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MOLECULAR METABOLISM

Leptin and insulin engage specific PI3K subunits in hypothalamic SF1 neurons

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ABSTRACT

Objective: The ventromedial hypothalamic nucleus (VMH) regulates energy balance and glucose homeostasis. Leptin and insulin exert metabolic effects via their cognate receptors expressed by the steroidogenic factor 1 (SF1) neurons within the VMH. However, detailed cellular mechanisms involved in the regulation of these neurons by leptin and insulin remain to be identified.
Methods: We utilized genetically-modified mouse models and performed patch-clamp electrophysiology experiments to resolve this issue.
Results: We identified distinct populations of leptin-activated and leptin-inhibited SF1 neurons. In contrast, insulin uniformly inhibited SF1 neurons. Notably, we found that leptin-activated, leptin-inhibited, and insulin-inhibited SF1 neurons are distinct subpopulations within the VMH. Leptin depolarization of SF1 neuron also required the PI3K p110β catalytic subunit. This effect was mediated by the putative transient receptor potential C (TRPC) channel. On the other hand, hyperpolarizing responses of SF1 neurons by leptin and insulin required either of the p110α or p110β catalytic subunits, and were mediated by the putative ATP-sensitive K^+ (K_{ATP}) channel.
Conclusions: Our results demonstrate that specific PI3K catalytic subunits are responsible for the acute effects of leptin and insulin on VMH SF1 neurons, and provide insights into the cellular mechanisms of leptin and insulin action on VMH SF1 neurons that regulate energy balance and glucose homeostasis.

Keywords Cellular mechanism; Conditional knockout mouse; Patch clamp technique; Functional heterogeneity; Homeostasis

1. INTRODUCTION

The ventromedial nucleus of hypothalamus (VMH) has long been recognized as a brain site that regulates energy balance [1]. The expression of nuclear receptor, steroidogenic factor 1 (SF1), is highly restricted to the VMH and is critical for its development [2]. Notably, SF1-expressing neurons within VMH (VMH SF1 neurons) regulate energy balance and glucose homeostasis [3,4].

SF1 neurons express both leptin receptors (LepRs) and insulin receptors (InsRs). Deficiency of LepRs or InsRs selectively in SF1 neurons results in opposite body weight phenotypes. In particular, SF1-specific LepR deficiency results in obesity when challenged with high fat diet (HFD) [5,6]. Inversely, loss of InsR in SF1 neurons protects against HFD-induced obesity [7]. These results are somewhat paradoxical as both receptors are known to activate phosphatidylinositol-3-kinase (PI3K)-dependent intracellular signaling pathways [8–10]. These observations may be explained in part by the findings that leptin acutely activates (or depolarizes) while insulin acutely inhibits (or hyperpolarizes) SF1 neurons [5,7]. Moreover, we cannot exclude the role of PI3K-dependent regulation of genomic pathways (e.g. FOXO1 signaling), which are similarly activated by leptin and insulin and may also play a determining role in chronic metabolic phenotypes [11]. Notably, the acute (and possibly chronic) effects of leptin and insulin are segregated to distinct subpopulations of VMH SF1 neurons [7], supporting functionally heterogeneous SF1 neurons, which independently may contribute to different facets of metabolism. However, detailed cellular mechanisms that underlie the distinct actions of leptin and insulin on VMH SF1 neurons still remain to be identified.

PI3K functions as heterodimers that consist of the 110 kDa catalytic subunits (p110α and p110β) and the 85 kDa regulatory subunits (p85α and p85β) [12]. Deletion of the regulatory PI3K subunits (p85α and p85β) in the arcuate pro-opiomelanocortin (POMC) neurons blunts the acute effects of both leptin and insulin [13]. Moreover, p110β isoform has a dominant role over p110α in mediating the acute effects of leptin and insulin in POMC neurons as well as in maintaining energy balance [14]. However, contribution of specific PI3K catalytic subunit

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isoforms to the acute effects of leptin and insulin on VMH SF1 neurons remains undefined. In this study, we characterized the acute effects of leptin and insulin on VMH SF1 neurons and identified the role of specific PI3K catalytic subunit isoforms (p110α and p110β) in this response.

2. METHODS

2.1. Mice

Male (4–16 weeks old) pathogen-free SF1-cre mice [5] were crossed with either the Z/EG (GFP) reporter mice (Jackson Laboratory, # 003920) or the tdTomato reporter mice (Jackson Laboratory, #007908) to identify VMH SF1 neurons. For some experiments, SF1-cre::GFP or SF1-cre::tdTomato mice were crossed with either $p110\alpha^{flox/flox}$ mice [15] or $p110\beta^{flox/flox}$ mice [16] to delete p110α or p110β specifically in SF1 neurons. All mice used in this study were housed in a light–dark (12 h on/off; lights on at 7:00 A.M.) and temperature-controlled environment with food and water available *ad libitum* in the University of Texas Southwestern Medical Center and Korea Advanced Institute of Science and Technology (KAIST) facilities. All experiments were performed in accordance with the guidelines established by the National Institute of Health Guide for the Care and Use of Laboratory Animals, as well as with those established by the University of Texas and KAIST Institutional Animal Care and Use Committee.

2.2. Electrophysiology

Whole-cell patch-clamp recordings from SF1 neurons maintained in hypothalamic slice preparations and data analysis were performed as previously described [13,17]. Briefly, 4- to 16-week-old male mice were deeply anesthetized with i.p. injection of 7% chloral hydrate or isoflurane inhalations and transcardially perfused with a modified ice-cold artificial CSF (ACSF) (described below), in which an equiosmolar amount of sucrose was substituted for NaCl. The mice were then decapitated, and the entire brain was removed and immediately submerged in ice-cold, carbogen-saturated (95% O_2 and 5% CO_2) ACSF (126 mM NaCl, 2.8 mM KCl, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 5 mM glucose). A brain block containing the hypothalamus was made. Coronal sections (250 μm) were cut with a Leica VT1000S or VT1200S Vibratome and then incubated in oxygenated ACSF at room temperature for at least 1 h before recording. Slices were transferred to the recording chamber and allowed to equilibrate for 10–20 min before recording. The slices were bathed in oxygenated ACSF (32°C–34 °C) at a flow rate of ~2 ml/min. The pipette solution for whole-cell recording was modified to include an intracellular dye (Alexa Fluor 594 or Alexa Fluor 488) for whole-cell recording: 120 mM K-gluconate, 10 mM KCl, 10 mM HEPES, 5 mM EGTA, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 2 mM MgATP, 0.03 mM Alexa Fluor 594 or Alexa Fluor 488 hydrazide dye, pH 7.3. Epifluorescence was briefly used to target fluorescent cells, at which time the light source was switched to infrared differential interference contrast imaging to obtain the whole cell recording (Zeiss Axioskop FS2 Plus equipped with a fixed stage and a QuantEM:512SC electron-multiplying charge-coupled device camera or Nikon Eclipse FN1 equipped with a fixed stage and an optiMOS scientific CMOS camera). Electrophysiological signals were recorded using an Axopatch 700B amplifier (Molecular Devices), low-pass filtered at 2–5 kHz, and analyzed offline on a PC with pCLAMP programs (Molecular Devices). Recording electrodes had resistances of 2.5–5 MΩ when filled with the K-gluconate internal solution. Input resistance was assessed by measuring voltage deflection at the end of the response to a

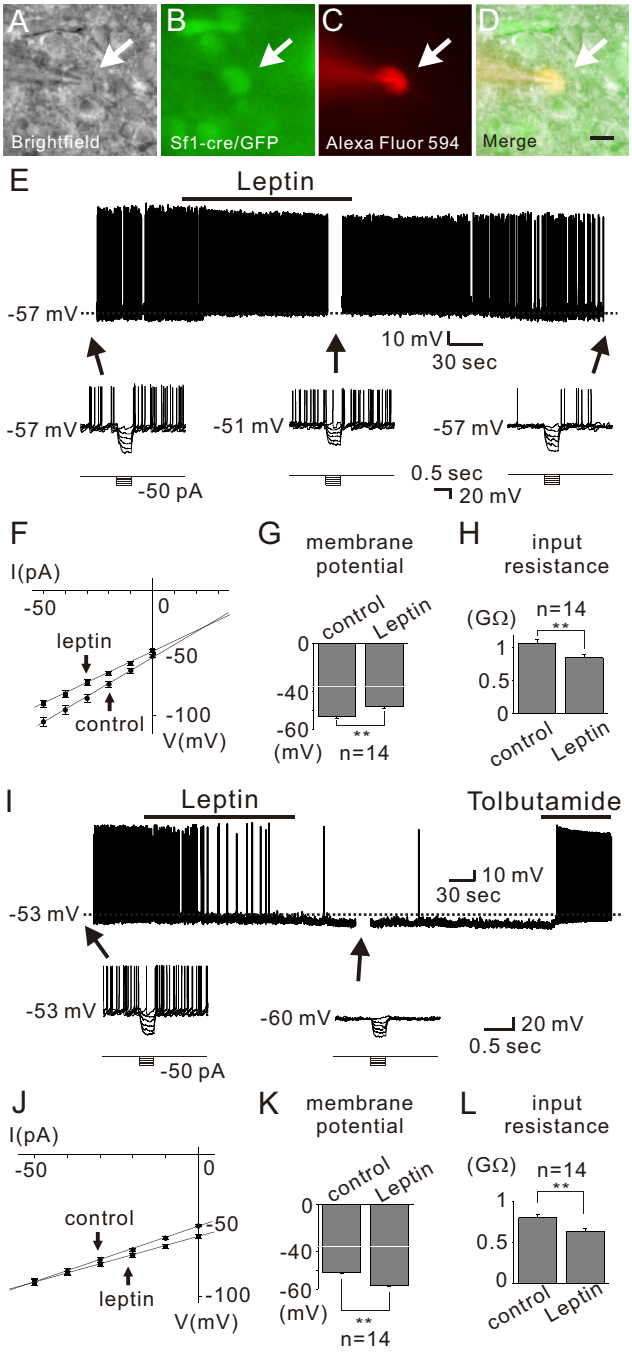

Figure 1: VMH SF1 neurons are either depolarized or hyperpolarized by leptin. (A–D) Brightfield illumination (A), fluorescent (FITC) illumination (B), fluorescent (TRITC) illumination, and merged image of targeted VMH SF1 neuron (D) Arrows indicate the targeted cell. Scale bar = 10 μm. (E) Image demonstrates a leptin-induced depolarization of VMH SF1 neurons (upper panel). Dashed line indicates the resting membrane potential. Lower panel demonstrates voltage responses to hyperpolarizing current steps applied before, during, and after leptin application. (F) IV relationship demonstrates leptin-induced decrease in input resistance. (G–H) Effects of leptin on membrane potential and input resistance. Results are shown as mean ± SEM. ** indicates $p < 0.01$. (I) Image demonstrates a leptin-induced hyperpolarization of VMH SF1 neurons (upper panel). Lower panel demonstrates voltage responses to hyperpolarizing current steps in the course of recording at time points indicated with arrows. (J) IV relationship demonstrates leptin-induced decrease in input resistance. (K–L) Effects of leptin on membrane potential and input resistance. Results are shown as mean ± SEM. ** indicates $p < 0.01$.

