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'As the spectrum of biological activities of ghrelin expands, potential clinical targets, mimetics and perhaps antagonists of grehlin are beginning to emerge.'

Physiological, pathological and potential therapeutic roles of ghrelin

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Ghrelin, a hormone that is produced mainly by the stomach, was identified originally as the endogenous ligand of the growth hormone secretagogue (GHS) receptor. Ghrelin might also be synthesized in other organs, where it might have autocrine or paracrine effects. GHS receptors are present in tissues other than the hypothalamus and pituitary, which indicates that ghrelin has other effects in addition to stimulating the release of growth hormone. Recently, it has been suggested that ghrelin might be involved in the pathogenesis of many diseases and be a therapeutic target in these diseases. Here, we provide an overview of the physiological effects of ghrelin and of its pathological and potential therapeutic roles.

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Introduction

Ghrelin is a recently discovered hormone that, through a process of reverse pharmacology, has been shown to be a natural ligand of the orphan growth hormone secretagogue (GHS) receptor type 1a (GHS-R1a) [1]. Similar to synthetic GHSs (e.g. GHRP-6, MK0667 and hexarelin), ghrelin strongly stimulates the release of growth hormone (GH) in both animals and humans [2]. GHS receptors are present in the hypothamulus and pituitary, which is consistent with the GH-releasing effect of ghrelin [3]. However, GHS receptors are also present in other areas of the CNS and in peripheral tissues, which indicates that ghrelin has effects in addition to the release of GH [4–9]. Indeed, ghrelin modulates appetite, energy and glucose homeostasis, gastrointestinal, cardiovascular, pulmonary and immune functions, cell proliferation and bone physiology [4–9].

In addition, based on the alteration of either ghrelin concentration or expression of its receptor, studies implicate ghrelin in the pathogenesis of many diseases, including GH deficiency, cachexia, anorexia, obesity, diabetes, metabolic syndrome, polycystic ovary syndrome (PCOS), gastroparesis, gastric ulcer, atherosclerosis, hypertension, chronic heart failure, dilated cardiomyopathy, sepsis and osteoporosis [4–9]. In many of these diseases, modulation of ghrelin activity is beneficial, so it might represent a novel therapeutic target [4–9].

Here, we give an overview the physiological effects of ghrelin, relating these to pathological and potential therapeutic roles. As we begin to understand the biological and pathological activities of ghrelin better, additional clinical applications for ghrelin agonists and antagonists will emerge.

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Structure and production of ghrelin

The gene that encodes human preproghrelin, which is located on chromosome 3p25–26, consists of five exons and four introns [7–9]. Spliced ghrelin mRNA is translated to a 117-amino acid precursor preproghrelin, which is subsequently cleaved to yield ghrelin [7–9]. Ghrelin is a peptide of 28 amino acids that, under the influence of an unidentified acyl-transferase, is subject to a unique esterification. Typically this involves an octanoic acid at Ser3, although other types of esterification have been observed [1,7,8]. This esterification is essential for binding to GHS-R1a and it might also influence the transport of ghrelin across the blood–brain barrier [10,11]. Ghrelin is highly conserved in mammals, in particular the ten amino acids at the N terminus, and has significant homology with motilin [7–9]. Naturally occurring variants of ghrelin include des-Gln ghrelin, which lacks Gln at residue 14 and is produced by alternative splicing of the same gene, and des-acyl ghrelin, which lacks octanoic acid at Ser3 [12–14]. Des-Gln ghrelin is present in smaller amounts in the serum and is as active as full-length ghrelin [12]. Des-acyl ghrelin constitutes most circulating ghrelin and mediates no effects through GHS-R1a because it cannot bind to this receptor [13,14]. However, recent research shows that des-acyl ghrelin has physiological effects that are mediated by receptors other than GHS-R1a, including inhibition of glucose output by primary hepatocytes [15], inhibition of lipolysis [16], promotion of adipogenesis [17], inhibition of cell apoptosis [18], beneficial cardiotropic effects and vasodilation [19,20], stimulation of food intake [21], and relaxation of iris muscles [22]. Recently, obestatin has added further complexity to ghrelin physiology. Obestatin is a novel hormone that is derived from the gene that encodes ghrelin and seems to have functionally opposite effects to those of ghrelin on energy homeostasis, gastrointestinal function (although this has been questioned in recent studies), anxiety and hunger. However, both these related peptides increase memory retention [23,24].

Two-thirds of circulating ghrelin are produced by X/A-like cells of the oxyntic mucosa of the stomach and most of the remainder originates in X/A-like cells of the small intestine [25]. However, lower amounts of ghrelin are also produced in other organs, such as pancreas, kidney, placenta, lymphatic tissue, gonads, thyroid, adrenal, heart, lung, eye, pituitary and hypothalamus, and in various neoplastic tissues and cancer-cell lines [4–9,22,26]. The ghrelin produced in these tissues might have autocrine/paracrine effects, but endocrine effects are unlikely.

After collection of blood specimens, EDTA and aprotinin should be added and the plasma fraction separated by centrifugation and acidified immediately before freezing at −70 °C to ensure the stability of acylated ghrelin during storage [27]. Ghrelin concentration is usually measured by radioimmunoassay (RIA) [28]. Although the most commonly used RIA recognizes acylated and non-acylated ghrelin (which accounts for >90% of total circulating ghrelin), a RIA specific to acylated ghrelin is available [28]. Assays that measure specific forms of ghrelin might be more useful in determining its physiological role than those that detect both acylated and des-acylated forms, because the ratio of acylated:non-acylated ghrelin is modified in some physiological and pathological conditions [28].

Ghrelin receptor(s)

The best investigated ghrelin receptor, GHS-R1, has two variants, GHS-R1a and GHS-R1b, which result from alternative processing of pre-mRNA of the same gene [26]. Like GHS-R1a, GHS-R1b is expressed widely but, unlike GHS-R1a, it does not bind either ghrelin or synthetic GHSs, and its function is unclear [26,29]. However, a recent study has demonstrated that GHS-R1b influences signalling by GHS-R1a, which indicates that GHS-R1b might be biologically significant [30].

GHS-R1a is expressed highly in the hypothalamus and pituitary, which is consistent with its observed action on the pituitary and its role in the control of appetite, food intake and energy balance [3]. Interestingly, however, GHS-R1a expression is also reported in areas of the CNS that affect biological rhythms, mood, cognition, memory and learning, such as the hippocampus, pars compacta of the substantia nigra, ventral tegmental area, dorsal and medial raphé nuclei, Edinger-Westphal nucleus and pyriform cortex [31]. Moreover, GHS-R1a is expressed in the vagal nodose ganglion, activation of which might mediate many of the effects of ghrelin [32]. In addition, peripheral organs such the stomach, intestine, pancreas, thyroid, gonads, adrenal, kidney, heart and vasculature, bone, and various tumours and cell lines contain GHS-R1a [4–9,22,26]. These data are in agreement with the growing spectrum of actions of ghrelin. Recent experiments in GHS-R1a knockout mice show that GHS-R1a is essential for ghrelin-mediated stimulation of GH secretion and orexigenic effects [33].

GHS-R1a is a G-protein-coupled receptor, and the octanoyl group at Ser3 and the 4–5 amino acids at the N terminus are essential for its activation by ghrelin [14]. Binding of ghrelin to GHS-R1a activates multiple intracellular pathways that might be cell specific. In somatotrophic cells, stimulation of GHS-R1a results in Ca^{2+} mobilization, which is mediated through the G_{q11}–phosphatidylinositol–phospholipase C system [3]. In neurons that contain neuropeptide Y, activation of GHS-R1a results in mobilization of Ca^{2+} through the G_s–cAMP–protein kinase A (PKA) signalling pathway [34]. Ghrelin-induced activation of cAMP–PKA might also result from binding of ghrelin to receptors other than GHS-R1a [35]. GHS-R1a activation might also regulate the activity of 5′-AMP-activated protein kinase (AMPK), stimulating activity in rat hypothalamus and decreasing activity in rat liver [36]. AMPK might thus be involved in the orexigenic, lipogenic and glucogenic effects of ghrelin. In addition, ghrelin induces proliferative activity by activation of the mitogen-activated protein kinase cascade in, for example, adrenal cells [37], preadipocytes [38], osteoblasts [39], hepatoma [40] and pancreatic adenocarcinoma cells [41].

Recently, it has been demonstrated that, *in vitro*, GHS-R1a is constitutively active to ~50% of its maximal capacity [42]. The importance *in vivo* of this ligand-independent signalling is mostly unclear. However, recent studies have identified mutations in the ghrelin receptor that are associated with a selective loss of constitutive activity but do not affect ghrelin affinity, potency and efficacy. In families, these mutations segregate with the development of short stature and obesity, which indicates that selective lack of constitutive signalling by the ghrelin receptor leads to a syndrome that is characterized by short stature and obesity and apparently develops during puberty [43,44].

Recent studies have shown that ghrelin binds to and is active in some cell lines that are devoid of GHS-R1a. In these studies, ghrelin and other GHSs have different binding and activity profiles in the same system, which reinforces the idea that GHS-R1a is not the sole receptor for ghrelin and/or GHSs. Based on binding studies and

functional studies, the list of potential receptors is growing and includes receptors for des-acyl ghrelin, receptors other than GHS-R1a for ghrelin, common receptors for ghrelin and des-acyl ghrelin that have either the same or antagonistic effects in response to these two ligands, and receptors that bind peptidyl GHS (hexarelin) specifically [29]. Only the receptor that binds peptidyl GHS has been characterized; in the coronary vasculature, this receptor has been identified as CD36, a multifunctional B-type scavenger receptor [45]. Understanding that GHS-R1a-activated pathways and ghrelin receptors might be tissue- and effect-specific is of paramount importance in terms of the therapeutic potential of ghrelin because it might mean that it is possible to selectively modify specific effects of ghrelin.

Regulation of ghrelin activity

Mechanisms that regulate the effects of ghrelin have received much interest because these might clarify the pathological role and therapeutic potential of ghrelin. The mechanisms studied most extensively include: (i) expression and secretion of ghrelin; (ii) expression of ghrelin receptor(s) in target tissues; and (iii) sensitivity of ghrelin receptor(s) to ghrelin. The factors that influence the systemic concentration of ghrelin and GHS-R1a are summarized in Table 1.

In rats and humans, ghrelin secretion varies markedly throughout the day, with peaks preceding food intake [46–48]. The nocturnal increase in plasma ghrelin concentration is blunted in obese subjects and by sleep deprivation [49].

The circulating concentration of ghrelin is responsive to acute and chronic energy imbalance. In animals and humans the ghrelin concentration shows preprandial increases and rapid postprandial decreases with every meal [46–48]. The mechanisms responsible for preprandial surges in ghrelin are not known fully, but they are probably triggered by the sympathetic nervous system [50]. It seems that the depth and duration of prandial suppression of ghrelin concentration is related to the number of calories ingested, and that ingested lipids suppress ghrelin levels less effectively than either carbohydrates or proteins [51]. Prandial suppression of ghrelin does not require luminal nutrient exposure in the stomach or duodenum, the principal sites of ghrelin production, but might be mediated by signals that originate in the intestine and by postabsorptive events [51,52].

Circulating ghrelin levels are associated negatively with body mass index [53]. Thus, ghrelin secretion increases in anorexia nervosa [54] and cachexia [55], is reduced in obesity [56], and normalizes following recovery to ideal body weight. Thus, the changes observed in ghrelin concentration in response to variations in nutritional state are opposite to those of leptin, and it has been suggested that both hormones might act as signals of the metabolic balance to the CNS [57]. The exception to the negative association between body mass index and ghrelin secretion is in Prader–Willi syndrome, where obesity is associated with hypersecretion of ghrelin [58]. Although it is not clear how ghrelin-producing cells in the gut sense changes in energy stores, evidence indicates that they respond, at least in part, to adiposity-associated fluctuations in levels of insulin but not leptin [57]. These changes indicate that ghrelin might contribute to these situations as either a compensatory or a causative element.

Various hormones and drugs also affect ghrelin levels. Insulin [59], either oral or intravenous glucose [60], somatostatin and its natural analogue cortistatin [61], and GH [62] suppress systemic concentrations of ghrelin. Together with the stimulatory effect of ghrelin on GH secretion, the suppressive effect of GH on ghrelin levels indicates negative-feedback between the stomach and the pituitary [62]. Other factors that reduce ghrelin expression and/or secretion include peptide YY (PYY)3–36 [63], oxyntomodulin [64] and urocortin-1 [65] (all of which are potent anorexigenic peptides) and thyroid hormones [66]. However, administration of leptin does not modify the concentration of ghrelin in the circulating [67]. By contrast, ghrelin secretion is stimulated by acetylcholine stimulation [68], combined administration of GH-releasing hormone (GHRH) and arginine [69], testosterone (in hypogonadal men) [70], and oestrogen and insulin-like growth factor 1 (IGF-1, in patients with anorexia nervosa) [71].

Ghrelin concentrations are also altered in many clinical conditions. Ghrelin concentration decreases after gastric bypass [72] and gastrectomy [73], in chronic gastritis associated with *Helicobacter pylori* [74] and in hyperthyroidism [75], which indicates that reducing the concentration of ghrelin might contribute to the weight loss that is normally observed in these patients. Plasma ghrelin concentration also decreases in patients with short bowel syndrome [76] (probably because of loss of ghrelin-producing

TABLE 1

Factors influencing the systemic concentration of ghrelin and expression of GHS-R1a[a]

Factor	Increased ghrelin concentration	Decreased ghrelin concentration		Increased GHS-R1a concentration	Decreased GHS-R1a concentration
Clinical condition	Fasting Anorexia nervosa Cardiac cachexia Cancer cachexia Prader–Willi syndrome Ghrelinoma Bulimia nervosa	Feeding Obesity Type 2 DM Type 1 DM Binge-eating disorders Total gastrectomy	Gastric bypass HP-gastritis Hyperthyroidism Hypogonadism PCOS Cushing's syndrome	Fasting and chronic food restriction	N/A
Hormones, drugs and nutrients	Acetylcholine agonists GHRH Testosterone Oestrogen IGF-1	Glucose load Oral protein load Insulin Somatostatin Cortistatin	PYY Oxyntomodulin Urocortin Thyroid hormones Glucocorticoids	GHRH plus arginine	GH Leptin IGF-1 Glucocorticoids Sex steroids

[a] Abbreviations: DM, Diabetes mellitus; HP-gastritis, *H. pylori*-associated chronic gastritis.

tissues), binge-eating disorders [77], male hypogonadism [70], PCOS [78] and Cushing's syndrome [79]. It has been reported that low concentrations of ghrelin are associated independently with type 1 [80] and type 2 [81] diabetes mellitus, indicating that ghrelin might have a role in the aetiology of diabetes. By contrast, ghrelin levels increase in patients with bulimia nervosa [82] and ghrelinoma [83]. Based on the data available currently, ghrelin seems to be part of the interface between the energy homeostasis, glucose metabolism, and physiological processes that are regulated by the classical endocrine axes.

Regulation of the expression of GHS-R1 also has a key role in the actions of ghrelin in target tissues. A heterologous group of endocrine signals regulate expression of the gene that encodes GHS-R1 in the pituitary and hypothalamus. Thus, expression of mRNA that encodes GHS-R1 is inhibited by GH [84] and upregulated by GHRH [85]. Additional evidence indicates that the anorexigenic hormone, leptin, reduces the expression of *GHS-R1* mRNA in the arcuate nucleus [86]. Similarly, levels *GHS-R1* mRNA in the pituitary are reduced by IGF-1 [87]. Glucocorticoids [88], thyroid hormones [89] and sex steroids [90] are also involved in regulating GHS-R1 expression.

An important characteristic of GHSR-1a is the tissue-specific regulation of its response to ghrelin. Exposure of GHS-R1a to ghrelin increases receptor responsiveness in the arcuate nucleus [86] and attenuates receptor responsiveness in the pituitary [85]. The former seems to be GH-dependent [86] whereas the latter is a consequence of a combination of both uncoupling of the receptor from heterotrimeric G proteins and the internalization of the surface receptor–ghrelin complex [91]. Recycling of GHS-R1a has received much attention [91]. In this process, following internalization into vesicles mediated by clathrin-coated pits, ghrelin is degraded and GHS-R1a sorted into endosomes to be recycled back to the plasma membrane, restoring cell responsiveness to ghrelin. Recycling of GHS-R1a, which takes 360 min, thus regulates cell responsiveness to ghrelin by influencing the number of surface receptors and the amount of ghrelin to which the cells are exposed.

There is also growing interest in alternative ligands of GHS-R1a that might modulate its responsiveness to ghrelin. Studies that refer to the activation of GHS-R1a by adenosine are controversial, with some suggesting that adenosine is a partial agonist [92] and others rejecting this hypothesis [93]. More recently, it has been reported that GHS-R1a is bound and inhibited by another endogenous molecule called cortistatin, a neuropeptide that is homologous to somatostatin [94]. These findings support the hypothesis that natural ligands other than ghrelin modulate the activity of GHS-R1a.

Ghrelin: physiological, pathological and therapeutic roles

The spectrum of known biological activities of ghrelin is growing. This has led to studies on the pathological role of ghrelin and therapeutic potential of ghrelin and ghrelin mimetics and antagonists in many diseases. Table 2 summarizes the physiological role of ghrelin and the therapeutic potential of ghrelin receptor agonists and antagonists. Nonetheless, it should be pointed out that the distinction between the physiological and pharmacological actions of ghrelin is not entirely clear at present. In this regard, the development of specific ghrelin receptor antagonists is likely to help distinguish these roles, and is thus of major importance to clinical investigators.

GH secretion

As expected, ghrelin possesses a strong, dose-related, GH-releasing effect [95]. In addition, ghrelin potentiates GHRH-dependent secretion of GH [96]. The GH-releasing effect of ghrelin seems to result from the binding of ghrelin to GHSR-1a on somatotrophic cells in the pituitary [95], ghrelin-induced activation of GHRH-containing

TABLE 2

Effects of ghrelin and the therapeutic potential of ghrelin agonists and antagonists

Function	Physiological or pathological role	Therapeutic potential
GH-release	Stimulates GH-release	Ghrelin agonists in GH-deficiency states such as aging and short stature
Appetite and body weight	Stimulates appetite and food intake and increases body weight	Ghrelin agonists in cachexia and anorexia Ghrelin antagonists in obesity and Prader–Willi syndrome
Metabolism	Inhibits insulin secretion and action; induces hyperglycaemia; stimulates lipogenesis and proliferation of adipocytes; and inhibits lipolysis	Ghrelin agonists in metabolic syndrome
Reproduction	Inhibits LH secretion, embryo development, spermatogenesis, Leydig-cell development and testosterone secretion	Unknown
Gastrointestinal	Stimulates gastric secretion; offers epithelial protection; and stimulates motility	Ghrelin agonists in gastroparesis and in gastric ulcer and colitis
Cardiovascular	Decreases blood pressure; improves endothelial function; increases stroke volume; decreases inotropism; decreases cardiomyocytes apoptosis; and protects against ischaemia/reperfusion injury	Ghrelin agonists in atherosclerosis, hypertension, chronic heart failure, dilated cardiomyopathy, sepsis and cardiopulmonary bypass surgery
Pulmonary	Unknown	Ghrelin agonists in pulmonary hypoplasia and hypertension
Cell proliferation	Stimulates the proliferation of several normal cell lines	Ghrelin agonists or antagonists, depending on the tumour. Ghrelin agonists in diseases associated with abnormal angiogenesis
Immunology	Enhances immune cell proliferation and inhibits secretion of proinflammatory cytokines	Ghrelin agonists in either immunodeficiency or inflammatory states, such as wasting diseases and sepsis
Bone	Stimulates proliferation and function of osteoblasts	Ghrelin agonists in osteoporosis and metabolic bone disease

neurons and inhibition of somatostatin-containing neurons in the hypothalamus [97], and activation of vagal afferents [32].

Stimulating the release of GH might have several implications for the physiological, pathological, therapeutic and diagnostic effects of ghrelin. (i) *In vivo*, it is often difficult to separate the direct effects of ghrelin from those related to GH secretion. Moreover, although ghrelin and GHS-R1a knockout animals have a similar appearance to wild-type animals, indicating that ghrelin does not have a significant role in determining growth, the presence of compensatory mechanisms cannot be ruled out as an explanation for these findings [33,98]. Recently, studies have documented a positive correlation between ghrelin and anthropometrical parameters in the first months of life, which strengthens the hypothesis that ghrelin exerts an influence on growth [99]. (ii) Studies in humans indicate that disruption of ghrelin signalling might be involved in some clinical conditions associated with GH deficiency, such as aging and short stature. Reduced expression of GHS receptors in the hypothalamus [100] and reduction in systemic concentration of ghrelin have been reported in elderly patients [101]. Thus, impairment of the ghrelin system has, at least theoretically, a role in the changes in body composition and function that result from an age-related decrease in GH [102]. In addition, recent studies report an association between familial short stature and mutations in GHS-R1a [43,44]. (iii) In GH-deficiency states, long-acting, orally active ghrelin analogs might also represent a more physiological approach to increasing the pulsatile release of endogenous GH than a single daily dose of recombinant human GH. At present, there is no definite evidence of the therapeutic efficacy of ghrelin analogs in the treatment of GH-deficiency states, although some benefits in osteoporosis have been reported in elderly subjects [103]. (iv) Ghrelin might have diagnostic potential, based on the strong, reproducible, GH-releasing effects of ghrelin. Particularly when combined with GHRH, ghrelin is one of the most potent, reliable stimulation tests to evaluate the capacity of the pituitary to release GH for the diagnosis of GH deficiency. This might be an alternative to the insulin-tolerance test, which is the gold standard test for diagnosing GH deficiency [104].

Appetite and body weight

Several studies have implicated ghrelin in the regulation of both appetite and body weight. Considerable evidence supports a role for ghrelin in mealtime hunger and meal initiation. As stated earlier, the circulating concentration of ghrelin increases before meals and decreases postprandially [46–48]. Prandial changes in plasma ghrelin levels occur in association with changes in hunger scores, independently of external cues [46]. In animals and humans, infusion of ghrelin stimulates the spontaneous intake of food [105,106]. Recent studies have clarified the cellular mechanism underlying the orexigenic effect of ghrelin. By either activating vagal afferents [107] or through the bloodstream [108], the signal reaches the arcuate nucleus of the hypothalamus. Here, neurons that contain the orexigenic peptides neuropeptide Y and agouti-related peptide are activated, whereas neurons containing the anorexigenic peptides cocaine and amphetamine-related transcript and pro-opiomelanocortin are inhibited [109]. Leptin has opposite effects on the same neurons, which indicates that ghrelin and leptin are complementary components of a regulatory system that informs the CNS about acute and chronic energy balance [110]. The arcuate neurons project to other nuclei, including orexin-containing neurons in the lateral hypothalamic area, to stimulate appetite [111].

In addition to a probable role in meal initiation, ghrelin seems to be an adiposity-related hormone that is involved in the long-term regulation of body weight. Ghrelin levels correlate inversely with energy stores and manifest compensatory changes in response to body-weight alterations. Indeed, ghrelin levels increase in response to the weight loss that results from low-calorie diets [112], mixed life-style modifications and exercise [113], Huntington's disease [114], anorexia nervosa [54], bulimia nervosa [82], cancer anorexia/cachexia [115], and chronic failure of the heart [55], lungs [116], liver [117] and kidneys [118]. Similarly, the expression of ghrelin receptors in the hypothalamus increases markedly with either fasting or chronic food restriction [119], as does the hypothalamic response to a ghrelin-receptor agonist [120], which is consistent with a feed-forward loop that enhances ghrelin-mediated stimulation of appetite during energy deficit. Conversely, circulating levels of ghrelin decrease with weight gain caused by forced overfeeding [121], high-fat diets [122], excessive glucocorticoids [79] and successful treatment of coeliac disease [123] and anorexia nervosa [124]. Long-term administration of ghrelin promotes weight gain by stimulating food intake and regulating other aspects of energy homeostasis. Indeed, ghrelin increases the preference for dietary fat [125] and promotes adipogenesis directly [17]. It also decreases energy expenditure [126], lipolysis [16,127], adipocyte apoptosis [38], sympathetic nervous system activity [126], body temperature [128], proinflammatory cytokine production [129] and locomotor activity [130].

Alteration of ghrelin action might contribute to the change in body weight that accompanies many diseases. Decreased ghrelin levels have been associated with the weight loss that follows either partial or total gastrectomy [73]. Regarding *H. pylori*-associated chronic gastritis, ghrelin seems to rise following infection treatment and thus might contribute to the increased incidence of obesity, gastroesophageal reflux disease and its sequelae after eradication of *H. pylori* [74,131]. Following gastric bypass, ghrelin levels also decrease, which indicates that ghrelin might be involved in weight loss after bypass, although this is controversial [72,132,133]. Postprandial suppression of serum ghrelin is less robust in obese humans, possibly contributing to the pathogenesis of obesity [49]. Recent genetic studies in humans support a vital role for ghrelin in body-weight regulation; some large studies show linkage between polymorphisms in the gene encoding human preproghrelin and protection against obesity [134] and related sequelae, and others show a linkage between mutations in GHS-R1a and short stature and obesity [43,44].

Ghrelin is an especially appealing candidate for the treatment of cachectic patients because it is active when given systemically and affects food intake and many other processes that are involved in energy expenditure and fuel utilization, all of which promote weight gain (see earlier). Indeed, recent studies indicate that treatment with ghrelin might help to treat the cachexia that is associated with congestive heart failure [135], chronic obstructive pulmonary disease [136], sepsis [137] and cancer [138]. Plasma ghrelin levels in patients with anorexia nervosa are high (consistent with a state of negative energy balance) and return to control levels after weight gain by renutrition [124]. Although useful theoretically, administration of ghrelin to these patients does

not result in a significant improvement in food intake, which indicates that these patients might be relatively insensitive to the orexigenic effects of ghrelin [139].

Recent evidence supports the use of ghrelin antagonists in the treatment of positive energy-balance states. All published animal studies report that ghrelin blockade (using anti-ghrelin antibodies, ghrelin-receptor antagonists and antisense oligonucleotides) decreases spontaneous food intake, leading to weight loss in the longer studies [140–142]. Moreover, recent studies show that knockout of the genes that encode either ghrelin or GHSR-1a in mice results in a normal body weight and protects against obesity induced by a high-fat diet [33,98]. However, baseline ghrelin levels are low in obese individuals (see earlier), so further pharmacological reduction of ghrelin signalling in this setting might not have a major impact on body weight. However, anti-ghrelin pharmacotherapeutics might help to prevent the regain of body weight that has already been lost because weight reduction causes a rise in circulating levels of ghrelin and an increase in ghrelin receptors in the hypothalamus, as well as increased sensitivity to the orexigenic actions of ghrelin [143]. In contrast to other forms of obesity, in Prader–Willi syndrome, obesity is associated with chronic hyperghrelinaemia [58] and it has been hypothesized that ghrelin might contribute this. This is based on similarities between the clinical features of this syndrome and those predicted from overstimulation of neuropeptide Y by ghrelin (e.g. hyperphagic obesity, hypogonadotropic hypogonadism and dysregulation of GH) and the correlation between ghrelin levels and hyperphagia and excessive obesity, in these patients [58]. Indeed, the high ghrelin levels in obese people with Prader–Willi syndrome make these individuals logical first-line candidates for testing the weight-reducing effects of ghrelin-blocking agents.

Together, most available studies indicate that ghrelin has an important role in the response to alterations in body weight, that it might contribute to the change in body weight that accompanies some diseases, and that either potentiation or inhibition of the orexigenic action of ghrelin might be useful in conditions that are associated with weight loss and weight gain, respectively.

Metabolism

Recent research has focused on the metabolic effects of ghrelin, showing that it might have important effects on the metabolism of glucose and lipids.

Glucose metabolism. Recently, ghrelin has been implicated in the regulation of both insulin action and glucose homeostasis. GHS-R1a and GHS-R1b are present in animal and human endocrine pancreas [26,144]. Ghrelin is also present in pancreas, and epsilon pancreatic cells have been suggested to be a putative ghrelin-expressing cell type, although this is controversial [145].

Depending on the experimental conditions, ghrelin is reported to either inhibit or stimulate insulin secretion in animals and humans [146–148]. Nevertheless, most available data indicate a negative association between systemic ghrelin and insulin levels [80,81]. Insulin decreases ghrelin levels [59] and ghrelin inhibits insulin secretion both *in vitro* and in most human and animal studies [147,148]. In addition, ghrelin might also regulate some of the peripheral effects of insulin. Thus, ghrelin stimulates hepatic glucose production [40] and hampers the ability of insulin to suppress endogenous production glucose. In addition, it reinforces the action of insulin on glucose disposal in mice [149], inhibits secretion of the insulin-sensitizing protein adinopectin from adipocytes [150] and stimulates secretion of the counter-regulatory hormones, including GH [95], cortisol [95], adrenaline [95] and possibly glucagon [146]. Notably, in agreement with the evidence of binding sites in the human pancreas for both acylated and non-acylated ghrelin with similar affinity, non-acylated ghrelin counteracts the effects of acylated ghrelin on insulin secretion and hepatic insulin action [149].

As expected, acute administration of ghrelin to humans increases plasma glucose levels and amplifies the hyperglycaemic effect of arginine [148]. The hyperglycaemic effect of ghrelin might result from the endocrine effects of ghrelin as well as from direct effects on hepatocytes in which it modulates glycogen synthesis and gluconeogenesis [40]. By contrast, glucose load reduces ghrelin levels [60]. Insulin and glucose both negatively influence the secretion of ghrelin, so the overall picture indicates a functional link between ghrelin, the endocrine pancreas and glucose metabolism. However, the physiological and pathological importance of this link remains mostly unclear.

Recently, the role of ghrelin in diabetes has been investigated: low ghrelin levels are associated with insulin resistance, hypertension and the prevalence of type 2 diabetes [81]; polymorphisms of the ghrelin gene are associated with the risk of diabetes [151]; low levels of ghrelin have also been demonstrated in patients with type 1 diabetes [80]; ghrelin promotes regeneration of β cells in streptozocin-treated newborn rats, preventing the development of diabetes in disease-prone animals after β-cell destruction [152]; and ghrelin antagonists partially reverse hyperphagia in uncontrolled, streptozocin-diabetic rats [153]. All these data indicate that ghrelin might have a role in the pathogenesis and therapy of diabetes, contributing to either the impairment of insulin sensitivity or to the restraint of body-mass gain. Nonetheless, because of the controversy about the cause-and-effect relationship between ghrelin levels and diabetes, further investigations are needed to elucidate the precise role of ghrelin (and its variants) in the development and treatment of diabetes. In addition, low plasma concentrations of ghrelin are associated with several components of the metabolic syndrome (obesity, insulin resistance and blood pressure) and with metabolic cluster *per se*, which indicates that ghrelin might be a useful biomarker and medication tool for the metabolic syndrome [154]. Even so, enthusiasm for the use of ghrelin in metabolic syndrome might be tempered by its hyperglycaemic effects.

Lipid metabolism. Ghrelin has also been implicated in lipid metabolism. In the liver, ghrelin-induces lipogenic patterns of gene expression and triglyceride content, whereas the activity of the stimulator of fatty acid oxidation, AMPK, is reduced [155]. In the gastrocnemius muscle, ghrelin reduces triglyceride content, increases activity of mitochondrial oxidative enzymes and increases mRNA that encodes uncoupling protein 2, independent of changes in expression of genes that are involved in fat metabolism and phosphorylation of AMPK [155]. Peroxisome proliferator-activated receptor γ (PPARγ), activation of which reduces the fat content of muscle, is also increased selectively in skeletal muscle [155]. Thus, ghrelin favours triglyceride deposition in liver rather than skeletal muscle. Moreover, ghrelin also acts directly on differentiated adipocytes to stimulate lipogenesis *in*

vitro and *in vivo* by increasing the levels of PPARγ and the insulin-induced uptake of glucose [17,156], antagonizes lipolysis, reducing isoproterenol-stimulated lipolysis *in vitro* [16], and stimulates the differentiation and proliferation of preadipocytes [38]. In summary, although many aspects of the metabolic effects of ghrelin remain to be clarified, there is increasing evidence to indicate that these represent the most important actions of this peptide.

Reproduction

To date, the expression of ghrelin and its receptor have been documented in various reproductive organs, such as placenta, testis Leydig cells, rat ovary, mouse embryo and endometrium, which indicates that ghrelin might have a role in regulating reproductive function [157–159]. Indeed, available data indicate that ghrelin regulates several aspects of reproductive physiology, at least partially, in a paracrine/autocrine manner.

In the pituitary, ghrelin decreases the frequency of pulsatile luteinizing hormone (LH) secretion, leading to a decrease in LH concentrations [160]. Addition of ghrelin to culture media inhibited the development of two-cell embryos to the hatched blastocysts and decreased the total cell number of blastocysts, indicating that ghrelin might also regulate the development of preimplantation embryos [157].

In males, ghrelin has an additional inhibitory role, decreasing human chorionic gonadotropin (hCG)- and cAMP-stimulated testosterone secretion [158] and the expression of the gene encoding stem cell factor. This factor is a key mediator of spermatogenesis and a putative regulator of Leydig-cell development [161].

Overall, these findings show a predominantly inhibitory role for ghrelin in reproductive function. Together with the increase in systemic and uterine ghrelin in fasting states [159], these observations indicate that ghrelin might be responsible for inhibiting reproductive function in states of malnutrition to avoid the excess metabolic demands that are imposed by pregnancy.

In addition, given the widespread effects of ghrelin in gonadal function, several recent studies have investigated the relationship between circulating ghrelin concentrations and the hormonal and metabolic features in women with PCOS. In some studies, both circulating and ovarian ghrelin decrease in PCOS [162]. Although PCOS-associated hyperandrogenaemia and 17-OH-progesterone levels are inversely related to ghrelin levels, anovulation and polycystic ovary morphology are associated with higher concentrations [162,163]. Thus, it has been hypothesized that different clinical and biochemical manifestations of the syndrome might be associated with different concentrations of ghrelin. However, the pathological and therapeutic importance of this association is unclear.

In contrast to PCOS, in hypogonadal males, a positive correlation between ghrelin and androgens persists after testosterone-replacement therapy [70]. Nevertheless, taken together, these findings indicate that there might be a mutual influence between sex hormones and ghrelin in humans.

Gastrointestinal function

Available data indicate that ghrelin affects many aspects of gastrointestinal function, including exocrine secretion, epithelial protection and motility.

Although X/A cells of the acid-producing part of the stomach are the largest source of ghrelin, which indicates that ghrelin might regulate gastric-acid secretion, few studies have as yet dealt with this issue, and the results are equivocal because stimulation (probably by stimulation of parietal cells by vagal pathways) [164], inhibition [165] and lack of effect [166] have been reported. It has been suggested that the conflicting data might reflect the presence of both stimulatory and inhibitory pathways, and that experimental conditions and models might determine the balance between these. The stimulatory action of ghrelin on gastric secretion might be important in preparing the stomach to process food. Ghrelin also regulates secretion from the exocrine pancreas, stimulating pancreatic protein secretion through central pathways [167].

Ghrelin might also be involved in cell proliferation and differentiation of the gastrointestinal epithelium. A gastroprotective effect has been demonstrated in various models, which depends mainly on vagal activity, sensory nerves and hyperaemia mediated by nitric oxide synthase–nitric oxide and cyclooxygenase–prostaglandin systems [168,169]. The protective effect is not limited to the stomach because it has also been demonstrated in experimental colitis [170]. Although the physiological role of ghrelin in gastroprotection remains unclear, increases in both mucosal expression and plasma concentration of ghrelin have been reported in ulcers induced by stress [168], ethanol [171] and cysteamine [172].

Structural relationships between ghrelin and motilin have led to evaluation of the motility effects of ghrelin [173]. Indeed, it has been demonstrated that ghrelin, like motilin, induces the migrating motor complex and the acceleration of gastric emptying, in both humans and rodents [174,175]. Ghrelin also accelerates colonic motility [176]. The prokinetic actions of ghrelin are mediated by the acetylcholine system, through central mechanisms and probably the myenteric plexus [177,178]. The physiological and pathological importance of ghrelin-stimulated motility is mostly unclear. The fact that ghrelin-null mice have normal gastrointestinal motility, apart from subtle changes in food intake, indicates that ghrelin might not be a crucial physiological regulator of gastrointestinal motility, although its loss might be compensated by other redundant mechanisms [179]. By contrast, in humans the half-time of gastric emptying correlates positively with fasting plasma levels of ghrelin [180]. In addition, reduced density of ghrelin-immunoreactive cells has been demonstrated in animal models of human diabetes type 1 and type 2, and this might explain the slow gastric emptying and slow intestinal transit in diabetes gastroenteropathy [181]. Therefore, the effects of ghrelin might have therapeutic potential. Indeed, administration of ghrelin accelerates gastric emptying and improves meal-related symptoms in several types of gastroparesis (drug-induced, diabetic and idiopathic), which indicates that these patients are potential candidates for treatment with ghrelin [182–184].

Cardiovascular function

Increasing evidence supports a role of ghrelin in the direct regulation of cardiovascular function. Expression of mRNA that encodes ghrelin and its receptor has been observed in the heart and vasculature [20,26]. As anticipated (see earlier), numerous recent studies indicate that animal and human myocardium possess

multiple subtypes of GHS receptors that are different from the classical GHSR-1a, have different affinities for the various GHSs and might contribute independently to the wide array of cardiovascular activities of GHSs. Finally, ghrelin administration affects several aspects of vascular and cardiac function in different experimental conditions.

Regarding the macrocirculation, the vasoactive effects of ghrelin depend on the vascular territory. Ghrelin has a systemic vasodilatory effect that is endothelium-independent [20] and might involve peripheral and central mechanisms [185]. Indeed, intravenous administration of ghrelin in humans significantly decreases mean arterial pressure, but does not change heart rate [186]. Recently, an association between polymorphisms in the gene that encodes ghrelin and blood pressure [187], and low levels of ghrelin and hypertension has been demonstrated [81], which indicates that ghrelin might have a role in hypertension. By contrast, ghrelin increases coronary perfusion pressure in rat hearts perfused using the Langendorf system and significantly increases pressure-induced myogenic tone in coronary arterioles [188]. Whether the vasoactive effect of ghrelin in the coronary territory is mediated by activation of GHS-R1a remains to be investigated. In the microcirculation, ghrelin increases vascular flow, which might affect other physiological functions of ghrelin [189].

Ghrelin also improves endothelial function by inhibiting basal and tumour necrosis factor α (TNF-α)-induced production of chemotactic cytokines, increasing nitric oxide bioactivity and inhibiting angiotensin II-induced migration of human aortic endothelial cells [190–192]. Hence, ghrelin might oppose endothelial dysfunction and be useful for treating conditions that are associated with endothelial dysfunction such as atherosclerosis, endotoxic shock and congestive heart failure (CHF). Several studies reinforce the hypothesis that this peptide has a modulatory role in atherosclerosis, especially in obese patients in whom ghrelin levels are reduced. For example, expression of GHS-R1a is upregulated in atherosclerotic regions [193]; there is a positive association between the concentration of ghrelin in the plasma and carotid artery atherosclerosis [194]; ghrelin improves endothelial function in metabolic syndrome by increasing the bioactivity of nitric oxide [195]. Salutary effects of ghrelin administration have also been demonstrated in several aspects of endotoxic shock and CHF (see later).

Salutary cardiotropic effects of ghrelin have been demonstrated in several experimental models. These might result from an increase in GH, appetite and vasodilation, and a decrease in cytokine production, as well as from direct effects of ghrelin on cardiomyocytes.

In vitro, administration of ghrelin decreases inotropism [19,196] and lusitropism [196], inhibits apoptosis of cardiomyocytes [18], improves left ventricle function during ischemia–reperfusion injury (although less than synthetic peptidyl GHS) paralleling upregulated expression of myocardial ghrelin receptors [197] and reduces infarct size [198]. In healthy volunteers [186], and patients with CHF [199], ghrelin decreases systemic vascular resistance, which results in increased cardiac output, as shown by increased cardiac index and stroke-volume index. An association has been demonstrated between ghrelin systemic levels and cardiovascular indexes [200].

Based on the widespread actions of ghrelin, which include appetite stimulation, endothelial function improvement and inflammation inhibition, and its salutary cardiovascular effects, several studies have confirmed the potential role of ghrelin and its analogs in pathogenesis and therapy of cardiac diseases such as CHF, dilated cardiomyopathy and sepsis. Indeed, it has been demonstrated that administration of ghrelin to CHF patients improves cardiac function and structure, and attenuates cachexia [135]. In animal models of dilated cardiomyopathy, synthetic GHS derivatives improve left ventricle function and dilation, and decrease mortality [201,202]. Recent studies also attribute an important role to ghrelin in the pathogenesis and therapy of sepsis. Thus, vascular expression of ghrelin receptors is augmented in the hyperdynamic phase of sepsis, whereas ghrelin levels decreased in the early and late stages of sepsis, which indicates that decreasing cardiovascular responsiveness to ghrelin might be related to the transition between hyperdynamic and hypodynamic phases of sepsis [203]. In addition, administration of ghrelin improves haemodynamic, metabolic and inflammation parameters, and tissue perfusion and mortality in endotoxic shock [204,205]. These data indicate that ghrelin might be a future therapeutic target in these three cardiovascular diseases. Because of their effects on *in vitro* models of cardiac ischemia–reperfusion injury, synthetic GHS might also have a role in preventing the ischemia-reperfusion injury that follows cardiopulmonary bypass in cardiac surgery.

Pulmonary effects

Although some studies have demonstrated that lungs synthesize ghrelin, few note the effects of ghrelin on pulmonary function [26]. Recently, we have studied the pulmonary effects of ghrelin in two experimental models: monocrotaline-induced pulmonary hypertension [206] and nitrofen-induced congenital diaphragmatic hernia [207]. In the first model, chronic administration of ghrelin attenuates monocrotaline-induced pulmonary hypertension, pulmonary vascular remodelling and right ventricle hypertrophy, indicating a potential therapeutic role for ghrelin in pulmonary hypertension [206]. In the second model, overexpression of ghrelin was demonstrated in hypoplastic lungs, and exogenous administration of ghrelin attenuated pulmonary hypoplasia secondary to nitrofen-induced congenital diaphragmatic hernia, which indicates a potential therapeutic role for ghrelin in pulmonary hypoplasia [207].

Cellular proliferation

Recent evidence supports a role for ghrelin in the regulation of the proliferation of both normal and neoplastic cell lines. Ghrelin stimulates the proliferation of the H9c2 cardiomyocyte cell line [18], human adrenal zona glomerulosa cells [37], 3T3-L1 preadipocytes [38] and osteoblastic cells [208]. Thus, the peripheral actions of ghrelin result from modulation of function, and from regulation of the survival and proliferation of target cells.

Several endocrine and nonendocrine neoplastic cells (pituitary adenomas [209], gastroenteropancreatic and pulmonary carcinoids [210,211], thyroid tumours [212], testicular tumours [213], and prostate [214,215], lung [216], breast [217] and pancreatic [41] carcinomas) and their related cell lines synthesize ghrelin. Many of these neoplasms contain GHS-R1a and/or binding sites that recognize ghrelin either independently of its acylation or only synthetic peptidyl GHS. Evidence that ghrelin, ghrelin receptors and GHS receptors are coexpressed in several tumours and related

cell lines indicates that the ghrelin system is likely to have an important autocrine/paracrine role in the development of neoplasms.

Experiments *in vitro* demonstrate that ghrelin either stimulates or inhibits the proliferation of several human tumour cell lines. An antiproliferative effect of ghrelin has been shown in breast cancer cell lines [217], human thyroid carcinoma cell lines [212] and prostate neoplasms and related cell lines [214], whereas a proliferative effect has been shown in human hepatoma (HepG2) cells [40], in the PC3 prostatic carcinoma cell line [215] and in pancreatic carcinoma [41]. An explanation for the dual action of ghrelin and GHSs on the viability of neoplastic cells is not known, although it might be related, at least partially, with ghrelin concentration. In addition, ghrelin might also regulate neoplasm proliferation by exerting an inhibitory effect on angiogenic factors [218]. In assays developed with endothelial cells (HUVEC cells), ghrelin inhibits fibroblast growth factor 2-induced cell proliferation and Matrigel tube formation. This action seems to be modulated by an autocrine/paracrine mechanism because ghrelin and GHS-R1a are present in HUVEC cells. In addition, this effect seems to be restricted to proliferating endothelial cells, which might indicate high selectivity as an antivascular agent.

These findings support the possibility that ghrelin is involved in the pathogenesis of processes in which angiogenesis plays a key role, and support the potential of ghrelin-system modulators in the treatment of various neoplasms. Nevertheless, despite the potential proliferative effect on some neoplastic cell types, ghrelin is being investigated currently as an anticachectic agent in tumour-bearing animal models, where it seems to exert anabolic actions [138].

Immunomodulation

Ghrelin might also modulate immune function by enhancing immune-cell proliferation and inhibiting the secretion of proinflamatory cytokines from immune cells. Chronic administration of a ghrelin mimetic to old mice stimulates growth, differentiation and cellularity of the thymus, in addition to increasing T-cell production [219]. This results in enhanced resistance to the initiation of tumours and subsequent metastasis in animals that are inoculated with lymphoma cells and an improved thymic engraftment in bone marrow-transplant recipients. Hence, ghrelin mimetics might provide a novel therapeutic strategy in subjects with compromised immune functions.

Ghrelin also modulates the production of proinflammatory cytokines. In rodents, ghrelin attenuates endotoxin-induced anorexia, reduces cytokine production and improves mortality associated with lipopolysaccharide (LPS)-induced endotoxic shock [204]. Recently, ghrelin and GHS receptors have been demonstrated in human T cells and monocytes, where ghrelin specifically inhibits chronic, LPS- and leptin-induced synthesis of proinflammatory anorectic cytokines such as leptin, interleukin (IL)-1β, IL-6 and TNF-α [129]. The anti-inflammatory property of ghrelin might have widespread implications in the treatment of wasting diseases, aging and frailty (see earlier). A recent study has demonstrated that, by inhibiting neutrophil action, ghrelin ameliorates pancreaticobiliary inflammation and associated remote organ injury induced by pancreaticobiliary obstruction [220].

Bone physiology

Recent studies indicate that ghrelin is also involved in the regulation of bone growth and metabolism as one of its peripheral effects. It has been demonstrated that primary osteoblasts and osteoblastic cell lines in various species contain GHS-R1a [208]. There is also evidence that ghrelin treatment directly stimulates osteoblast proliferation and differentiation, alkaline phosphatase activity and Ca^{2+} accumulation in the matrix [208]. The role of ghrelin in bone physiology is reinforced by three additional observations: indices of systemic ghrelin secretion correlate with bone mineral density (BMD) in healthy adolescents [221]; decreased systemic ghrelin might be associated with osteopenia after gastrectomy and this might be attenuated by either ghrelin treatment or retaining part of the oxyntic gland area [222]; ghrelin increases BMD in both normal and GH-deficient rats [223]. These observations show that ghrelin stimulates bone formation directly and that it might be useful in the treatment of either osteoporosis or metabolic bone disease.

Other effects

Numerous other effects have been attributed to ghrelin. The continuously increasing list includes stimulation of prolactin and adrenocorticotrophic hormone [186], promotion of slow-wave sleep [224], memory retention [225] and anxiety-like behaviour [225], and stimulation of milk secretion [226]. Recently, we have reported that ghrelin is synthesized in the iris and induces relaxation of iris muscles [22]. The physiological and pathological importance of most of these effects remains to be clarified and thus they deserve more investigation.

Conclusion

The broad spectrum of biological activities that have been associated with ghrelin continues to expand. Hence, potential clinical targets for ghrelin, its mimetics and, perhaps, ghrelin antagonists are beginning to be identified. Nonetheless, many issues remain, including the regulation of ghrelin secretion, role of non-acylated ghrelin and obestatin, ghrelin receptor(s) and their downstream pathways, GHS-R1a ligands, the autocrine/paracrine role of ghrelin, and the distinction between physiological and pharmacological effects of ghrelin. In the next few years these issues are likely to be answered, making it possible to clarify the precise physiological, pathological and therapeutic roles of ghrelin.

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