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Hypothalamic peroxisome proliferator-activated receptor gamma regulates ghrelin production and food intake

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ABSTRACT

Peroxisome proliferator-activated receptor-γ (PPARγ) regulates fatty acid storage, glucose metabolism, and food intake. Ghrelin, a gastric hormone, provides a hunger signal to the central nervous system to stimulate appetite. However, the effects of PPARγ on ghrelin production are still unclear. In the present study, the effects of PPARγ on ghrelin production were examined in lean- or high-fat diet-induced obese (DIO) C57BL/6J mice and mHypoE-42 cells, a hypothalamic cell line. 3rd intracerebroventricular injection of adenoviral-directed overexpression of PPARγ (Ad-PPARγ) reduced hypothalamic and plasma ghrelin, food intake in both lean C57BL/6J mice and diet-induced obese mice. These changes were associated with a significant increase in mechanistic target of rapamycin complex 1 (mTORC1) activity. Overexpression of PPARγ enhanced mTORC1 signaling and suppressed ghrelin production in cultured mHypoE-42 cells. Our results suggest that hypothalamic PPARγ plays a vital role in ghrelin production and food intake in mice.

1. Introduction

Peroxisome proliferator-activated receptor-γ (PPARγ) has been reported to serve as an intracellular fuel sensor to regulate cell metabolism such as fatty acid and glucose metabolism, thereby converting nutritional signals into metabolic consequences (Ahmadian et al., 2013; Semple et al., 2006; Sharma and Staels, 2007). PPARγ is the target of the thiazolidinediones (TZDs) class of insulin-sensitizing drugs, which have been widely prescribed to combat type 2 diabetes mellitus (Monsalve et al., 2013). A common side effect of treatment with TZDs is weight gain (Ahmadian et al., 2013). Accumulation of body weight induced by TZDs was due to the enhanced food intake and feed efficiency in both rodents and human beings (Alemán-González-Duhart et al., 2016; Garretson et al., 2015; Ryan et al., 2011; Shimizu et al., 1998). Although appetite was reported to be stimulated after administration of the thiazolidinedione, change of food intake in PPARγ knockout mice is controversial. For example, food intake and body weight were significantly decreased in brain-specific PPARγ null mice (Lu et al., 2011) but increased in systemic PPARγ null mice (Yang et al., 2012) and adipose-specific PPARγ knockout mice (Jones et al., 2005) compared to control littermates. The molecular mechanism underlying PPARγ regulates appetite is still unclear, which is, at least, partially mediated through the regulation of hormonal production.

Ghrelin, is an orexigenic hormone mainly produced by gastric X/A cells (Date et al., 2000; Müller et al., 2015; Stengel et al., 2010). Preproghrelin undergoes endoproteolytic processing and posttranslational modification to produce acyl-ghrelin and des-acyl ghrelin (Kojima and Kangawa, 2005). Des-acyl ghrelin has the same amino acid sequence as ghrelin, but its third amino acid (serine 3) is not acylated (Hosoda et al., 2000). Posttranslational modification of ghrelin by octanoylation of its third amino acid serine residue is the key to ghrelin function. The enzyme which mediates octanoylation of ghrelin has been identified as a conserved orphan membrane-bound O-acyl transferase (MBOAT) and named ghrelin O-acyl transferase (GOAT) (Gutierrez et al., 2008; Yang et al., 2008). Ghrelin has been reported to exercise a broad array of functions including control of food intake (Wren et al., 2000; Wren et al., 2001) and glucose metabolism (Heppner and Tong, 2014; Tong et al., 2010). Plasma ghrelin levels increase with fasting and decrease after feeding, indicating that ghrelin is involved in meal initiation (Cummings et al., 2001). Ghrelin levels are negatively correlated with body mass index (Tschöp et al., 2001). Investigation of the molecular mechanisms by which ghrelin-producing cells regulate the synthesis and secretion of ghrelin will yield new insights relevant to treatment strategies for human obesity and diabetes.

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Previous studies have demonstrated that PPARγ modulates appetite in both rodents and human beings through its central effects, especially in the hypothalamic ARC (Garretson et al., 2015; Lu et al., 2011; Ryan et al., 2011; Sarruf et al., 2009). Ghrelin acts on hypothalamic brain cells to increase hunger, gastric acid secretion and gastrointestinal motility to prepare the body for food intake (Kojima and Kangawa, 2005; Ueno et al., 2005). It remains unknown whether PPARγ can directly act on ghrelin-producing cells to regulate its synthesis and secretion. In the present study, we report that activation of PPARγ significantly decreased the synthesis and secretion of ghrelin in both hypothalamus and mHypoE-42 cells, a hypothalamic cell line in which ghrelin is abundantly expressed (Hong et al., 2016). Our results suggest that direct activation of hypothalamic PPARγ may inhibit ghrelin synthesis and food intake in mice.

2. Materials and methods

2.1. Materials

Total ghrelin enzyme immunoassay kit was purchased from Phoenix Pharmaceuticals, Inc. (Burlingame, CA). Rabbit anti-PPARγ, anti-Raptor, anti-phospho-mTOR (Ser2448), anti-phospho-p70 S6 Kinase (Thr389), anti-phospho-S6 (Ser235/236), anti-mTOR, anti-p70 S6 Kinase, anti-S6, anti-phospho-AKT (Ser473), anti-AKT and mouse anti-β-actin were from Cell Signaling Technology (Beverly, MA). Mouse anti-ghrelin was from Abcam Inc. (Cambridge, MA). Trizol reagent and reverse transcription (RT) system were from Promega Inc. (Madison, WI). Horseradish peroxidase-conjugated, donkey anti-rabbit IgG and donkey anti-mouse IgG were purchased from Jackson ImmunoResearch (West Grove, PA). Immobilon western chemiluminescent HRP substrate was purchased from Millipore (Temecula, CA).

2.2. Animal experiments

Male C57BL/6J mice were housed in standard cages and maintained at a regulated environment (24 °C, 12 h light, 12 h dark cycle with lights on at 7,00 and off at 19:00) with ad libitum access to a normal chow diet (control diet, D12450; Research Diets) or high-fat diet (60% of kcal as fat, D12492, Research Diets) for 12 weeks. Animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996).

A 26-gauge stainless steel guide cannula (Plastics One, Roanoke, VA) projecting into the third cerebral ventricle was implanted into each mouse using flat-skull coordinates from bregma (antero-posterior, −0.825 mm; medio-lateral, 0 mm; dorso-ventral, −4.8 mm) (Hong et al., 2016; Dalvi et al., 2012). All surgeries were performed under aseptic conditions and with the mice under isoflurane (as inhalant: 5% induction; 1–2% maintenance) anesthesia. One week before surgery, mice were housed in individual plastic cages. Mice were trained and mold injection provided for 3 days before the experiment in order to acclimate the animals to the injection and the stress. All animal protocols were approved by the Animal Care and Use Committee of Jinan University.

2.3. Intracerebroventricular (icv) microinjections

After 12 h fasting, C57BL/6J mice received either Ad-PPARγ (10^9 pfu) or Ad-GFP (10^9 pfu) in a total volume of 2 μl by slow infusion. Ad-PPARγ and Ad-GFP adenovirus were micro injected into the third ventricle 1 h before the onset of the dark phase. The mice were returned to their cages with free access to a premeasured amount of chow and water, and the effect of central PPARγ on feeding was determined. Food intake and body weight were measured at selected time points post-treatment.

2.4. Cell culture and transfection

Embryonic mouse hypothalamic cell line N42 (mHypoE-42) (Cellutions Biosystems Inc., Burlington, NC, USA), a hypothalamic cell line in which ghrelin is abundantly expressed, was used for in vitro analysis of ghrelin modulation. mHypoE-42 cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 and maintained in high glucose DMEM medium supplemented with 10% FBS. Cells were plated at optimal densities and grown for 48 h, then infected with Ad-PPARγ (10^6 pfu) or Ad-GFP (10^6 pfu) for 48 h.

2.5. Western blotting analysis

The tissues and cultured cells were quickly harvested, rinsed thoroughly with PBS, then homogenized on ice in the lysis buffer. Protein concentration was measured by Bradford's method. Proteins were loaded onto SDS-PAGE gels, and then transferred to nitrocellulose membranes. For ghrelin analysis, a tricine gel was utilized. The membranes were incubated for 1 h at room temperature with 5% fat-free milk in Tris-buffered saline containing Tween 20, followed by incubation overnight at 4 °C with the primary antibodies. The antibodies were detected using 1:10,000 horseradish peroxidase-conjugated, donkey anti-rabbit IgG and donkey anti-mouse IgG (Jackson ImmunoResearch, USA). A western blotting luminol reagent was used to visualize bands corresponding to each antibody. The band intensities were quantitated by Image J software.

2.6. RNA extraction, quantitative real-time PCR

For gene expression analysis, RNA was isolated from mouse tissues or mHypoE-42 cells using TRIzol and reverse-transcribed into cDNAs using the First-Strand Synthesis System for RT-PCR kit. SYBR Green-based real-time PCR was performed using the Mx3000 multiplex quantitative PCR system (Stratagene, La Jolla, CA). Triplicate samples were collected for each experimental condition to determine relative expression levels. Sequences for the primer pairs used in this study follow (Table 1).

2.7. Measurements of ghrelin

Blood samples were collected after anesthesia in the presence of aprotinin (2 μg/ml) and EDTA (1 mg/ml). HCl was added into plasma and cell culture medium for a final concentration of 0.1 mol/l to

Table 1
List and sequences of primers used in RT-PCR experiments.

	Upstream primer (5′-3′)	Downstream primer (5′-3′)	Accession number(s)
Mouse ghrelin	CCATCTGCAGTTTGCTGCTA	GCAGTTTAGCTGGTGGCTTC	No·NM_021488
Mouse ghrelin O-acyltransferase	GTGAGTGCTGGAGCTGGACTG	TGAGCCACAGAGCTGTGCTTC	No. EU518496
Mouse β-actin	ATCTGGCACCACACCTTC	AGCCAGGTCCAGACGCA	No. NM_ 007393.5
Mouse AgRP	AACCTCTGTAGTCGCACCTAGC	AAACCGTCCCATCCTTTATTCT	No. NM_ 001271806.1
Mouse NPY	AGGCTTGAAGACCCTTCCAT	GATGAGGGTGGAAACTTGGA	No. NM_ 023456.3
Mouse POMC	GAGCTGATGACCTCTAGCCTCT	ATCAGAGCCGACTGTGAAATCT	No. NM_ 001278584.1
Mouse PPARγ	TCAGCTCTGTGGACCTCTCC	ACCCTTGCATCCTTCACAAG	No. NM_ 017321456.1