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Research Paper

Catecholamines Facilitate Fuel Expenditure and Protect Against Obesity via a Novel Network of the Gut-Brain Axis in Transcription Factor *Skn-1*-deficient Mice

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ABSTRACT

Taste signals and nutrient stimuli sensed by the gastrointestinal tract are transmitted to the brain to regulate feeding behavior and energy homeostasis. This system is referred to as the gut-brain axis. Here we show that both brush cells and type II taste cells are eliminated in the gastrointestinal tract of transcription factor *Skn-1* knockout (KO) mice. Despite unaltered food intake, *Skn-1* KO mice have reduced body weight with lower body fat due to increased energy expenditure. In this model, 24-h urinary excretion of catecholamines was significantly elevated, accompanied by increased fatty acid β-oxidation and fuel dissipation in skeletal muscle and impaired insulin secretion driven by glucose. These results suggest the existence of brain-mediated energy homeostatic pathways originating from brush cells and type II taste cells in the gastrointestinal tract and ending in peripheral tissues, including the adrenal glands. The discovery of food-derived factors that regulate these cells may open new avenues the treatment of obesity and diabetes.

Research Context: Taste signals and nutrient stimuli sensed by the gastrointestinal tract are transmitted to the brain to regulate feeding behavior and energy homeostasis along the gut-brain axis. We propose the concept that taste-receiving cells in the oral cavity and/or food-borne chemicals-receiving brush cells in the gut are involved in regulation of the body weight and adiposity via the brain. The discovery of food-derived factors that regulate these cells may open new avenues for the treatment of obesity and diabetes.

1. Introduction

Taste signals and nutrient stimuli sensed by the gastrointestinal (GI) tract are transmitted to the central nervous system, including the nucleus of the solitary tract and hypothalamus, via afferent neurons and humoral mediators, thereby controlling feeding behavior and energy homeostasis. This system is referred to as the gut-brain axis (Cummings and Overduin, 2007; Furness, 2012). This information is subsequently conveyed from the brain to peripheral tissues via efferent sympathetic and parasympathetic nerves including preganglionic sympathetic splanchnic input to the adrenal glands. For example, gut incretins such as glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), which are released from the intestinal enteroendocrine cells in response to nutrient ingestion, potentiate glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells (Wu et al., 2015). GLP-1 also activates the GLP-1 receptors expressed on vagal afferent nerve terminals in the portal vein and in the brain across

Abbreviations: BAT, brown adipose tissue; ChgA, chromogranin A; CT, computed tomography; Dbh, dopamine-β-hydroxylase; Dclk1, doublecortin-like kinase 1; Ddc, dopa decarboxylase; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; GSIS, glucose-stimulated insulin secretion; HFD, high-fat diet; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; KO, knockout; NEFA, non-esterified fatty acid; OGTT, oral glucose tolerance test; Pnmt, phenylethanolamine *N*-methyltransferase; RER, respiratory exchange ratio; SCC, solitary chemosensory cells; T3, triiodothyronine; T4, tetraiodothyronine; TG, triacylglycerol; Th, tyrosine hydroxylase; Trpm5, transient receptor potential melastatin 5; TSH, thyroid stimulating hormone; Ucp3, uncoupling proteins 3; WAT, white adipose tissue.

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the blood-brain barrier, which is involved in the regulation of appetite (Katsurada et al., 2014; Kinzig et al., 2002; Vahl et al., 2007). Gavage of agonists for bitter taste receptor increases food intake via the secretion of ghrelin, a hormone that potentiates hunger sensation (Janssen et al., 2011).

The *Skn-1* (also known as *Pou2f3*) gene, which encodes the POU homeodomain transcription factor, was originally identified as a regulator of the differentiation of epidermal keratinocytes (Andersen et al., 1993, 1997). We previously reported that *Skn-1a* is expressed in sweet, umami (savory), and bitter-sensing taste cells, which are referred to as type II taste cells (Matsumoto et al., 2011). Type II taste cells are completely eliminated in *Skn-1* knockout (KO) mice, resulting in loss of electrophysiological and behavioral responses to sweet, umami, and bitter tastes. Thus, *Skn-1a* is critical for generating type II taste cells. *Skn-1a* is also required for the generation of *Trpm5*-expressing solitary chemosensory cells (SCCs) in the nasal respiratory epithelium and microvillous cells in the main olfactory epithelium (Ohmoto et al., 2013; Yamaguchi et al., 2014). *Skn-1a* is expressed in several tissues including the stomach, but not in the brain (Yukawa et al., 1993).

There are four major cell types in the small intestine: enterocytes and Goblet, Paneth, and enteroendocrine cells (van der Flier and Clevers, 2009). In addition to these cell types, brush cells (also referred to as tuft cells or caveolated cells) constitute a minor fraction (0.4%) of the adult mouse intestinal epithelium (Gerbe et al., 2012). Brush cells are supposed to be chemosensory cells (Young, 2011) and express transient receptor potential melastatin 5 (*Trpm5*), Doublecortin-like kinase 1 (*Dclk1*), and choline acetyltransferase (Bezencon et al., 2008; Gerbe et al., 2009, 2011; Saqui-Salces et al., 2011; Schütz et al., 2015). The mechanisms of differentiation and the function of brush cells remain elusive.

In the present study, we found that *Skn-1a* regulates differentiation of *Trpm5*-expressing brush cells in the GI tract. *Skn-1* KO mice exhibited reduced body weight with lower body fat than wild-type (WT) littermates. Despite unaltered food intake, *Skn-1* KO mice exhibited increased energy expenditure, caused by augmented catecholamine secretion. Our work raises the concept that taste cells receiving sweet, bitter, and umami tastes as well as brush cells receiving food-borne chemicals are involved in regulating body weight and body fat. Collectively, the present study provides new insights into the regulation of energy homeostasis originating from brush cells and taste cells in the GI tract and signaling to peripheral tissues including the adrenal glands, via the brain.

2. Material and Methods

2.1. Experimental Animals

All animal experiments were approved by the Animal Care and Use Committee at The University of Tokyo. *Skn-1/Pou2f3*-deficient mice with a mixed 129 × C57BL/6J background were generated as previously described (Matsumoto et al., 2011) and then backcrossed to C57BL/6J for >10 generations. Male *Skn-1* KO mice and their WT littermates generated by crossing the heterozygous mice were used in the present study. Mice were fed a normal chow diet (Lab MR Breeder, Nosan Co., Yokohama, Japan) after weaning or High-Fat Diet 32 (CREA Japan Inc., Tokyo, Japan) from 4 weeks of age for >12 weeks to induce dietary obesity. Mice were maintained in a room with a constant temperature of 22 ± 1 °C under a 12-h light-dark cycle (lights on at 8 a.m.). Tissue samples were collected from 12- to 16-week-old mice that were fasted for 18 h.

2.2. X-ray Computed Tomography (CT) Scan Analysis

Mice older than 16 weeks were anesthetized using an isoflurane nebulizer (Muromachi Kikai Co. Ltd., Tokyo, Japan); a Latheta LCT-200 CT instrument (Aloka-Hitachi LCT-200, Tokyo, Japan) was then used to scan abdominal fat and muscle mass and extract thighbone density.

2.3. Blood Chemical Parameters

Blood was collected from approximately 20-week-old mice by cardiac puncture at 10 a.m. under ad libitum feeding condition and after 18-h fasting. Serum chemical parameters and hormones (PTH, T3, and T4) were analyzed by Nagahama Life Science Laboratory (Shiga, Japan). Plasma insulin and leptin were measured using the Luminex 200™ System (Luminex, Austin, TX, USA) by GeneticLab Co., Ltd. (Sapporo, Japan). Plasma adiponectin and FGF21 were measured using mouse adiponectin ELISA (BioVender, Brno, Czech Republic) and mouse and rat FGF-21 ELISA (BioVender), respectively.

2.4. Indirect Calorimetry

Indirect calorimetry was performed on 20- to 28-week-old mice using an indirect calorimeter (ARCO-2000, Arco Systems Inc., Chiba, Japan) and spontaneous motor activity was measured using a pyroelectric infrared ray sensor (NS-AS01, Neuroscience Inc., Tokyo, Japan) for 3–4 days after 2 days of habituation essentially as described by Tschop et al. (2011) or with an indirect calorimeter (MK-5000RQ, Muromachi Kikai Co. Ltd.) as previously described (Watanabe et al., 2014). The respiratory exchange ratio (RER) was calculated as the molar ratio of VCO_2/VO_2. Energy expenditure (kcal per hour) was calculated as heat (kcal/h) = $(3.815 + 1.232 \times \mathrm{RER}) \times VO_2$.

2.5. Mitochondrial DNA Copy Number

To measure the mitochondrial DNA copy number, the ratio of mitochondrial DNA to genomic DNA was calculated by a previously described method (Watanabe et al., 2014). In brief, total DNA was extracted from the gastrocnemius of 14- to 19-week-old mice that were fasted for 16 h using a QIAamp DNA mini kit (QIAGEN Ltd., Tokyo, Japan) and used for qPCR (Applied Biosystems Japan Ltd., Tokyo, Japan). We used the TaqMan Gene Expression Assay (Applied Biosystems Foster City, USA; ACTB, Mm00607939_s1; CYTB, Mm044225274). The PCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The mitochondrial DNA copy number is presented relative to nuclear DNA following amplification of the mitochondrial gene region (cytochrome *b* vs. the nuclear endogenous control region, β-actin).

2.6. DNA Microarray

DNA microarray analysis using total RNA extracted from the gastrocnemius of 12- to 16-week-old mice that were fasted for 18 h was performed as previously described (Nakai et al., 2008) with minor modifications. In brief, Total RNA samples (100 ng each) were prepared and processed for microarray analysis using a 3′ IVT Express kit and GeneChip® Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's standard protocols. The raw microarray data (CEL files) were quantified by the Factor Analysis for Robust Microarray Summarization (FARMS) (Hochreiter et al., 2006) using the statistical language R (Team and Computing, 2005) and Bioconductor (Gentleman et al., 2004). To identify differentially expressed genes, the rank products (RP) method (Breitling et al., 2004) was applied to the FARMS-quantified data. All microarray data are MIAME compliant and have been deposited in a MIAME-compliant database, the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/, GEO Series accession number GSE76936), as described in more detail on the MGED Society web site (http://www.mged.org/Workgroups/MIAME/miame.html).