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Neuronal O-GlcNAc transferase regulates appetite, body weight, and peripheral insulin resistance

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ABSTRACT

The *ogt* gene encodes O-linked N-acetylglucosamine transferase (O-GlcNAc transferase [OGT]) that catalyzes the transfer of β-N-acetylglucosamine (GlcNAc) from the uridine-diphosphate-GlcNAc to the hydroxyl group of serine or threonine residues of nucleocytoplasmic proteins. This process is a common protein posttranslational modification, called protein O-GlcNAcylation, which is a known intracellular sensor of glucose metabolism and plays an important role in regulating cellular signaling, transcription, and metabolism. However, little is known about the function of OGT in the brain. Here, we report that the CaMKIIα promoter-dependent neuronal knockout (KO) of OGT in adult mice led to short-term overeating, body weight gain, and peripheral insulin resistance. These phenotype changes were accompanied by marked elevation of serum insulin and leptin levels and neuronal cell death, including the loss of leptin receptor–expressing neurons, in the hypothalamus. The neuronal OGT KO exacerbated obesity and insulin resistance induced by high-fat diet. Surprisingly, the peripheral insulin resistance induced by neuronal OGT KO was reversed at its own 2–3 months after OGT KO, and the mice even showed increased insulin sensitivity several months later. These findings reveal an important role of neuronal OGT in the regulation of feeding behavior, body weight, and peripheral insulin sensitivity.

1. Introduction

Obesity is one of the biggest drivers of preventable chronic diseases and health care costs (Cawley and Meyerhoefer, 2012). It is linked to increased risk for type 2 diabetes, hypertension, hypercholesterolemia, chronic heart disease, stroke, asthma, arthritis, cancer, and neurodegenerative diseases (Hammond and Levine, 2010; Sellbom and Gunstad, 2012). Obesity results from an imbalance between energy expenditure and dietary intake. Eating and drinking more calories than burning cause weight gain, overweight, and obesity. Genetics, eating habits, how and where people live, attitudes and emotions, life habits, and high income can lead to energy imbalance and weight gain (Berthoud and Morrison, 2008; Morton et al., 2006). Accumulated evidence also supports the central role of the brain in the regulation of food intake and the pathogenesis of obesity.

Energy homeostasis is controlled mainly by neuronal circuits in the hypothalamus and brainstem, whereas reward and motivation aspects of eating behavior are controlled by neurons in the limbic regions and cerebral cortex of the brain (Ahima and Antwi, 2008). Peripheral signals from the gastrointestinal tract, pancreas, and adipose tissue are sent to the hypothalamus and brainstem via the vagus nerve and hormonal mediators, such as ghrelin, insulin, and leptin. Depending on the information received, the brain initiates adjustments to the body's energy intake or use by altering appetite or satiety signals, altering physical activity, or even altering cellular energy use (Balthasar, 2009; Morton et al., 2006). However, the control and regulation of energy intake and use in the brain at the molecular level is much less understood.

One of the genes most commonly linked to human obesity is Gnpda2, which affects flux through the hexosamine biosynthesis pathway (HBP) (Gutierrez-Aguilar et al., 2012; Speliotes et al., 2010; Wolosker et al., 1998). The HBP produces uridine-diphosphate N-acetylglucosamine (UDP-GlcNAc), which is the donor substrate for the enzyme O-GlcNAc transferase (OGT). OGT catalyzes the transfer of GlcNAc from UDP-GlcNAc to the hydroxyl group of serine or threonine residues of nucleocytoplasmic proteins. This process is called protein O-GlcNAcylation. Approximately 2%–5% total

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intracellular glucose enters the HBP, and the ultimate product UDP-GlcNAc is synthesized based on nutrient concentration and the availability of nutrient-processing enzymes. Thus, O-GlcNAcylation is a nutrient-sensitive modification poised to dynamically integrate metabolic signals (Bond and Hanover, 2013; Bullen et al., 2014; Hanover et al., 2012).

Many proteins are modified by O-GlcNAc in the mammalian brain. In a proteomic study, we identified 274 proteins modified with O-GlcNAc and mapped 458 O-GlcNAc sites in the mouse brain (Alfaro et al., 2012). Nearly double numbers of O-GlcNAcylated proteins and O-GlcNAcylation sites are identified in the human brain using a similar approach (Wang et al., 2017). OGT is also expressed abundantly in the brain (Cole and Hart, 2001; Liu et al., 2012). OGT participation in intracellular O-GlcNAcylation is essential for embryonic cell viability because systematic OGT knockout (KO) is lethal in mouse ontogeny (Shafi et al., 2000). Constitutive KO of OGT in neurons leads to neuronal apoptosis and early postnatal death (O'Donnell et al., 2004).

To investigate the function of neuronal OGT in the mature brain, we generated inducible CaMKIIα promoter-dependent neuronal OGT KO mice. We found that OGT KO induced in the mature brains increased feeding behavior during the first 4 weeks and led to obesity with higher serum levels of insulin and leptin, reversible insulin resistance, and neuronal loss in the hypothalamus. The OGT KO also exacerbated obesity and insulin resistance induced by high-fat diet (HFD). Our findings indicate an important role of neuronal OGT in the regulation of feeding behavior and peripheral energy metabolism.

2. Materials and methods

2.1. Reagents and antibodies

Tomoxifen (T5648), corn oil (C8267), D-(+)-Glucose (G7528), and anti-OGT antibody (TI-14, O6014) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Monoclonal anti-O-GlcNAc antibody (RL2, MA1-072) and rabbit anti-leptin receptor antibody (PA1-053) were purchased from Thermo Fisher Scientific Company (Waltham, MA, USA). Antibody against glyceraldehyde 3-phosphate dehydrogenase was the product of Santa Cruz Biotechnology (Dallas, TX, USA). Alexa 488-conjugated goat anti-rabbit secondary antibody was from Molecular Probes (Carlsbad, CA, USA). Humulin R (U-100) was from Eli Lilly Company (Indianapolis, IN, USA). HFD (D12492; 20% protein, 20% fat, 60% carbohydrate) and control diet (CD) (D12450 J; 20% protein, 10% fat, 70% carbohydrate) were products of Research Diets, Inc (New Brunswick, NJ, USA).

2.2. Mice

The CaMK2α-Cre$^{(+)}$;Ogt$^{loxp(+)/loxp(+)}$ mice were generated by crossing the hemizygous B6.129S6-Tg (Camk2a-cre/ERT2)1Aibs/J Cre-expressing mice with the B6.129-Ogttm1Gwh/J OGT-floxed mice, both of which were initially purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and back crossed for at least 10 generations in our animal facility. The neuronal KO of OGT in the male CaMKIIα-Cre$^{(+)}$;Ogt$^{loxp(+)/loxp(+)}$ mice was induced by intraperitoneal (i.p.) injection of tamoxifen (75 mg/kg/d) for 4 consecutive days. The control mice for this study were either male CaMKIIα-Cre$^{(+)}$;Ogt$^{loxp(+)/loxp(+)}$ mice injected with vehicle (corn oil) or male CaMKIIα-Cre$^{(-)}$;Ogt$^{loxp(+)/loxp(+)}$ mice injected with tamoxifen. No difference between the 2 control groups was observed.

Mice were housed (4–5 animals per cage) with a 12/12 hours light/dark cycle and with ad libitum access to food and water. The housing, breeding, and animal experiments were in accordance with the approved protocol from the Institutional Animal Care and Use Committee of the New York State Institute for Basic Research in Developmental Disabilities and were according to the PHS Policy on Human Care and Use of Laboratory animals (revised March 15, 2010).

2.3. Reverse transcription-polymerase chain reaction analysis

The total RNA from frozen mouse brain tissue was extracted by using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and reversed transcribed using SuperScript III first-strand synthesis system for reverse transcription-polymerase chain reaction (Thermo Fisher Scientific) according to the manufacturer's instructions. The primer for OGT (Forward, 5-GATTTGTACTGTGTTCGCAGTGACCTG-3; reverse, 5-TGTGTTATAACAATCTTCTGCTTCAGCAAC-3) was located at exon 4 and 8 of the mouse *ogt* gene, respectively. Using the Mastercycler Nexus Thermal Cycler (Eppendorf, Hamburg, Germany), reactions were carried out at 94 °C for 5 minutes for initial denaturation and then at 94 °C for 30 seconds, 57 °C for 30 seconds, and 72 °C for 45 seconds. After 38 cycles of amplification, additional extensions were done at 72 °C for 5 minutes. Products were resolved and examined by 2% agarose gel electrophoresis.

2.4. Western blot analysis

Mouse brain tissue was homogenized in prechilled buffer containing 50 mM Tris–HCl (pH 7.4), 50 mM GlcNAc, 100 mM sodium fluoride, and 2.0 mM sodium orthovanadate, 1.0 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 20 μM UDP, 0.5 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 2.0 μg/mL aprotinin, 10 μg/mL leupeptin, and 2.0 μg/mL pepstatin A. The homogenate samples were boiled in Laemmlie's buffer for 5 minutes, and the protein concentrations were measured by using the Pierce 660 nm protein assay (Thermo Fisher Scientific). The samples were resolved in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred onto Immobilon-P membrane (Millipore, Bedford, MA, USA). The blots were then probed with primary antibodies and developed with the corresponding horseradish peroxidase–conjugated secondary antibody and ECL kit (Pierce, Rockford, IL, USA). Densitometric quantification of protein bands in Western blots were analyzed by using the Multi Gauge V3.0 software (Fuji Photo Film Co., Ltd).

2.5. Immunohistochemistry

Mouse brains were immersion-fixed in 4% paraformaldehyde at 4 °C for 24 hours, followed by dehydration in 30% sucrose at 4 °C for 48 hours. Sagittal sections of 40-μm thickness were cut by using a freezing sliding microtome. The sections were stored in antifreeze solution, consisting of glycerol, ethylene glycol, and phosphate-buffered saline (PBS) at the ratio of 3:3:4 at −20 °C till immunofluorescence staining.

Sagittal mouse brain sections proximal to the middle line, which contain the arcuate nucleus (ARC) of the hypothalamus, were chosen for immunofluorescence analysis. Briefly, tissue sections were incubated in PBS containing 0.5% Triton-X100 for 15 minutes, followed by blocking in 5% normal goat serum containing 0.05% Triton X-100 for 45 minutes. After incubation with anti-leptin receptor antibody (1:100) at 4 °C overnight, the sections were washed and then incubated with Alexa 488-conjugated goat anti-rabbit second antibody (1:500) for 2 hours. The sections were finally washed in PBS for 3 times and mounted on microscope slides with ProLong Gold antifade reagent (Invitrogen, Eugene, OR, USA). High-quality TIFF images (resolution: 1024 × 1024; field size: 318.3 μm × 318.3 μm) were achieved using 40 × oil objective lens of a Nikon 90i fluorescent microscope equipped with Nikon C1 3-laser confocal system and a Nikon DS U1 digital camera. The number of immune positive cells was counted by using Image J software package (Image J 1.50i, NIH).