GOAT: A Stomach Enzyme That Whets Our Appetite

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The discovery of ghrelin O-acetyltransferase (GOAT) is the last – but not least – chapter of a long story which started around 1980, when Cyril Bowers and colleagues [1] discovered that certain opioid peptide derivatives had weak growth hormone (GH) releasing activity. Further studies led to the identification of several synthetic peptides that potently stimulated GH release, although the receptor responsible for mediating these effects remained unknown. This receptor, named growth hormone secretagogue receptor (GHS-R), was finally discovered in 1996 by Roy Smith and colleagues [2] and identified as a typical G-protein-coupled receptor.

With the use of the ‘orphan receptor strategy’, the endogenous ligand for the GHS-R1a was subsequently identified by Kojima et al. [3] in 1999 and named ‘ghrelin’. Ghrelin demonstrated GH-releasing properties similar to the synthetic GHSs. However, what has brought this hormone and the GHS-R to the forefront of metabolic research are the additional properties of ghrelin to stimulate food intake and promote adiposity [4]. Thus, the endogenous ghrelin system today is less of a target for GH-related therapies, but is an important basis for potential drugs regulating energy metabolism and body mass. Genetic models support those pharmacological data as it has been described that both ghrelin and GHS-R ‘knock-out’ mice are resistant to high fat diet [5–7].

Ghrelin is a 28-amino acid peptide hormone mainly secreted by the stomach, which has two unique characteristics: It is the only circulating peptide hormone to increase food intake, and its serine-3 residue has a hydroxyl group that requires acylation by n-octanoic acid in order to allow ghrelin to bind and activate its only known receptor [3]. This acylation has been shown to be necessary for most of the biological actions of ghrelin. Although the major active form of ghrelin possesses an octanoylation at the serine-3 position, the vast majority (80–90%) of circulating ghrelin has been found to be non-acylated. However, this result may be biased by the less than opti-

mal sample collection methods and imperfect ghrelin immunoassays. Based on available information, the predominant form of serum ghrelin is desacyl ghrelin. Neither its putative functions nor a possibly existing specific receptor for this form of ghrelin are known [8]. An extensive search for the enzyme responsible for the acylation and activation of ghrelin had been ongoing in numerous laboratories since ghrelin was discovered in 1999. Very recently, two independent teams of scientists using two similar approaches were successful and have identified GOAT as the specific acyl transferase that activates ghrelin.

Yang et al. [9] generated several cell cultures producing only desacyl-ghrelin by cDNA transfection. In a second step they co-transfected these cells with cDNA of 16 members of a family of related enzymes, the membrane-bound O-acyltransferases (MBOATs) which catalyze O-acylation reactions related to Wnt signaling. They measured whether the cells were able to produce acyl-ghrelin after being transfected with one of the candidates’ genes and demonstrated that MBOAT 4 was the only candidate producing acylated ghrelin at the expected serine-3 position in the cell culture. Thus, MBOAT 4 was re-named GOAT.

At the same time, Gutierrez et al. [10] used gene silencing technology to identify the acyl transferase. They took advantage of the fact that TT cells derived from human medullary carcinoma cells were able to produce acyl-ghrelin when octanoic acid is added to the culture medium. The group designed silencing RNAs (siRNAs) for 12 possible candidate genes to knock down each candidate at a time in the TT cells. When treating the cell culture with siRNA of MBOAT 4, but not other candidates, a significant decrease in ghrelin acylation occurred, indicating that this enzyme was crucial for the modification of ghrelin, which they also re-named GOAT. Using human embryonic kidney (HEK-293) cells co-transfected with GOAT and ghrelin they also showed that GOAT is

Fig. 1. Scientific history of the ghrelin system.

able to produce ghrelin that is modified with an octanoic acid at exactly the 3rd serine residue. Further, the generation of GOAT deficient mice verified that GOAT is the only enzyme capable of acylating ghrelin since acyl-ghrelin is completely absent in GOAT 'knock-out' mice.

Like most new and exciting observations, the current studies prompt many questions. One of the most basic questions is how GOAT expression and activity may be regulated under physiological conditions. It is known that long-term fasting inhibits ghrelin acylation, even though total ghrelin levels are increased. Further studies must address whether GOAT is influenced by hunger and nutrient availability and if its activity follows ghrelin's distinct secretion pattern. Another issue that is not clear yet is the precise location within the cell where GOAT becomes active. All members of the superfamily

MBOAT have membrane-spanning regions. Thus it is plausible to speculate that GOAT is located in the endoplasmic reticulum, but this hypothesis still needs to be tested. A very interesting observation in the paper of Gutierrez et al. [8] is that in humans GOAT expression in the pancreas exceeds its levels in the stomach while GOAT can hardly be detected in the pancreas of mice. If further studies corroborate that human GOAT activity correlates with its expression, this data would suggest that the activation of ghrelin might occur mainly in the pancreas. Although there are several studies suggesting that ghrelin influences insulin secretion [8]. This issue remains a controversial topic with numerous unanswered questions as some reports have shown that desacyl-ghrelin may also holds some physiologic functions in the regulation of insulin secretion or action. Studying the regulation and physio-

logical function of GOAT in the pancreas under several conditions, including insulin deficiency or insulin resistance, will help to clarify the role of ghrelin in glucose homeostasis. Indeed, the identification of GOAT will address some of those questions about the different roles of acyl- versus desacyl-ghrelin. In this sense, the characterization of the GOAT 'knock-out' mouse, which is basically an acyl-ghrelin deficient mouse, represents an excellent approach to differentiate between the actions of acyl- and desacyl-ghrelin, particularly when comparing this model to ghrelin and GHS-R deficient mice.

There are also some new hopes about potential pharmacological opportunities arising from the discovery of GOAT. Several approaches involving ghrelin and its receptor, such as a ghrelin vaccine [11] and GHS-R antagonists [8], are being investigated by for-profit enterprises. If octanoylated ghrelin is responsible for the increase in feeding behavior and adiposity, would GOAT inhibitors represent an elegant approach to suppress food consumption and/or decrease fat mass storage? One of the advantages of GOAT is that ghrelin is the only one peptide involved in energy balance that needs to be octanoylated for its biological functions. Therefore, GOAT is expected to be a specific target. This makes GOAT a unique and readily

accessible peripheral target as most of the pharmacological targets in the treatment of obesity are involved in different functions and different molecular mechanisms or located in the central nervous system. Even with the help of GOAT, the research community still has a long way to go in order to understand how the ghrelin system works or if a pharmacological cure for the metabolic syndrome is possible. Until then, GOAT will continue to whet the scientific appetite of researchers studying obesity, diabetes, and nutrition related diseases.

Acknowledgements

The authors are indebted to Matthias Tschöp, Carlos Dieguez and Miguel Lopez for their suggestions and criticisms of this paper. H.K is funded by a fellowship of the International Leibniz Graduate College. R.N. is currently recipient of a Marie Curie Outgoing International Fellowship.

Conflicts of Interest

None.

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On the Contents of This Issue

The third issue of *OBSESITY FACTS* covers a broad range of topics. First of all, let's speak about financial aspects. Højgaard et al. [1] calculated the effect of an increased waist circumference at baseline for health care costs during the following 7 years. Not unexpectedly, waist circumference at baseline predicted higher future health care costs. Every centimeter in males and females resulted in increased health costs of 2.08 and 1.25%, respectively. Several similar studies are available for BMI; it would have been of interest to determine to what extent waist circumference predicts future health costs upon adjustment for BMI.

The major complex disorder that is associated with obesity is type 2 diabetes mellitus (T2DM). Hans-Georg Joost [2] takes an in-depth look at the pathogenesis of T2DM. Based on the extensive review of the etiological factors, Joost addresses both risk assessment and prevention. He correctly points out that genotyping the currently known single nucleotide polymorphisms (SNPs), which predict an increased risk for T2DM, is of limited value only in older individuals; however, if phenotypic risk factors and biomarkers have not become evident, genotyping can allow a risk assessment and theoretically a personalized prevention. It will be necessary to determine if informed individuals are able to decrease their risk of T2DM. An increased intraabdominal fat mass leading to an increased waist circumference is the major phenotypical risk factor; quite evidently, T2DM is responsible for a substantial proportion of the health care costs due to increased waist circumference [1].

Mutations in the melanocortin-4 receptor gene (MC4R) can be found in 1–6% of individuals with obesity. The differing rates are seemingly due to samples of individuals with more or less severe obesity; furthermore, current evidence indicates that rates are higher among children than among adults. We are somewhat hesitant to speak of a monogenic form of obesity; male mutation carriers have a 4.5 kg/m² higher BMI than their wild type family members; the effect is seemingly twice as strong in females (9 kg/m² [3]). Tarnow et al. [4] have performed an in-depth in vitro study of the complete loss-of-function mutation Ser136Phe,

which was identified in an Austrian patient. Based on assessment of dimerization investigated by ELISA and FRET, the authors conclude that this mutation shows a dominant negative effect, further substantiating that dominant negativity is one of the effects MC4R mutations can induce.

Kring et al. [5] performed extensive molecular genetic studies of known candidate genes for obesity. For this purpose, 234 obese men and a non-obese group (n = 323) were genotyped. The fascinating aspect of this study is that these individuals were originally assessed at their draft board examination and then re-examined at the end of their forties. Apart from BMI, several obesity-related phenotypes were assessed. For three candidate genes, association to specific phenotypes was detected. Because none of these effects was significant upon correction for multiple testing, the authors recommend independent studies based on the hypotheses derived from these explorative analyses.

For the first time, we include a book review in this current issue. Please feel free to send us book reviews, which you deem appropriate for the readership of *OBSESITY FACTS*.

And last but not least, we thank Henriette Kirchner and Ruben Nogueiras for their stimulating editorial on GOAT. We encourage you to contact us if you wish to write an editorial on novel groundbreaking research.

Johannes Hebebrand, Essen

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