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Central action of xenin affects the expression of lipid metabolism-related genes and proteins in mouse white adipose tissue

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ABSTRACT

Xenin is a gastrointestinal hormone that reduces food intake when administered centrally and it has been hypothesized that central action of xenin participates in the regulation of whole-body metabolism. The present study was performed to address this hypothesis by investigating the central effect of xenin on the expression of genes and proteins that are involved in the regulation of lipid metabolism in white adipose tissue (WAT). Male obese *ob*/*ob* mice received intracerebroventricular (i.c.v.) injections of xenin (5 μg) twice 12 h apart. Food intake and body weight change during a 24-h period after the first injection were measured. Epididymal WAT was collected at the end of the 24-h treatment period and levels of lipid metabolism-related genes and proteins were measured. Xenin treatment caused significant reductions in food intake and body weight compared to control vehicle treatment. Levels of fatty acid synthase (FASN) protein were significantly reduced by xenin treatment, while levels of adipose triglyceride lipase (*Atgl*) and beta-3 adrenergic receptor (*Adrb3*) mRNA and phosphorylated hormone sensitive lipase (Ser^{660}-pHSL and Ser^{563}-pHSL) were significantly increased by xenin treatment. These findings suggest that central action of xenin causes alterations in lipid metabolism in adipose tissue toward reduced lipogenesis and increased lipolysis, possibly contributing to xenin-induced body weight reduction. Thus, enhancing central action of xenin and its downstream targets may be possible targets for the treatment of obesity by reducing the amount of stored fat in adipose tissue.

1. Introduction

Hormones produced by the gastrointestinal tract play a role in the regulation of whole body energy balance. Gut hormones such as cholecystokinin (CCK), peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) are released into the circulation in response to nutrient ingestion, and have been implicated in the control of food intake by limiting the size of individual meals. Ghrelin on the other hand is the only orexigenic gut hormone with high plasma levels before meals (Kairupan et al., 2016). Xenin, another gut hormone is a 25-amino acid peptide that is produced by a subset of intestinal K cells. It is secreted into the circulation in response to food ingestion, suggesting that xenin acts as an endogenous satiety factor (Anlauf et al., 2000; Feurle et al., 1992; Feurle et al., 2003). Consistent with this assumption, both peripheral and central administrations of xenin reduce food intake in rodents and chicks (Alexiou et al., 1998; Cline et al., 2007; Cooke et al., 2009; Leckstrom et al., 2009). Xenin reduces food intake partly through reduced gastric emptying rate without causing taste aversion (Cline et al., 2007; Kim and Mizuno, 2010b; Leckstrom et al., 2009). Intraperitoneal (i.p.) injection of xenin increases the number of Fos-immunoreactive cells, a marker of cell activation, in several hypothalamic nuclei and the nucleus of solitary tract of the brainstem that are known to be involved in the regulation of energy balance (Cline et al., 2007; Kim and Mizuno, 2010b; Leckstrom et al., 2009). Extracellular signal-regulated kinase (ERK) signaling pathway is also activated in hypothalamic neurons after i.p. xenin administration (Kim et al., 2016). Moreover, feeding-suppressing effect of xenin has also been shown to be associated with hypothalamic appetite-regulating neuropeptide systems (Cooke et al., 2009; Kim and Mizuno, 2010a; Kim et al., 2014; Nandar et al., 2008; Schusdziarra et al., 2004). These findings support the hypothesis that xenin inhibits feeding at least partly through the activation of specific cells in the central nervous system (CNS) involving the hypothalamus and the brainstem.

Although the effect of centrally administered xenin on food intake has been studied, little is known about the role of xenin in the regulation of body weight and adiposity. Feeding-suppressing effect of xenin is mediated via neurotensin receptor 1 (Ntsr1) (Kim and Mizuno, 2010a). Ntsr1-deficient mice are characterized by hyperphagia and mild obesity, implicating the signaling through Ntsr1 in feeding and body weight regulation (Opland et al., 2013; Remaury et al., 2002). Thus, activation of Ntsr1 by xenin may influence not only food intake but also body weight. If an overall effect of xenin is catabolic, it should promote

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metabolic alterations that favor increased energy utilization and/or decreased energy storage. Therefore, we hypothesized that increased central action of xenin will lead to changes in the expression of lipid metabolism-related genes and proteins in the adipose tissue toward reducing the amount of stored fat. To address this hypothesis, we examined the effect of intracerebroventricular (i.c.v.) xenin treatment on the expression levels of lipogenic and lipolytic genes and proteins in white adipose tissue (WAT) of obese mice in the present study.

2. Materials and methods

2.1. Animals

Male 4-month-old *ob*/*ob* mice (C57BL/6J background) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Animals were maintained under a 12:12 light/dark cycle (lights on at 0600 h) with free access to standard rodent chow pellets (Prolab RMH 3000, 4.5% fat by weight, Ralston Purina) and water throughout the experiment. All studies were approved by the Institutional Animal Care and Use Committee (University of Manitoba).

2.2. I.c.v. cannulation

Mice were anesthetized with an i.p. injection of ketamine (100 mg/kg b.w.)/xylazine (10 mg/kg b.w.). The topical anesthetic bupivacaine (1 mg/kg b.w.) was applied to the skin before making an incision. A stainless steel guide cannula (outer diameter: 0.64 mm, inner diameter: 0.33 mm) was stereotaxically implanted into the lateral ventricle with the following coordinates: 0.4 mm posterior to the bregma, 1.0 mm lateral from the midline and 1.8 mm deep to the dura in accordance with the atlas of Paxinos and Franklin (2001). The cannula was fixed to the skull with dental cement and stainless steel screws. The mice received subcutaneous injection of buprenorphine (0.1 mg/kg b.w.) 3 times per day for 3 days post-surgery. Localization of the cannula was verified by assessing drinking behavior responses to the i.c.v. administration of angiotensin II (100 ng in 1 μl). Only mice responding with a robust drinking behavior were used for the experiment. Localization of the cannula was also confirmed at the end of the experiment by injecting the dye through the cannula and only mice showing a spread of dye in the ventricle system were included in the experiment. Of the 15 mice that underwent stereotaxic surgery, one mouse died post-surgery from an unknown reason and two mice lost cement head caps before the i.c.v. treatment experiment. One mouse (out of 12) did not show the drinking response to angiotensin II and was removed from the study. Two mice (out of 11) did not show spread of the dye in the ventricular system after dye injection and were excluded from the study.

2.3. I.c.v. xenin treatment and tissue collection

To determine if the central action of xenin reduces body weight, we examined the effect of i.c.v. administration of xenin on food intake and body weight in *ob*/*ob* mice. *Ad libitum* fed mice received 2 i.c.v. injections of xenin (5 μg/injection, American Peptide Co., Sunnyvale, CA, USA) at 1000 h and 2200 h. Xenin was reconstituted in artificial cerebrospinal fluid (aCSF) consisting of 124 mM NaCl, 26 mM $NaHCO_3$, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$ and 10 mM $_D$-glucose. Control mice received two i.c.v. injections of aCSF. Drugs were injected in a total volume of 1 μl over 30 s. An injection pipe was left inside the cannula for another 30 s then removed from the cannula. Body weight and food weight were measured immediately prior to the first injection and 24 h after the first injection, and body weight change and food intake during a 24-h period were calculated. The mice were euthanized by CO_2 narcosis 12–14 h after the second injection (between 1000 h and 1200 h). Epididymal adipose tissues and skeletal muscles were collected for RNA and protein analyses.

2.4. RNA analysis

Total RNA was extracted from the tissue of each animal, cDNA was prepared, and mRNA expression levels were measured by real-time PCR using the ABI 7500 Fast thermal cycler (Applied Biosystems, Foster City, CA, USA) as described previously (Poritsanos et al., 2008). All the gene-specific primers (Table 1) were designed with Primer Express software (Version 3.0, Applied Biosystems). Levels of mRNA were normalized to *β-actin* or *cyclophilin* mRNA levels, and were expressed as a percentage of the aCSF-treated control group. All reactions were performed in triplicates and the coefficient of variation was <5% for each triplicate.

2.5. Western blot analysis

Tissues were lysed in protein lysis buffer (50 mM HEPES, 150 mM NaCl, 10% glycerol and 1% Triton X-100) and supplemented with EDTA-free proteinase inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). Total protein concentration was determined by Bradford assay. Proteins (20 μg) were separated using 10% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes (Westran, Whatman Schleicher & Schuell, Keene, NH, USA). After blocking with 5% non-fat milk in 1× Tris-buffered saline with 0.05% Tween 20 (TBST) for 1 h at room temperature, membranes were incubated overnight at 4 °C in primary antibodies for total hormone sensitive lipase (tHSL, #4107, 1:1000, Cell Signaling Technology, Danvers, MA, USA), phospho-Ser^{660} HSL (Ser^{660}-pHSL, #4126, 1:1000, Cell Signaling Technology), phospho-Ser^{563} HSL (Ser^{563}-pHSL, #4139, 1:1000, Cell Signaling Technology), adipose triglyceride lipase (ATGL, #2138, 1:500, Cell Signaling Technology), perilipin (#3470, 1:200, Cell Signaling Technology), comparative gene identification-58 (CGI-58, #H00051099-M01, 1:400, Abnova, Taipei, Taiwan), fatty acid synthase (FASN, #sc-20140, 1:500, Santa Cruz Biotechnology, Dallas, TX, USA) and α-tubulin (#sc-8035, 1:1000, Santa Cruz Biotechnology) diluted in blocking buffer, followed by washing in TBST. Appropriate horseradish peroxidase-conjugated secondary antibody was used at 1:10,000 dilution and incubated for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence system (Advansta Inc, Menlo Park, CA, USA). Densitometry of the bands on autoradiography film was analyzed using Quantity One software (Bio-Rad, Hercules, CA, USA). Signal intensity of Ser^{660}-pHSL and Ser^{563}-pHSL was normalized to tHSL signal intensity. Signal intensity of other proteins was normalized to α-tubulin signal intensity. Levels of protein were expressed as a percentage of the aCSF-treated control group.

2.6. Blood chemistry

Serum free fatty acids (FFA) and glycerol levels were determined by colorimetric assay with commercial kits (SFA-1, Zen-Bio, Research Triangle Park, NC, USA and 10,010,755, Cayman Chemical Co., Ann Arbor, MI, USA, respectively).

2.7. Statistical analysis

Data are presented as means ± standard error of the mean (S.E.M.). Comparisons between 2 groups were performed by Student's *t*-test (for parametric data) or Wilcoxon test (for nonparametric data). In all cases, differences were taken to be significant if *P*-values were below 0.05.

3. Results

3.1. Effect of i.c.v. xenin treatment on food intake, body weight, and serum levels of FFA and glycerol in ob/ob mice

I.c.v. xenin treatment significantly reduced 24-h food intake by 33.0% compared to the control aCSF treatment in obese *ob*/*ob* mice

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