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The nutrient sensor OGT in PVN neurons regulates feeding

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Abstract

Maintaining energy homeostasis is crucial for the survival and health of organisms. The brain regulates feeding by responding to dietary factors and metabolic signals from peripheral organs. It is unclear how the brain interprets these signals. O-GlcNAc transferase (OGT) catalyzes the posttranslational modification of proteins by O-GlcNAc and is regulated by nutrient access. Here, we show that acute deletion of OGT from α CaMKII-positive neurons in adult mice caused obesity from overeating. The hyperphagia derived from the paraventricular nucleus (PVN) of the hypothalamus, where loss of OGT was associated with impaired satiety. These results identify O-GlcNAcylation in α CaMKII neurons of the PVN as an important molecular mechanism that regulates feeding behavior.

Obesity is a major contributor to disease throughout the world (*1*). Currently, there is no successful and available treatment for the majority of obese patients. One of the genes most commonly linked to human obesity, *Gnpda2*, affects flux through the hexosamine biosynthesis pathway (HBP) (*2–4*). The HBP produces uridine-diphosphate: N-acetylglucosamine (UDP-GlcNAc), which is the donor substrate for the enzyme O-GlcNAc transferase (OGT). OGT cleaves UDP-GlcNAc and covalently attaches GlcNAc to the hydroxyl group of serine or threonine in nuclear and cytoplasmic proteins in β -linkage (O-GlcNAc). Nutrient access directly via the HBP, and metabolic hormones such as insulin regulate the activity of OGT (*5, 6*). Although OGT has been shown to be important for neuronal development, the role of OGT for mature brain function is almost completely

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S9

References (24–29)

unknown (7–9). To study the function of OGT in normal behavior, we created conditional OGT knockout mice by crossing floxed OGT mice (OGT^{Fl}) with mice expressing a tamoxifen-inducible version of Cre recombinase under the α CaMKII promoter (α CaMKII-CreER^{T2}). This enables acute brain-specific deletion of OGT in α CaMKII-expressing neurons in adult mice, which we confirmed by means of immunohistochemistry, Western blotting, and polymerase chain reaction (fig. S1). Knockout of OGT in other tissues and cells has been shown to lead to decreases in cell number, probably because of impaired mitosis, and in fact, constitutive knockout of OGT leads to early embryonic lethality (8, 10, 11). In contrast, in postmitotic neurons deletion of OGT did not affect cell number in vitro or in vivo (fig. S2, A and B).

Acute and brain-specific loss of OGT in adult mice caused rapid weight gain (Fig. 1, A and B, and fig. S3, A and B). Within 3 weeks, the amount of adipose tissue tripled, whereas the lean weight had not changed, as quantified with whole-body nuclear magnetic resonance (NMR) [fat mass, $n = 7$ wild-type (WT) mice, 2.5 ± 0.21 g; $n = 6$ OGT knockout mice, 8.3 ± 0.86 g] (fig. S3, C and D). The incorporation of fat was general and not particular to any specific body region (fig. S3, E to G). Obesity can result from either excessive caloric intake or insufficient energy expenditure. Daily food intake rapidly increased upon knockout of OGT and plateaued at a level more than twice as high (Fig. 1C). If access to food was restricted to the same amount consumed by WT mice, the OGT knockout mice retained normal body weight. When free access to food was reintroduced, the OGT knockout mice quickly approached the weight of OGT knockout littermates who had been fed ad libitum throughout the experiment (Fig. 1D). To quantify food intake and energy expenditure simultaneously in real time, we used comprehensive laboratory animal monitoring system (CLAMS). CLAMS confirmed that loss of OGT leads to hyperphagia (fig. S3H). Energy expenditure was actually increased (fig. S3I). The accelerated energy expenditure resulted from, at least in part, hyperactivity (fig. S3J). As expected from a combination of hyperphagia and hyperactivity, knocking out OGT caused higher vO_2 and vCO_2 , leading to a respiratory exchange ratio (RER) above 1 ($n = 17$ WT mouse days (6 mice), 0.94 ± 0.004 ; $n = 20$ OGT knockout mouse days (6 mice), 1.04 ± 0.007) (fig. S3, K to M).

Daily food intake is a factor of both the size of each meal and meal frequency. A normal diurnal rhythm was preserved in OGT knockout mice (Fig. 1E and fig. S4, A and B). Although there was no difference in meal frequency, loss of OGT increased meal size as well as meal duration (meal size, $n = 268$ WT mouse meals (6 mice), 0.25 ± 0.02 g; $n = 330$ OGT knockout mouse meals (7 mice), 0.42 ± 0.02 g) (Fig. 1E and fig. S4, C to E). The point at which a meal is terminated depends on the total caloric content of the food but also noncaloric determinants of the food, mainly its volume and composition. When fed either regular carbohydrate-rich pellets or fat-based food paste with higher caloric density, the OGT knockout mice overate the same amount of calories (fig. S4F).

Immunohistochemistry for OGT showed that within the core feeding circuitry, the major loss of OGT in the knockout mice occurred in medial nuclei of the hypothalamus, most notably in the paraventricular nucleus (PVN) (Fig. 2A and fig. S5A). There was only minor loss of OGT from nuclei of the midbrain and the brainstem (Fig. 2A and fig. S5A). Using α CaMKII-CreER⁺ $\times$ Td^{Fl} mice and immunohistochemistry, we noticed that some

α CaMKII PVN cells expressed thyrotropin-releasing hormone (TRH) and some oxytocin (fig. S6A). Before any major weight gain, OGT deletion reduced the expression of TRH, as determined with in situ hybridization, in a subpopulation of cells in the PVN without affecting their viability, whereas several other known neuropeptides regulating feeding behavior were largely unaltered: proopiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), orexin, agouti-related peptide (AgRP), neuropeptide Y (NPY), vasopressin (AVP), and oxytocin (TRH, $n = 4$ WT mice, 40 ± 4.1 cells/ $150 \times 10^3 \mu\text{m}^2$; $n = 4$ OGT knockout mice, 23 ± 2.9 cells/ $150 \times 10^3 \mu\text{m}^2$) (Fig. 2B and figs. S6, B to E, and S7A) (12, 13). Next, we infected hypothalamic organotypic cultures with a virus that expresses green fluorescent protein (GFP) under the α CaMKII promoter and treated the explants with varying concentrations of glucose. The PVN retained its normal morphology and expression of neuropeptide during the extent of the experiment (~2.5 weeks) (fig. S8A). Immunohistochemistry for O-GlcNAc demonstrated that 1 hour of treatment with 5 mM glucose, simulating physiological and meal-derived fluctuations in glucose, increased O-GlcNAc in α CaMKII-positive cells [1 mM glucose, $n = 360$ cells, 134 ± 10 intensity/cell (arbitrary units, a.u.); 5 mM glucose $n = 330$ cells, 211 ± 12 intensity/cell (a.u.)] (Fig. 2C) (14–16). In contrast, there was no change in O-GlcNAc levels in neighboring α CaMKII-negative cells (fig. S8B). Incubation for 16 hours with 25 mM glucose raised the O-GlcNAc levels in α CaMKII cells to a larger extent [5 mM glucose, $n = 390$ cells, 156 ± 9 intensity/cell (a.u.); 25 mM glucose, $n = 375$ cells, 298 ± 14 intensity/cell (a.u.)] (fig. S8, C and D). Unlike cortex and the hippocampus, where O-GlcNAc levels mirror food intake, it has recently been reported that fasting increased O-GlcNAc in AgRP neurons, which has been proposed to be due to stimulation of OGT expression by ghrelin in these cells (6, 7, 15). In the PVN, fasting decreased O-GlcNAc, but only in α CaMKII-positive cells [α CaMKII-positive cells, fed mice $n = 455$ cells, 158 ± 5 intensity/cell (a.u.); starved mice $n = 259$ cells, 81 ± 6 intensity/cell (a.u.)] (fig. S8E). In addition, we compared the expression of the immediate early gene c-Fos in α CaMKII PVN cells in vivo in mice fed ad libitum or refed upon starvation. This enabled us to characterize cellular responses to food intake as mice start eating after a period of starvation. Food intake appeared to activate α CaMKII cells in the PVN, and loss of OGT blocked this activation completely (fig. S8, F and G).

O-GlcNAc is highly expressed in neuronal synapses in the brain (17). We crossed α CaMKII-CreER⁺ $\times$ OGT^{F1/WT} $\times$ TdTF1 mice to assess whether OGT regulates excitatory synaptic input onto α CaMKII PVN cells. If deleting OGT attenuates excitatory input, it would explain, at least in part, how OGT regulates the activity of these cells. The mean capacitance of labeled cells averaged ~15 pF and did not differ between WT and knockout mice (fig. S9A). The mean miniature excitatory post-synaptic current (mEPSC) frequency decreased by 72% in OGT knockout cells ($n = 6$ WT cells, 1.75 ± 0.11 Hz; $n = 6$ OGT knockout cells, 0.50 ± 0.10 Hz) (Fig. 3, A and B). The sharp decrease in mEPSC frequency suggests that OGT is essential for maintaining the number of functional excitatory synapses onto α CaMKII PVN neurons.

The mean mEPSC amplitude was also decreased, but to a much smaller degree ($n = 6$ WT cells 19.7 ± 1.2 pA; $n = 6$ knockout cells, 15.6 ± 0.9 pA) (Fig. 3, A and C). Neither the rise time nor the decay time of the EPSCs changed upon deletion of OGT (fig. S9, B and C).

Pharmacological inhibition of glutamatergic signaling in the PVN has previously been shown to elicit intense feeding (18).

Selectively deleting OGT in the α CaMKII cells of the PVN by means of stereotactic virus injection caused concurrent obesity and hyperphagia (Fig. 4, A to C, and fig. S9D). Because deletion of OGT in α CaMKII cells in the PVN prevents their feeding-induced activation and leads to hyperphagia, we predicted that stimulating those cells would decrease food intake. Activating α CaMKII PVN cells optogenetically decreased cumulative food consumption over 24 hours (baseline, $n = 11$ experiments (5 mice), 3.6 ± 0.14 g; 50 Hz, $n = 11$ experiments (5 mice), 2.6 ± 0.33 g) (Fig. 4, D and E) (19). The amount of food ingested per meal was smaller, whereas there was no significant effect on meal frequency (meal size, baseline, $n = 125$ meals, 0.25 ± 0.014 g; 50 Hz, $n = 110$ meals, 0.20 ± 0.014 g) (Fig. 4F and fig. S9E). In a second experiment, we started the stimulation at the onset of darkness after fasting the mice during the light phase. Fasting increased food intake, and 20 Hz optogenetic stimulation for 4 hours produced a significant decrease in food intake, whereas 50 Hz stimulation for 1 or 4 hours produced large, significant drops in feeding (fig. S9F). The decrease in meal size with 50 Hz stimulation was evident in the first meal, without any change in latency to initiation of feeding (fig. S9, G and H).

Together, our data favor a model in which the function of OGT is to couple energy intake with energy need, at least in part, by regulating the excitatory synaptic input in α CaMKII PVN cells (Fig. 4G). The select effect on meal termination suggests that OGT regulates satiation. α CaMKII neurons are often excitatory. Although vGlut2-positive excitatory neurons in the PVN decrease food intake, there appears to be only partial over-lap between α CaMKII and vGlut2 expression in the PVN (20, 21). Because glucose remains elevated in the cerebrospinal fluid more than 1 hour upon eating, the feeding-related changes in O-GlcNAc integrates nutrient availability on a time scale longer than a single meal (14, 22, 23). The brain promotes satiation by coordinating adipokines, reflecting body energy depots, and acute food-derived signals into circuits that turn off feeding. Rather than constituting a link in meal-to-meal satiety feedback loops, our observations suggest that OGT controls the threshold of such loops through its regulation of excitatory synaptic transmission onto α CaMKII PVN neurons. The observation that OGT knockout mice quickly reached a plateau in daily food intake supports this idea. Thresholding satiation between meals confers the behavioral advantage of stabilizing caloric intake over time so that the previous meal informs on the caloric need of the next. These data do not exclude additional, faster regulation of OGT activity and levels via metabolic hormones. These findings identify the regulation of excitatory synapses onto α CaMKII PVN neurons by OGT as an important mechanism underlying satiation, representing a potential medicinal target for human obesity.

Supplementary Material

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O.L., G.W.H., and R.L.H. designed all experiments; the optogenetics experiments were also designed by Y.A. and J.E.S., and the electrophysiological experiments were also designed by I.H. O.L. performed all experiments and

analyzed all data but the electrophysiology (I.H.) and the optogenetics (J.E.S.). The optogenetics data were analyzed by J.E.S., Y.A., and O.L. O.L., G.W.H., and R.L.H. wrote the manuscript. We thank G. Schütz for providing the $\alpha\text{CaMKII-CreER}^{T2}$ mice, J. L. Bedont for help with in situ hybridization experiments, and R. H. White for assistance with mating and genotyping. All data necessary to understand and assess the conclusions of the manuscript are in the body of the paper and in the supplementary materials. All primary data are archived on a secure server located in the Department of Neuroscience at Johns Hopkins University (JHU). All data will be made available upon request. G.W.H. receives a share of royalty received by the university on sales of the CTD 110.6 antibody, which are managed by JHU. The research was supported by NIH (grant R01NS036715 to R.L.H. and grants R01DK6167, N01-HV-00240, and P01HL107153 to G.W.H.) and the National Institute on Drug Abuse Intramural Research Program (Y.A.).

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Fig. 1. Acute deletion of OGT in α CaMKII-positive neurons causes hyperphagia-dependent obesity

(A) Photo of mice 4 weeks after OGT deletion. (B) Body weight time course [$n = 8$ WT mice, $n = 8$ OGT knockout mice; repeated-measures two-way analysis of variance (ANOVA) with post hoc Bonferroni test, $P < 0.05$]. (C) Daily food intake time course ($n = 6$ WT mice, $n = 6$ OGT knockout mice; repeated-measures two-way ANOVA with post hoc Bonferroni test, $P < 0.05$). (D) Body weight time course upon pair-feeding ($n = 4$ free mice, $n = 4$ restricted mice, $n = 3$ WT mice). (E) Food intake every 15 min from sample mice. Quantifications represent mean $\pm$ SEM.

Fig. 2. OGT-mediated hyperphagia is associated with feeding circuitry function in the PVN (A) α CaMKII expression within feeding circuitry (schematized from fig. S5A). (B) TRH expression. (Left) Image of probe staining. (Right) Quantification ($n = 4$ WT mice, $n = 4$ OGT knockout mice; two-tailed t test: $P < 0.05$). (C) (Left) GFP and O-GlcNAc in the PVN. (Right) Quantification of O-GlcNAc intensity in GFP-positive cells (1 mM, $n = 360$ cells, 5 mM, $n = 330$ cells; two-tailed t test: $P < 0.05$). Quantifications represent mean $\pm$ SEM.

Fig. 3. OGT regulates excitatory synaptic function in α CaMKII PVN neurons
 (A) Sample traces from WT and OGT knockout cells. (B) mEPSC frequency and (C) amplitude in α CaMKII-positive PVN neurons ($n = 6$ WT cells, $n = 6$ OGT knockout cells; two-tailed t test, $P < 0.05$). Quantifications represent mean $\pm$ SEM.

Fig. 4. OGT regulates feeding behavior in α CaMKII neurons of the PVN

(A) (Top) Schematic of stereotactic PVN injection. (Bottom) GFP expression in the PVN after stereotactic injection. (B) Body weight and (C) daily food intake time course ($n = 5$ WT mice, $n = 7$ OGT knockout mice; repeated-measures two-way ANOVA with post hoc Bonferroni test, $P < 0.05$). (D) Schematic of optogenetic setup with α CaMKII-driven expression of channelrhodopsin-2 in the PVN with the abutting laser. (E and F) Food intake after optogenetic stimulation. (E) 24 hours cumulative food intake. Bars represent average intake over all mice, and lines represent average intake per individual mouse (baseline, $n = 11$ experiments (5 mice); stimulation, $n = 11$ experiments (5 mice); two-tailed t test, $P < 0.05$). (F) Average meal size (baseline, $n = 125$ meals (5 mice); stimulation, $n = 110$ meals (5 mice); two-tailed t test, $P < 0.05$). (G) Model of OGT-dependent hyperphagia. Quantifications represent mean $\pm$ SEM.