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Review

The discovery of ghrelin — A personal memory

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Abstract

The endogenous ligand for growth-hormone secretagogue receptor (GHS-R) was purified from the stomach and named it "ghrelin," after the word root "ghre" in Proto-Indo-European languages meaning "grow", since ghrelin has potent growth hormone (GH) releasing activity. Ghrelin plays important roles for maintaining growth hormone release and energy homeostasis in vertebrates. In this essay, I described my personal memory on the discovery of ghrelin in the year 1999.
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Keywords: Ghrelin; Stomach; Acyl-modification; Octanoic acid; GHS-R

Contents

1. A novel hormone from stomach

It was on the early morning of October 11, 1999, a national holiday in Japan, that I received the fax from Nature office announcing the acceptance of our paper on ghrelin. The news that our paper on ghrelin would soon be published for the world filled me not with joy, but relief. The reviewers and editors at Nature ranked our paper highly and welcomed the opportunity to publish details on our discovery of ghrelin, a novel growth-hormone-releasing peptide. However, every research paper has its own laborious history before publication.

Ghrelin was discovered from stomach by us as the endogenous ligand for the GHS-R (growth hormone secretagogue receptor), an orphan receptor at the time when we had been searching for its ligand [1,2]. The structure of ghrelin is unprecedented; ghrelin is a peptide hormone, in which the serine 3 (Ser3) is *n*-octanoylated and this modification is essential for ghrelin's activity (Fig. 1). Ghrelin is secreted from the stomach and circulates in the blood to activate the release of growth hormone from pituitary and stimulate appetite by acting on the hypothalamic arcuate nucleus [3–5]. Our Nature paper reports two important points. First, it reports a novel growth-hormone-releasing peptide from stomach, an organ with no previously recognized role in the control of GH release. Second, it reports that the activity of ghrelin is entirely dependent on the octanoyl-modification of ghrelin. The puzzles leading to these discoveries were the biggest hurdles in our research, and hence the crux of the essay you are now reading.

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2. Ligand search

From the beginning of 1998 we began searching for the yet-to-be discovered endogenous ligands of several orphan GPCRs. Among those we sought, we looked upon GHS-R (growth hormone secretagogue receptor) as somehow an exception. While most of these orphan receptors lacked specific activator, however, GHS-R had GHSs, a group of specific activators composed of synthetic compounds that stimulate GH release [6]. This means that we can monitor the GHS-R assay system whether this system works well or not. We and probably other research groups elsewhere in the world had been searching for the ligand from brain. This was the natural site to target, as GHS-R is expressed in both the hypothalamus and pituitary. While we had succeeded in identifying several peptides that activate GHS-R, however, these peptides were invariably protein fragments such as purkinje cell protein 2 or myelin basic protein. Their activities were far too low to qualify them as actual ligands for GHS-R.

By January 1999, almost a year after beginning our hunt for the GHS-R ligand, we still hadn't come up with any leads. I and Hiroshi, a graduate student who had been working with me from 1998, had labored step by step with our chromatography and completed more than 500 assays without any hint of the ligand. We began to toy with the notion that we should change the target tissue from brain to other tissues. But this required courage: we, and perhaps others, had assumed from the distribution pattern of the GHS-R that the brain tissue was the probable production site for the endogenous ligand.

Another group of researchers had recently discovered GPR38, a GPCR with a high homology to GHS-R [7]. GPR38 was initially reported as a kind of orphan GPCR, though later it was identified as the motilin receptor [8]. Caught in a total deadlock, I speculated that the endogenous ligands of GHS-R and GPR38, receptors with highly similar amino acid structures, might cross react. If I could find the GPR38 ligand, I ventured, perhaps I could glean hints on where and how to find the GHS-R ligand. With luck, I could discover the GHS-R ligand from a homology search on genome or EST databases.

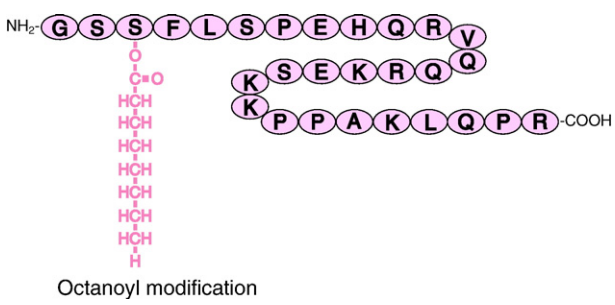

Fig. 1. Structure of human ghrelin. Human ghrelin is a 28-amino acid peptide hormone, in which the 3rd amino acid Ser is modified by *n*-octanoic acid. Misleadingly, the size of the figure gives the impression that the octanoyl-modification is very large. The octanoic acid, which has 8 carbons, is actually identical in size to a single amino acid such as leucine, which has 6 carbons.

3. Stomach

GPR38 is expressed at high levels in the stomach and thyroid tissues. Noting this, we began to assay for GHS-R expressing cells with stomach extract. Fig. 2 shows the result of our first assay of gel-filtration chromatography using a peptide extract from the stomach, performed on February 9, 1999. We observed abundant levels of intracellular calcium release in the all fractions. The results were curious, however, we thought that the cells had undergone some deleterious change by the stomach samples. In a similar experiment performed just a week earlier using another batch of orphan GPCR expressing cells, all of the assay fractions exhibited elevated calcium concentrations due to cell puncture. Speculating that an active peptide in the stomach may have damaged the cells, we put aside the stomach extracts and began targeting other tissues and organs. Success continued to elude us, however. Unable to detect activity wherever we looked, we pored over our daily results and ceaselessly re-examined the data from earlier assays.

Working on a hunch that the stomach may have the ligand, we tried again three weeks later, but with only a tenth of the stomach sample we had used before. Again we failed. Dejectedly, we set the sample aside. Surely, we concluded, the stomach contained an unknown peptide that damaged the cells.

A month later, in the middle of March, just as we were about to give up our search, I decided to re-examined all of our data on the stomach assay to search fro a clue. It occurred to me then that the stomach might contain very high amounts of the ligand for the GHS-R. The best way to test the notion, I realized, was to repeat the assay with a still lower amount of stomach extract.

Finally, we discovered that the calcium increasing activity was focused to a single region at a molecular weight of 3000–4000 (Fig. 3). In the previous assay we had used far too much stomach sample, pushing the activity in all of the fractions outside the measurable range. Unexpectedly, the stomach contained excessive levels of the endogenous ligand. Only several mg of the stomach extract was sufficient for detecting the activity. With great surprise we had discovered a novel growth-hormone-releasing peptide in the stomach! Admittedly, our surprise was mixed with some vexation. It was disheartening to see in retrospect how our judgments of assays had been clouded by two mistaken notions: first, that the ligand would exist only at very low concentration; second, that GH release was controlled only in the brain.

Encouraged anew, we happily set about purifying the ligand and soon succeeded. On May 30, after a mere 10 days, the ligand had been purified from just a few milligrams of stomach tissue. We fretted that other groups had surely succeeded in the same feat already: the high concentrations of the ligand in the stomach would have made that easy. With trepidation, we scanned the contents for mentions of the GHS-R ligand in the latest journals. Before setting off for my lab every morning, I went online to check the electronic editions of scientific magazines such as Nature, Science and Cell. If another group published the same discovery, our effort would have been in vain.

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