Gastric *O*-acyl transferase activates hunger signal to the brain

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Some of us may remember from school biology lessons that goats are classified as ruminants because they have four stomachs. However, recent findings now demonstrate that most mammals have GOAT in their stomach. In this issue of PNAS, Gutierrez *et al.* identified and characterized the enzyme ghrelin *O*-acyl transferase (GOAT) that adds a fatty acid moiety to the gastric hormone ghrelin (1). This important discovery marks a major step forward in understanding the ghrelin signaling system and could have significant implications both for body weight regulation and nutrient–gene interactions.

About Ghrelin

Ghrelin is an orexigenic peptide hormone secreted mainly from the stomach and proximal small bowel (2, 3). It is cleaved from a larger precursor, preproghrelin, which bears a signal sequence dictating secretion into the circulation (2). Uniquely, ghrelin requires posttranslational modification in which the serine-3 hydroxy-containing residue is covalently linked to a medium-chain fatty acid, typically octanoate, through an ester bond. This is required for the peptide to bind to its receptor, GHSR1a. Most biological actions ascribed to ghrelin require this acylation (4), whereas desacyl ghrelin has as yet no defined biological action (2, 5, 6). Thus, GOAT may play a key role in the molecular regulation of energy metabolism, and also represent a prime drug target for the treatment of obesity and diabetes.

Discovery of GOAT

The discovery of GOAT can be traced back to the identification of a family of acyl transferases named MBOATs for membrane-bound *O*-acyl transferases that catalyze *O*-acylation reactions related to Wnt signaling (7). Wnt-secreting cells are known to require the action of a gene product called *porcupine*, which exhibits structural similarities to MBOATs and transfers acyl groups, such as palmitic acid, to a conserved cysteine residue (8, 9). Knowledge on Wnt signaling provided the essential framework and direction guiding scientists toward the discovery of GOAT. The development of a ghrelin octanoylation cell culture system by using the human medullary thyroid carcinoma cell line (TT cell line) (10), which secretes acylated ghrelin when supplemented with octanoic acid, was another crucial step for the successful identification of GOAT. The authors then used gene-silencing technology to identify the specific ghrelin acyl transferase from a pool of candidate genes with structural and functional features shared by known acylating enzymes. Knockdown of candidate 7, later confirmed as a member of the MBOAT family of acyl transferases, but not other candidate genes, greatly diminished octanoyl ghrelin synthesis. The predicted protein encoded by the longer transcript of candidate 7 was renamed the ghrelin *O*-acyl transferase, GOAT. Next, the ability of GOAT to octanoylate ghrelin was confirmed by transfection studies in human embryonic kidney (HEK-293) cells by demonstrating that cotransfection with GOAT and ghrelin led to secretion of octanoylated ghrelin. This octanoylation, confirmed by mass spectrometry fragmentation to occur exclusively at serine 3, appeared to be unique and specific to GOAT and could not be shown for other members of the MBOAT family of enzymes. Another interesting observation relates to the limited, but existing, capacity of GOAT to acylate ghrelin with other medium-chain fatty acids besides octanoate, ranging from acetate to tetradecanoic acid. Furthermore, the fact that GOAT is conserved across vertebrates and that mouse, rat, or zebrafish GOAT were all functionally able to faithfully octanoylate human ghrelin, further suggests that GOAT has an important physiological function in the regulation of ghrelin signaling. The detection of significant levels of GOAT transcripts primarily in stomach and pancreas with very low levels in other tissues of humans further connected GOAT with ghrelin production and secretion. However, the most convincing evidence that GOAT is essential for ghrelin acylation *in vivo* was provided by the finding of complete absence of octanoylated ghrelin in mice with a deletion of the GOAT gene.

Evidence suggests that long-term fasting inhibits ghrelin acylation.

In the February 2008 issue of *Cell*, Yang *et al.* (11) reported mouse MBOAT4 as the enzyme that octanoylates ghrelin GOAT. The authors provided evidence for the specificity of GOAT for ghrelin octanoylation and suggest that this enzyme is located within the endoplasmic reticulum (ER). The relative difference of the amount of GOAT mRNA and preproghrelin mRNA is ≈200-fold, which is consistent with the relative ratio of an enzyme and its substrate. It is of interest that GOAT mRNA was not found in mouse pancreas in that study.

Future Implications

Now, since the GOAT has been driven out of the woods, its appearance in the garden of obesity research brings new questions and opportunities. Ghrelin is not only the lone circulating factor to markedly trigger hunger, but it also represents the only hormone that is known to require *O*-acylation with octanoate to be biologically active. The physiologically relevant functions of acylated ghrelin and the putative absence of such for des-acylated ghrelin remain to be confirmed and clarified in detail. The GOAT knockout mouse presented by Gutierrez and colleagues is by definition an octanoyl-ghrelin-deficient mouse, but one that still has large amounts of des-octanoyl ghrelin. A careful comparison with the total ghrelin knockout mice could therefore provide insight into a possible physiological role for nonacylated ghrelin.

Other important open questions are how, by which mechanism(s), and in

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which physiologic situations GOAT is activated or blocked? It is well known that ghrelin levels rise before meals (12, 13) and decrease with food intake (14). Very recent evidence suggests that long-term fasting inhibits ghrelin acylation but not total secretion, whereas feeding suppresses both the acylated and des-acylated forms of ghrelin (15). It is plausible that metabolic conditions such as fasting and satiation modulate the activation and activity of GOAT by controlling its substrate (e.g., octanoic acid) and thereby the physiologic function of ghrelin.

A basic question arising from the works of Gutierrez *et al.* presented in this issue (1) and earlier from Yang and coworkers (11) is whether GOAT gets its substrate from diet-derived fatty acids or fatty acids mobilized from adipose stores. *De novo* synthesis of medium-chain fatty acids is minimal in most mammals. Therefore, it is likely that dietary lipids are a principle source of octanoate in the stomach, consistent with the findings of Nishi *et al.* (16). The results of Gutierrez and colleagues demonstrating that, like specific gastric cells, pancreatic tissues also express transcripts for ghrelin and GOAT in humans, raise the possibility of an endogenous production of octanoate in the GOAT-expressing or their surrounding cells. Another intriguing possibility would be that white adipose tissue mobilizes and releases fatty acids of suitable length for GOAT to activate ghrelin during fasting, which would be consistent with the known fact that circulating ghrelin levels increase during food deprivation.

Mice deficient for ghrelin or its receptor have been reported to show an energy balance relevant phenotype only on a high-fat diet, whereas ghrelin/ghrelin-receptor double-knockout mice show a leaner and more metabolically fit phenotype even on a standard diet (17–20), suggesting the potential existence of a second relevant ghrelin receptor. If this hypothesis is correct, traditional receptor antagonism may not be a superior strategy for altering endogenous ghrelin signaling. Pharmacologic interventions specifically targeting GOAT or ghrelin activation, on the other hand, may present a novel, elegant, and complete way to prevent and treat obesity by indirectly interfering with ghrelin signaling.

The role of ghrelin in the regulation of insulin secretion and insulin action remains a controversial topic. It has not been proven clearly that absence of ghrelin signaling alone improves insulin secretion or glucose homeostasis (20) and des-acylated ghrelin has recently been proposed to play an important role in pancreatic function (21). The loss-of-function GOAT mutants will provide a unique tool to delineate the important physiologic functions of the different isoforms of ghrelin in the pancreas as well as other tissues.

Another intriguing question that deserves attention is why ghrelin, among all other peptides, may be the only hormone that requires octanoylation to activate its receptor. Is this a specific modification important for the orexigenic properties of this gut hormone? What is the significance of the medium-chain fatty acid specificity in ghrelin octanoylation? Furthermore, is octanoylation the key mechanism by which ghrelin activity or tissue distribution is controlled? The discovery of GOAT marks a new beginning of an exciting time for ghrelin research. With better characterization of this enzyme and the availability of GOAT mutant models, our understanding of the physiologic functions of ghrelin, especially des-acylated ghrelin, should be making considerable progress.

It will take time and further study to determine whether MBOAT4 will be a promising target for drugs to treat obesity. Until then however, at least we now know a scapeGOAT we can blame for our next midnight binge.

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