (Zhao et al., 2001), whereas the p110β allele remained intact in tissues that do not express SF1 (e.g. the cortex and

brainstem) (Supple Fig. 1a). Further, we found that p110β RNA was significantly reduced in the VMH from p110β$^{lox/lox}$/SF1-Cre mice, while expression of p110α, another p110 isoform present in the hypothalamus (Cantley, 2002), was not changed (Supple Fig. 1b). Finally, we crossed a FoxO1GFP reporter allele (Fukuda et al., 2008) to the mice lacking p110β in SF-1 neurons. This allowed assessment of PI3K activity specifically in SF1 neurons by monitoring FoxO1GFP translocation between the nucleus and cytoplasm (Fukuda et al., 2008). We found that while FoxO1GFP was localizifically in SF1

energy required for physical activities, basal metabolism, and thermogenesis evoked by stimuli such as food intake (Butler and Kozak, 2010; Castaneda et al., 2005). Since neither ambulatory movements nor rearing activities were significantly changed in p110$\gamma^{lox/lox}$/SF1-Cre mice (Figs. 2f and 2g), we conclude that the decreases in energy expenditure observed in p110$\gamma^{lox/lox}$/SF1-Cre mice are due to reduced basal metabolic rate and/or diet-induced

in the regulation of energy homeostasis. However, the critical brain sites where the PI3K pathway mediates leptin signal to regulate energy homeostasis have not been fully explored. We found that in p110$^{\check{z}lox/lox}$ control mice, intracerebroventricular (i.c.v.) injections of leptin significantly inhibited food intake by decreasing both meal size and meal frequency over a 24 hr period (Figs. 4a–4c). In contrast, in p110$^{\check{z}lox/lox}$/SF1-Cre mice leptin-induced inhibition of food intake was significantly blunted (Fig. 4a). Leptin-induced reduction in meal size treaded to be blunted (Fig. 4b), while effects of leptin on meal frequency remained unchanged (Fig. 4c). Administration of leptin also promoted fat oxidation in control mice, demonstrated by decreased respiratory exchange rate (RER), while this effect was significantly blunted in p110$^{\check{z}lox/lox}$/SF1-Cre mice (Fig. 4d). Body weight of p110$^{\check{z}lox/lox}$ mice was significantly reduced 24 hr after leptin injections, whereas acute leptin administration did not reduce body weight in p110$^{\check{z}lox/lox}$/SF1-Cre mice (Fig. 4e). These results indicate that PI3K signalling in VMH neurons is required for mediating acute effects of exogenous leptin on energy homeostasis.

We have identified PI3K-p110$\check{z}$ as a mediator downstream of leptin receptor activation in SF1 neurons. However, it is notable that we observed differences between mice lacking PI3K in SF1 neurons compared to mice lacking leptin receptors in these neurons. For instance, p110$^{\check{z}lox/lox}$/SF1-Cre mice do not show hyperphagia, although the acute appetite-suppressing responses to exogenous leptin at a pharmacological dose are blunted in these mice, suggesting that other signaling pathways may exist, which compensate for impaired PI3K signaling under resting conditions. While mice lacking leptin receptors in SF1 neurons develop obesity on both regular chow and HFD (Bingham et al., 2008; Dhillon et al., 2006), p110$^{\check{z}lox/lox}$/SF1-Cre mice show increased sensitivity to diet-induced obesity but maintain normal body weight when fed with regular chow. Moreover, SF1-specific deletion of leptin receptors causes insulin resistance before onset of obesity (Bingham et al., 2008), whereas p110$^{\check{z}lox/lox}$/SF1-Cre mice do not show deficits in glucose homeostasis (Supple Fig. 2). Therefore, it is likely that leptin actions in SF1 neurons are also partly mediated by other signalling pathways, such as the Jak-Stat3 or ERK pathways (Robertson et al., 2008). Indeed, SF1-specific deletion of suppressor of cytokine signaling-3 (Socs-3), a potent feedback inhibitor of the leptin-induced Jak-Stat3 pathway (Bjorbaek et al., 1998; Howard et al., 2004), leads to improved glucose homeostasis (Zhang et al., 2008), supporting the hypothesis that the Jak-Stat3 pathway may mediate leptin actions in the SF1 neurons to control glycemic balance. In summary, our results indicate that PI3K activity in VMH neurons plays a physiologically relevant role in the regulation of energy expenditure which may play a key role in the physiological response to excess intake of calories. Moreover, it will be interesting to assess whether this response is altered during the development of obesity.

Experimental Procedures

Animal Care

Care of all animals and procedures were approved by the UT Southwestern Medical Center. Mice were housed in a temperature-controlled environment in groups of two to four at 22°C–24°C using a 12 hr light/12 hr dark cycle. The mice were fed either standard chow (4% fat, #7001, Harlan-Teklad, Madison, WI) or HFD (42% fat, #88137, Harlan Teklad)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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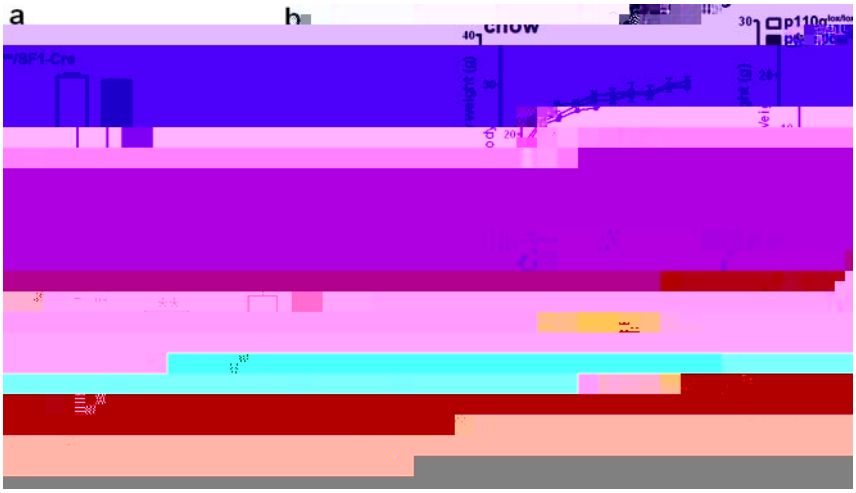

Fig. 1.

Deletion of p110 in SF1 neurons increases sensitivity to diet-induced obesity. (a) Weekly body weight was measured in group housed male mice weaned on regular chow (n=12/genotype). (b) Body composition was measured in 15-week old male mice fed with regular chow (n=12/genotype). (c) Weekly body weight was measured in group housed male mice weaned on HFD (n=16 or 23/genotype). (d) Body composition was measured in 18-week old male mice fed with HFD (n=10/genotype). (e) Serum leptin levels were measured in 7-month old male mice at both fed and fasted conditions (n=6/genotype). Data are presented as mean ± SEM, and *P<0.05 and **P<0.01 between $p110^{\gamma lox/lox}$/SF1-Cremice and $p110^{\gamma lox/lox}$ mice.

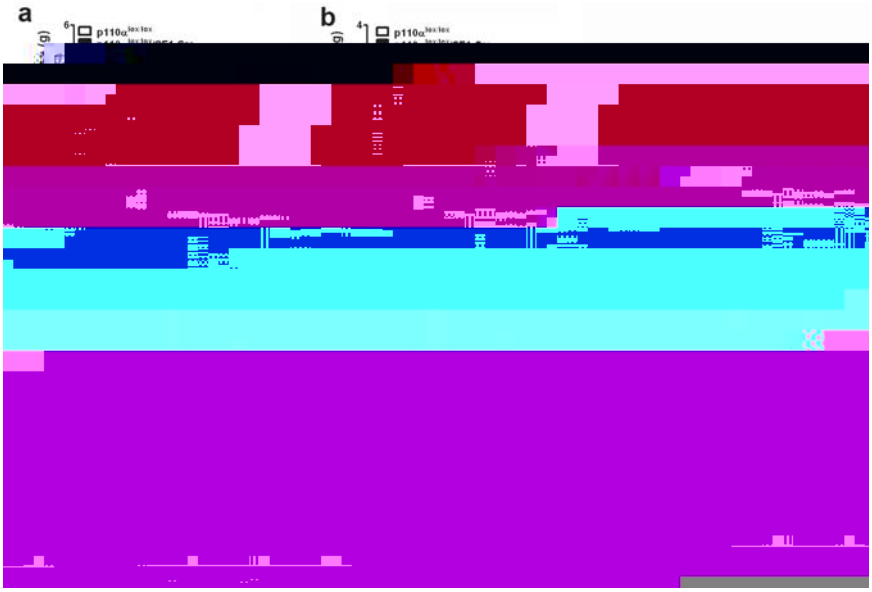

Fig. 2.

Deletion of p110α in SF1 neurons reduces energy expenditure. (a–b) Daily food intake was measured in 7-week old male mice with comparable body weight fed with regular chow (a) or with HFD (b) (n=11–15/genotype). (c–g) Seven-week old chow-fed male mice (n=11 or 16/genotype) were fed with HFD for 2 weeks and matched for body weight (p110α$^{lox/lox}$: 23.8±0.7 g vs p110α$^{lox/lox}$/SF1-Cre 24.9±0.7 g, P=0.31), followed by metabolic analyses using the TSE metabolic chambers. Data are presented as mean ± SEM. * P<0.05 and ** P<0.01 between p110α$^{lox/lox}$/SF1-Cre mice and p110α$^{lox/lox}$ mice.

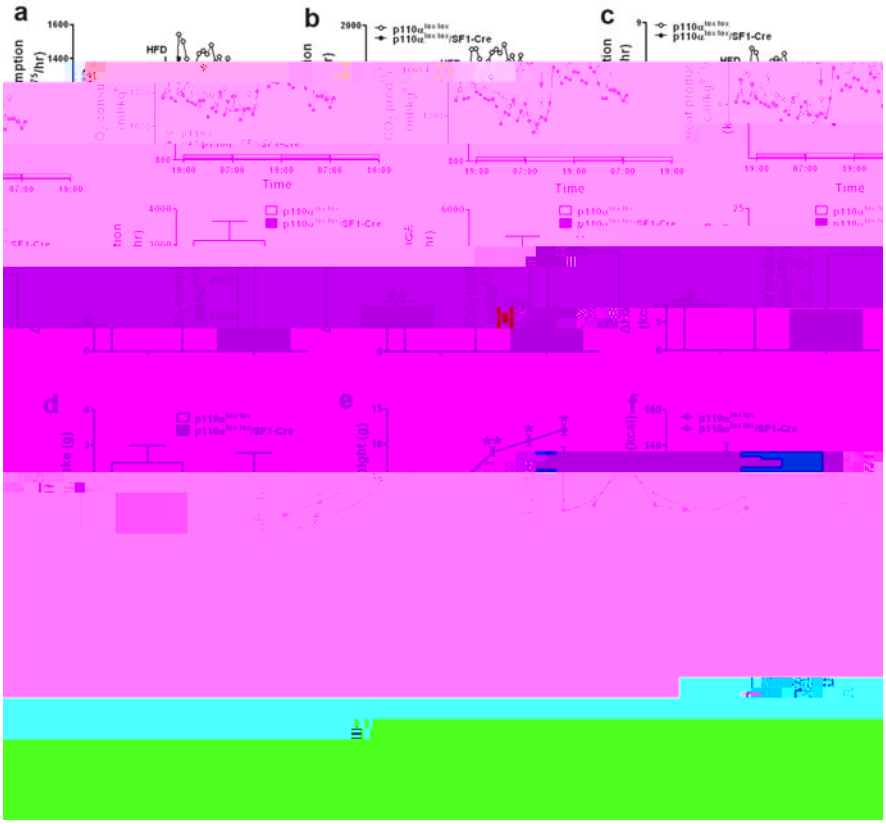

Fig. 3.

Deletion of p110α in SF1 neurons disrupts the thermogenic regulation in response to HFD feeding and fasting. (a–d) Six-month old chow-fed male mice (n=6/genotype) were matched for body weight ($p110\alpha^{lox/lox}$: 37.7±1.4 g vs $p110\alpha^{lox/lox}$/SF1-Cre 37.4±1.0 g, *P*=0.90) and adapted to the TSE metabolic chambers. The mice were provided with HFD at 17:00 (2 hr prior to dark cycle), and metabolic parameters were monitored from 24 hr before the HFD feeding till 24 hr afterwards using the TSE metabolic chambers. Upper panels: temporal levels of O_2 consumption (a), CO_2 production (b) and heat production (c). The arrow indicates the beginning of HFD feeding. Lower panels: changes in O_2 consumption (a), CO_2 production (b) and heat production (c) between the 12-hr dark cycle before HFD feeding and the 12-hr dark cycle afterwards in $p110\alpha^{lox/lox}$/SF1-Cre and $p110\alpha^{lox/lox}$ mice. (d) HFD intake during the 12-hr dark cycle in the TSE chambers. (e–f) The mice were maintained on HFD for 6 weeks and weekly body weight gain (e) and energy intake (f) were recorded. (g–h) Five-month old chow-fed male mice (n=6/genotype) were matched for body weight ($p110\alpha^{lox/lox}$: 31.5±0.6 g vs $p110\alpha^{lox/lox}$/SF1-Cre 32.4±1.1 g, *P*=0.51) and fasted for 24 hr. The body weight loss was measured (g) and heat production was recorded using the TSE chambers (h). (i) Fourteen-week old chow-fed mice were fed with HFD for 3 weeks (mean body weight: $p110\alpha^{lox/lox}$: 33.1±3.4 g vs $p110\alpha^{lox/lox}$/SF1-Cre 38.8±2.6 g, *P*=0.26), and BAT were collected after euthanasia. Messenger RNA levels of indicated BAT genes were quantified with real-time PCR (n=6 or 7/genotype). Data are presented as mean ± SEM, and * P<0.05 and ** P<0.01 between $p110\alpha^{lox/lox}$/SF1-Cre mice and $p110\alpha^{lox/lox}$ mice.

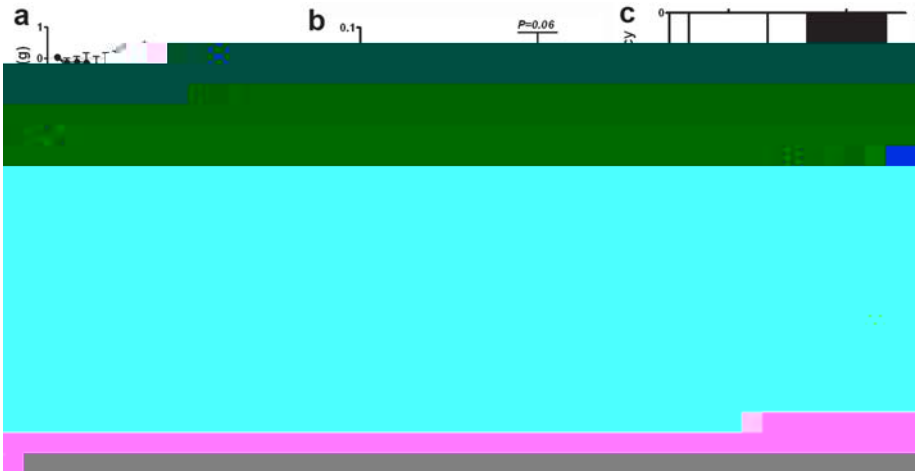

Fig. 4.

Deletion of p110ŭ in SF1 neurons blunts the anorexigenic effects of central leptin. Five-month old chow-fed male mice (n=4 or 6/genotype) were matched for body weight, and received saline (1εl, i.c.v.) at 16:00 followed by leptin (6εg in 1εl saline, i.c.v.) 24 hr later. Leptin-induced reductions in food intake (a), meal size (b), meal frequency (c) and RER (d) were monitored using the TSE metabolic chambers. (e) Leptin-induced weight loss was measured. Data are presented as mean ± SEM, and *P<0.05 and **P<0.01 between p110ŭ$^{lox/lox}$/SF1-Cremice and p110ŭ$^{lox/lox}$ mice.