Ghrelin is a newly discovered peptide hormone produced by the stomach that displays potent growth hormone-releasing activity and a stimulatory effect on food intake and digestive function while reducing energy expenditure. The isolation of ghrelin has led to new insights into how this gastric hormone links the endocrine control of nutritional homeostasis with growth hormone secretion and gastrointestinal motility through gut-brain interactions.

Unraveling the role of the brain signals that contribute to the regulation of body growth, food intake, and energy homeostasis has been the focus of intense investigation over the past several years. Growth hormone (GH), produced and released from the pituitary gland, is known as the master anabolic hormone that regulates the numerous phases of metabolism of proteins, fats, sugars, and minerals in mammals. Until recently, the secretion of GH was assumed to be physiologically regulated by the opposite actions of two key hypothalamic peptides: stimulation by GH-releasing hormone (GHRH) and inhibition by somatostatin (14). It is also well accepted that feeding behavior is regulated by complex mechanisms in the central nervous system, particularly at the level of the hypothalamus. Recently, a novel peptide hormone, ghrelin (a name derived from the Proto-Indo-European roots “ghre” for grow and “relin” for release), has been identified. The peptide is primarily synthesized in the stomach and released into the bloodstream to exert potent GH-releasing and appetite-promoting actions (10). The discovery of ghrelin provided new molecular insights into the physiological signaling mechanisms released by peripheral tissues connecting the gut to the brain to regulate GH secretion, feeding behavior, energy balance, and digestive function (5).

The topsy-turvy finding of an endogenous peptide ligand

In the course of searching for elusive endogenous GH-releasing factor, Cyril Bowers unexpectedly observed in 1977 that specific chemical modifications of the structure of the pentapeptide enkephalin could significantly improve the opioid peptide’s GH-releasing properties in vitro (3). In 1980, as a result of complex conformational energy calculations, Bowers’ group described the first so-called GH-releasing peptide (GHRP)-6, a synthetic hexapeptide that was a potent GH secretagogue (GHS) both in vitro and in vivo. GHRP-6 became the lead compound at the origin of the development of other GHSs. These are a family of small synthetic peptides and non-peptide molecules (MK-0677) that stimulate GH secretion in various animals (4).

The interest in GHSs temporarily dimmed in 1982 with the discovery of the hypothalamic peptide GHRH. GHRH and GHSs were found to operate through distinct G protein-coupled receptors and to display a synergistic rather than an additive effect on GH release. Signal transduction pathways activated by GHS ligands resulted in the increase of intracellular calcium ion concentration in pituitary cells, whereas GHRH increased cAMP (13). These findings gave rise to the hypothesis that a third control mechanism, independent of GHRH and somatostatin, might be involved in the regulation of GH secretion through activation of GHS receptors (5). At the end of 1999, Kangawa’s group developed a stable Chinese hamster ovary (CHO) cell line expressing the cloned GHS receptor to monitor changes in intracellular calcium concentration that were induced by rat tissue extracts from brain, lung, heart, kidney, intestine, and stomach. They observed that the intracellular calcium influx resulting from GHS receptor activation reached maximum values with the stomach extract. This extract was purified by gel filtration and HPLC chromatographic techniques and led to the isolation of a novel acylated peptide that was then named ghrelin. It was soon recognized throughout the scientific community that ghrelin undoubtedly corresponded to the endogenous GHS peptide at the origin of non-GHRH and nonsomatostatin GH regulation. Folwaczny et al. (6) simultaneously isolated a cDNA from a mouse stomach library and found a protein that they called prepromotilin-related peptide that was identical to the ghrelin precursor preproghrelin. Usually the development of analogs is guided by the discovery of new factors or hormones. The case of ghrelin is an example of reversed pharmacology initiated by the invention of synthetic analogs leading to the isolation of the natural ligand via the characterization of the natural receptor.

An octanoylated serine peptide and alternative splicing within the coding region: a world premiere

One exciting aspect of ghrelin is its unique structural characteristics. Ever since the first reports on the potent stimulatory action of GHRP-6 on GH release, it had been postulated that endogenous GHRP, if it really existed, was at least as hydrophobic a molecule as the synthetic peptides from the
The endocrine cells of the stomach are the main source of circulating ghrelin

The stomach is the major source of circulating ghrelin as determined by peptide quantification in the different regions of the gut and by the 70% reduction of plasma levels after gastrectomy in rodents and humans (7). Ghrelin expression is not restricted to the gastrointestinal tract and is present also in the pituitary, hypothalamus, heart, kidney, immune cells, and placenta, although in relatively low amounts (5). Gastric ghrelin is produced in the chromogranin A-immunoreactive X/A-like endocrine cells located in the mucosal layer of the fundus. These cells are closely associated with capillary networks, allowing secreted ghrelin to enter into the bloodstream and to exert a classical endocrine action. Before the discovery of ghrelin, the hormone released from X/A cells was unknown. Now X/A cells that produce ghrelin are called “grl cells” (5).
basal GH levels (13). Functional as well as anatomic studies support an inhibitory action of somatostatin on ghrelin/GHS-induced GH release exerted at both the hypothalamic and pituitary levels (19).

Ghrelin is a powerful orexigenic and adipogenic agent

In 1996, Okada et al. (12) were the first to report a short-lived increase in food intake after intracerebroventricular injection of the synthetic GHRP analog KP-102 in rats. With the discovery of ghrelin, it was logical to test the biological activity of the corresponding natural peptide on appetite and weight control. Experimental and clinical studies established that ghrelin administration induces a rapid increase in food intake in rodents and humans and initiates the sensation of hunger in humans (10). Although a number of other potent orexigenic peptides, including neuropeptide Y (NPY), agouti-related peptide (AgRP), and melanin-concentrating hormone have been previously characterized in the brain, ghrelin is the first food intake-stimulating signal originating from the stomach. Repeated injections of ghrelin cause weight gain in rodents that is not linked to changes in longitudinal skeletal growth or lean (muscle) mass and is linked to the increase in fat mass due to the reduction in fat utilization.

The physiological role of ghrelin is suggested by the reports that anti-ghrelin antibodies significantly inhibited starvation and natural, dark phase-induced food intake. In addition, suppression of the GHS receptors in the rat Arc nucleus reduced body weight and adipose tissue compared with controls (13). In adult humans, plasma ghrelin levels rise twofold before a meal and decline to trough levels within 1 h after eating. Likewise, ghrelin levels are elevated by fasting and decreased after refeeding in rats. In cases in which negative energy balance is normally observed, such as low-caloric diets, chronic exercise, cancer anorexia, anorexia nervosa, and Prader-Willi syndrome, levels of ghrelin were reported to be increased (10). In anorexic patients, ghrelin levels reached as much as twice the values found in normal patients. Partial weight gain in anorexic patients stabilizes ghrelin levels (16). In human obesity, ghrelin level is low, which may be related to the high caloric intake, whereas reduction of body weight in obese patients brings the ghrelin level up. Interestingly, although ghrelin levels are reported to be high in patients undergoing extreme diets, stomach bypass surgery decreased ghrelin levels, suggesting that the size of the stomach may be directly correlated to ghrelin levels (5).

Ghrelin coordinates stimulation of digestive function and enhanced appetite

The similarities of ghrelin with motilin at the ligand (36%) and receptor (50%) levels (6) led to studies on the effects of ghrelin on gastric motility (10). Intravenous injection of ghrelin in rats increases the amplitude of gastric motility. The gastric acid secretion is also stimulated by ghrelin injected both centrally and peripherally in rats. These effects are abolished by either atropine pretreatment or vagotomy, which are methods to chemically or surgically block vagal cholinergic input from the brain to the gastrointestinal tract. Circulating ghrelin levels are correlated with gastric emptying in humans, and ghrelin administration accelerates the gastric emptying of solid food after overnight fasting in mice and of artificial liquid food in rats. Ghrelin also normalizes gastric emptying delayed by abdominal surgery in rats. Although ghrelin increases GH release, there is no report that GH stimulates gastric function. Therefore, the increased gastric function after ghrelin injection is not secondary to increased GH release. Together, these observations suggest that the ghrelin hormone is a strong gastrokinetic agent (9) that links endocrine control of energy balance and growth with the regulation of digestive function.

Ghrelin as an endocrine signal to the hypothalamus

The Arc nucleus is a major hypothalamic site regulating food intake and body weight through the presence of popula-
tions of neurons containing orexigenic (NPY and AgRP) and anorexic (pro-opiomelanocortin and cocaine amphetamine-regulated transcript) peptides. In the brain, the GHS receptor gene is abundantly expressed in the hypothalamic Arc nucleus and ventromedial nucleus. Existing evidence supports a key role of the Arc nucleus in the orexigenic effects of ghrelin (Fig. 2). The chemical ablation of the Arc nucleus blocked ghrelin-induced food intake and attenuated the GH secretion. Selective disruption of the GHS receptors in the Arc by antisense GHS receptor mRNA had a similar blunting effect on ghrelin actions (15). Moreover, ghrelin injected centrally stimulated food intake in spontaneous dwarf rats, a GH-deficient model. These studies emphasized not only the importance of the Arc in the control of appetite but also that ghrelin’s regulatory effect on appetite is not essentially related to GH release. A key role of Arc NPY/AgRP neurons in the orexigenic effect of ghrelin is suggested by the expression of c-Fos and Egr-1, two markers of neuronal activity, in NPY-containing neurons after peripheral administration of the peptide (20) (Fig. 3). There is also an increase in both NPY and AgRP mRNA expression in rodents induced by peripheral injection of ghrelin. In addition, a nonpeptide NPY Y1 receptor antagonist and specific antiserum against NPY and AgRP (10) suppress ghrelin orexigenic action. However, this view is likely to be oversimplified, and additional mechanisms are also emerging in the context of the complex interplay between hypothalamic transmitters regulating food intake and energy homeostasis (10, 15).

The pathways through which gastric ghrelin released into the bloodstream signals the hypothalamus are still not clearly understood. There is anatomic evidence derived from studies on the capillary network in the Arc nucleus that part of the parenchyma in this nucleus can be exposed to circulating neurotransmitter substances. Pharmacokinetic studies also showed that human ghrelin crosses the blood-brain barrier as an intact molecule by a saturable transport in mice. Although passage for des-octanoyl mouse ghrelin was observed in the blood-to-brain direction, the octanoylated (bioactive) mouse ghrelin crosses the mouse barrier predominantly in the brain-to-blood direction (2). The extent and direction in which ghrelin can cross the blood-brain barrier is therefore influenced by at least two features of its primary structure: its posttranslationally added fatty acid side chain and its amino acid sequence.

**The clinical potential of ghrelin and related molecules**

Since their development, GHSs have raised interest due to their pharmacokinetic properties and high bioavailability following administration via oral or parenteral routes. Like GHSs, several ghrelin analogs have already been pinpointed with potential usefulness both as a treatment modality of GH deficiency and as an endocrine diagnostic tool. Activation of GHS receptors by ligands offers promising alternatives to exogenous GH in various clinical applications related to GH alterations, including those related to reversing the aging process. The mechanisms underlying the reduced GH secretion phenomenon in aging humans are complex. It seems, however, that impaired pituitary function does not play a major role and that the aged pituitary capacity to secrete GH can be restored under the influence of proper hypothalamic impulse (11). It is generally agreed that ghrelin, like GHRH analogs, can induce a more physiological profile of GH levels in plasma than subcutaneously administered recombinant GH. Furthermore, the synergistic effect of ghrelin coadministered with GHRH is currently being investigated to develop novel diagnostic and therapeutic tools (5).

As for a number of clinically relevant peptides, two main trends are underlying the quest for novel ghrelin analogs: modified peptides and nonpeptide analogs. Clinical use of the former implies the development of sophisticated and costly delivery systems with the purpose of overcoming the lack of stability and bioavailability of peptides. On the other hand, it is generally accepted that natural peptides and analogs are less questionable regarding their potential toxicity and side effects than related peptidomimetics might be. In that respect, ghrelin is a unique case among natural peptides, being more prone to clinical applications than other existing peptides. This is due to its unique alkylated serine residue in position which acts both as an effective shield against protease degradation and as a convenient integrated transport system. The properties of ghrelin would represent unprecedented cases for a peripheral peptide and stimulate research efforts to develop other peripheral administered, centrally acting peptides that could act as neuroactive drugs.

In summary, in light of the most recent findings, ghrelin is now emerging as a key player. The peptide integrates the control of food intake with digestive process as well as the assimilation of nutrients into fat coupled with the release of GH, an anabolic hormone involved in skeletal muscle growth and metabolic homeostasis. The fact that ghrelin is a circulating hormone produced by the stomach and regulated by the feeding status provides a new perspective on the existence of a gastric-hypothalamic-pituitary axis that relays information to maintain nutritional intake and energy balance. Further studies are needed to provide a link between ghrelin and other...
peptides involved in the complex process of feeding and metabolism. Obviously, the development of selective ghrelin antagonists currently represents an important venue to assess the physiological role of ghrelin under pathophysiological conditions and possible therapeutic venue for obesity.

References


