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Review

Yin and Yang - the Gastric X/A-like Cell as Possible Dual Regulator of Food Intake

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Ingestion of food affects secretion of hormones from enteroendocrine cells located in the gastrointestinal mucosa. These hormones are involved in the regulation of various gastrointestinal functions including the control of food intake. One cell in the stomach, the X/A-like has received much attention over the past years due to the production of ghrelin. Until now, ghrelin is the only known orexigenic hormone that is peripherally produced and centrally acting to stimulate food intake. Subsequently, additional peptide products of this cell have been described including desacyl ghrelin, obestatin and nesfatin-1. Desacyl ghrelin seems to be involved in the regulation of food intake as well and could play a counter-balancing role of ghrelin's orexigenic effect. In contrast, the initially proposed anorexigenic action of obestatin did not hold true and therefore the involvement of this peptide in the regulation of feeding is questionable. Lastly, the identification of nesfatin-1 in the same cell in different vesicles than ghrelin extended the function of this cell type to the inhibition of feeding. Therefore, this X/A-like cell could play a unique role by encompassing yin and yang properties to mediate not only hunger but also satiety.
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Key Words
Desacyl ghrelin; Eating; Ghrelin; Nucleobindin; Obesity; Obestatin; Stomach

Introduction

The stomach is the first site where food has a sustained contact with the gastrointestinal tissue. Therefore, it is not surprising that the stomach plays an important role in the regulation of hunger and satiety. In the past years specialized endocrine cells drew increasing attention and stimulated research in the field of physiology, behavioral medicine and obesity research. Different endocrine cells present in the stomach were shown to regulate physiological functions, predominantly gastric acid secretion. These cells encompass gastrin-producing cells, in low quantity serotonin-producing enterochromaffin cells, somatostatin-producing D cells (5%-10% of gastric oxyntic endocrine cells in rats, > 20% in humans) and histamine-containing enterochromaffin-like cells (65% in rats and 30% in humans).[1] Another cell type has been described that resembled the phenotype of pancreatic A cells although the function was unknown.[1] Therefore, this cell was termed X/A-like cell (in humans the same cell was named P/D_1 cell).[2,3] This cell type accounts for 20%-30% of the endocrine cell

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population in the gastric oxyntic mucosa and is distributed throughout the mucosal layer.[4] In addition to the stomach, X/A-like cells were also found in lower parts of the gastrointestinal tract with decreasing quantities.[4] Interestingly, whereas in the stomach the predominant type of X/A-like cells is of round shape without contact to the lumen (closed-type), with aboral distance from the stomach, the percentage of elongated cells with luminal contact (open-type) increases.[4] In 1999 the food intake stimulatory peptide hormone ghrelin was discovered,[5] and the identification of ghrelin in X/A-like cells[2,6] stimulated research on this particular cell and its peptide product(s). In the following years, additional peptide products of X/A-like cells have been identified, namely desacyl ghrelin, obestatin and nesfatin-1 which were also suggested to be involved in feeding regulation. This review will discuss the current knowledge on these peptides products and recent concepts on the involvement of this particular cell type in the regulation of not only hunger but also satiety.

Products of the X/A-like Cell

Besides ghrelin, desacyl ghrelin and *n*-decanoyl ghrelin[2,7] as well as obestatin[8] are derived from the same gene whereas nucleobindin2 (NUCB2)/nesfatin-1[9] is encoded by a different gene.

Ghrelin, Desacyl Ghrelin and *n*-Decanoyl Ghrelin

In 1999 Kangawa and Kojima discovered the growth hormone secretagogue, ghrelin as the endogenous ligand of the growth hormone secretagogue receptor 1a[5,10] which was later renamed ghrelin receptor (GRLN-R).[11] The 28 amino acid peptide has a unique fatty acid modification on the third amino acid. This n-octanoic residue is essential for the binding to the GRLN-R[5,12] by increasing the peptide's lipophilicity.[5] A recent study showed that medium chain fatty acids in the diet are the direct source for ghrelin's hydrophobic modification.[13] Interestingly, also the first 5 N-terminal amino acids can activate the receptor when they bear a fatty acid residue.[14] Recently, n-decanoyl ghrelin was identified as another acylated form and shown to represent a major circulating form of ghrelin in mice.[7]

The enzyme catalyzing the acylation of ghrelin was identified very recently in mice and humans as a member of the membrane-bound *O*-acyltransferases (MBOATs), MBOAT4 that was renamed ghrelin-*O*-acyltransferase (GOAT).[15,16] Both, C8 and C10 medium chain fatty acids are substrates for GOAT resulting in octanoyl and decanoyl ghrelin.[16] It is assumed that GOAT acetylates pro-ghrelin before its transport to the Golgi apparatus where pro-ghrelin is cleaved by prohormone convertase 1/3.[15] Interestingly, in addition to GOAT mRNA and protein expression in gastric X/A-like cells,[17,18] GOAT immunoreactivity was also identified in the circulation of rats and mice[18] raising the possibility of an extracellular acyl modification of ghrelin.

In contrast to ghrelin, desacyl ghrelin does not bear a fatty acid residue and therefore does not bind to the GRLN-R.[5] Both ghrelin and desacyl ghrelin are predominantly derived from gastric X/A-like cells and represent the major circulating forms with a proportion in the blood initially reported to range between 1:15[19] and 1:55.[20] However, recent advances in blood processing for labile peptides indicated a proportion of acyl/total ghrelin of 1:5 compared to 1:19 observed after standard blood processing (EDTA blood on ice).[21]

Obestatin

In 2005, a computer-based search for alternative splicing and post-translational processing predicted the cleavage site of pro-ghrelin leading to obestatin, a peptide assumed to curtail body weight and to antagonize ghrelin's orexigenic action.[8,22] Obestatin is also expressed in human gastric endocrine P/D1 cells[23,24] and rat gastric X/A-like cells[25] with a subcellular localization in secretory granules.[23,24]

Nucleobindin2/Nesfatin-1

The group of Mori recently reported the identification of NUCB2/nesfatin-1 in the rat hypothalamus.[26] Subsequent studies confirmed this finding[27-29] and extended its expression to the periphery with a 10-fold higher mRNA expression in gastric mucosa than in the brain.[9] Interestingly, ghrelin and nesfatin-1 immunoreactivity co-localized in the same oxyntic endocrine X/A-like cells as visualized by double immunofluorescence staining and high-resolution confocal microscopy from a single X/A-like cell indicated the occurrence of these 2 peptides in different subsets of vesicles.[9] Another finding indirectly supporting the production of ghrelin and nesfatin-1 in the same cell came from the observation of prohormone convertase 1/3 in X/A-like cells,[15] a hormone involved in the processing of both peptides.[15,30]

Peptide Release and Action on Receptors

Ghrelin and Desacyl Ghrelin

Ghrelin is mainly produced by gastric X/A-like cells[31] as in-

dicated by a pronounced decrease of circulating ghrelin levels after gastrectomy.[32] In addition, ghrelin is produced in the intestine,[2] pancreas[33] and other peripheral organs, namely kidney, liver, heart, testis, adipose tissue and skin,[34,35] although in much lower quantities compared to the gastric source. Circulating ghrelin levels are largely affected by the metabolic condition with an increase before meals and a decrease thereafter.[36,37] Moreover, fasting stimulates ghrelin mRNA expression,[38-40] whereas gastric ghrelin peptide content decreases pointing towards stimulated production and release of ghrelin under these conditions.[39,40] In addition to these short-term changes, circulating ghrelin concentrations depend on the metabolic status over time with increased levels under conditions of anorexia and cachexia and a decrease in overweight and obese subjects.[41-43] Similar to the observed changes of ghrelin, gastric GOAT mRNA as well as circulating GOAT protein levels were increased during fasting conditions.[18,44]

Ghrelin is affected by a multitude of hormones and transmitters which were tested in vitro and in vivo. The most established action is the inhibitory effect of somatostatin on circulating levels of ghrelin reported in experimental studies and humans,[45-47] likely mediated via the somatostatin receptor subtype 2 which is expressed on X/A-like cells (P/D1 cells) in animals[48] and humans.[49] Similarly, prostacyclin is likely to reduce circulating ghrelin levels[50] via direct interaction with the prostacyclin I_2 receptor expressed on X/A-like cells.[50] Moreover, glucagon-like peptide,[51-53] cholecystokinin-8,[54] insulin[46,55] and bombesin[45] reduce circulating levels of ghrelin although the mode of interaction remains to be established. On the other hand, central vagal cholinergic activation[56,57] and adrenaline as well as noradrenaline,[45,46,58] have been consistently shown to increase circulating levels of ghrelin in experimental animals. Stimulatory effects of dopamine have been reported in vitro[46] but not in vivo.[45] The ghrelin releasing effect of cannabinoids,[59] oxytocin and vasopressin,[46] secretin,[45] and endothelin 1 and 3[45,61] were reported either in vitro or in vivo and are yet to be further confirmed. However, one has to note that the underlying mechanisms regulating ghrelin production and release at the cellular and molecular levels are poorly characterized. Establishing pure preparations of isolated ghrelin cells using ghrelin promoter models coupled to fluorescent dye[62] or the recent use of the ghrelin-producing mouse cell line, MGN3-1[46] could be promising venues to conduct these investigations.

Interestingly, acyl and desacyl ghrelin are not uniformly regulated as shown by a recent study where lowering the gastric pH stimulated the release of desacyl ghrelin whereas acyl ghrelin was not affected.[3] In addition, also immunological[63] or physical[48] stressors lead to differential ratios of circulating levels of acyl and desacyl ghrelin indicative of a more rapid decrease of the acylated form, which could be attributed to a reduction in GOAT protein expression as recently suggested.[48] However, the exact mechanisms are unknown and warrant future investigation.

Ghrelin is the endogenous ligand of the GRLN-R which is expressed in the brain and peripheral structures and organs including the pituitary, vagal afferents, pancreas, spleen, myocardium, adipose tissue, thyroid gland, adrenal gland and gastric myenteric neurons.[34,64-66] This receptor is characterized by high constitutive activity[67] which is likely to hamper blockade of ghrelin signaling by ghrelin antagonists and may favor the development of inverse agonists.[68] In addition, the GRLN-R forms heterodimers with other receptors such as the cannabinoid 1 receptor[65] and the dopamine receptor 1[69] thought to result in increased signaling. Conversely, the GRLN-R desensitizes after stimulation through endocytosis via clathrin-coated pits and is characterized by slow dissociation with ghrelin,[70] likely preventing available GRLN-R available for binding.

Unlike ghrelin, desacyl ghrelin is unable to bind to the GRLN-R and the receptor for this peptide remains to be established. The existence of a specific desacyl ghrelin receptor is strongly suspected based on the findings that desacyl ghrelin exerts several biological actions in cells that do not express the GRLN-R, as shown by the inhibition of cell proliferation in breast cancer[71] and prostate cancer[72] cell lines and stimulation of insulin release from INS-1E cells.[73]

Obestatin

In contrast to the suggested role of obestatin as regulator of food intake, obestatin levels do not change dependent on metabolic status.[8,74] During fasting conditions gastric obestatin peptide content[25] and plasma obestatin levels[8] remain unaltered. One study reported a decrease of circulating obestatin during fasting,[75] a finding to be replicated. Initially, obestatin was described as the endogenous ligand of the 7 transmembrane domain G protein-coupled receptor, GPR39.[8] However, subsequent studies conducted by several independent groups[76-79] as well as the original investigators[80] did not reproduce the initial findings of binding of obestatin to recombinant GPR39 and activation of the receptor. However, recent studies by Zhang et al[81] provided new evidence for obestatin binding to GPR39 to regulate functions of diverse gastrointestinal and adipose tissues. One has to note that

the GPR39 displays high constitutive activity in the absence of ligand binding similar to the GRLN-R.[82] Taken together, there is need for further experiments in order to elucidate the endogenous ligand of the GPR39.

Nucleobindin2/Nesfatin-1

Plasma NUCB2/nesfatin-1 is regulated by nutritional status with a significant decrease after 24-hour fasting in rats and a return to baseline after refeeding.[9] However, so far these meal-related alterations have not been observed in humans under conditions where ghrelin levels are decreased.[83,84] Interestingly, a study in non-obese male subjects showed a negative correlation between body mass index (BMI) and fasting plasma levels of NUCB2/nesfatin-1.[83] In addition, other groups reported a positive correlation between NUCB2/nesfatin-1 plasma levels and BMI with lower levels in anorexic patients[85] and higher levels in obese subjects[86,87] giving rise to the regulation of NUCB2/nesfatin-1 by the amount of white adipose tissue, a tissue where NUCB2/nesfatin-1 was recently identified to be expressed.[86]

It is important to note that mature nesfatin-1 (10 kDa) has been described only in the initial report so far.[26] Subsequent studies only detected full length NUCB2 (47 kDa) in the brain,[28,88,89] gastric mucosa, pancreas, pituitary and adipose tissue,[9,86] whereas exogenous nesfatin-1 could be visualized with the techniques used.[9,89] This discrepancy raises the possibility of post-secretory processing of NUCB2 to nesfatin-1 in the blood or cerebrospinal fluid. This hypothesis is supported by the finding of processed nesfatin-1 in human plasma samples using a sensitive sandwich-type ELISA recognizing exclusively nesfatin-1 but not full length NUCB2.[83]

Despite the fact that our knowledge on the regulation of NUCB2/nesfatin-1 has increased over the past years, the receptor mediating NUCB2/nesfatin-1's actions remains to be identified. One study suggested an interaction with a G-protein-coupled receptor based on the finding that nesfatin-1 led to an increase of [Ca^{2+}] linked with protein kinase A signaling in isolated cultured rat hypothalamic cells.[27]

Effects on Food Intake and Energy Homeostasis

Although the physiological role of obestatin remains controversial[90] and those of desacyl ghrelin[91] and nesfatin-1[92] are still scarcely described, all these products of the X/A-like cell have been implicated in feeding regulation with ghrelin being the only stimulator of food intake and desacyl ghrelin and nesfatin-1 exerting anorexigenic actions.

Ghrelin, Desacyl Ghrelin and *n*-Decanoyl Ghrelin

Contrasting with the multitude of anorexigenic modulators of feeding,[93] ghrelin is the only known peripherally produced and centrally acting hormone that stimulates food intake in animals[94,95] and humans.[96] In line with the similar binding of octanoyl as well as *n*-decanoyl ghrelin to the GRLN-R, both forms stimulate feeding[7] and ghrelin-induced feeding is inhibited by various GRLN-R antagonists.[97] Further corroborating the key role of this receptor, injection of ghrelin did not result in an orexigenic response in GRLN-R knockout mice.[98,99] Ghrelin stimulates food intake via direct actions on the GRLN-R located on food regulatory brain nuclei after passage through the blood-brain barrier[100,101] and expressed on vagal afferents well established to convey gut peptide signaling influencing food intake.[102,103] Further supporting the importance of vagal signaling, subdiaphragmatic or gastric vagotomy prevented the orexigenic response to intravenous injection of ghrelin in rats.[102] However, another study reported a stimulation of food intake by intraperitoneally injected ghrelin in rats that underwent elective subdiaphragmatic vagal deafferentation.[104] These discrepant results may be related to different routes of administration of the peptide, higher doses recruiting alternative mechanisms and modalities of surgery that should be delineated in future studies.

As suggested by the negative correlation with BMI, ghrelin is not only affected by chronic changes in metabolic status but also involved in the long-term regulation of body weight homeostasis. Chronic infusion of ghrelin increases body weight gain in rodents which is due to increased appetite but also caused by the stimulation of fat storage and decrease of lipid mobilization resulting in enlargement of the fat depots.[41,105,106] In line with these findings, mice lacking both ghrelin and the GRLN-R have an increased energy expenditure associated with a decreased body weight,[107] whereas mice lacking either ghrelin[107,108] or the GRLN-R[107] do not show these alterations leading to the hypothesis of additional ligands and role of constitutive activity of receptors. In addition, ghrelin increased the mRNA expression of fatty acid synthase, acetyl-CoA carboxylase alpha, stearoyl-CoA desaturase-1 and lipoprotein lipase, all enzymes involved in the mediation of fat storage, whereas the mRNA expression of a major fat oxidation enzyme, carnitine palmitoyl transferase-1-alpha, was decreased.[109] Further supporting the physiological role of ghrelin in these processes, ghrelin knockout mice display

an opposite expression pattern of these enzymes.[109] Interestingly, in mice lacking β1-, β2- and β3-adrenoceptors and injected intracerebroventricularly with ghrelin these changes are not observed[109] pointing towards an involvement of the sympathetic nervous system in these homeostatic actions of ghrelin. Furthermore, the role of GOAT was investigated using genetic approaches in mice lacking GOAT or over-expressing ghrelin and GOAT.[110] GOAT knockout mice did not display alterations in body weight when fed a standard rodent diet but showed a reduction of body weight under conditions of high fat diet feeding compared to GOAT expressing wild type littermates.[110] Interestingly, substituting dietary medium-chain triglycerides results in a decrease of fat mass and body weight in mice lacking GOAT, leading to the speculation of GOAT acting as a lipid sensor.[110]

Besides its peripheral production, ghrelin is also expressed, although in lower quantities, centrally in the arcuate nucleus of the hypothalamus[111] and in neurons adjacent to the third ventricle.[112] The arcuate nucleus is crucially involved in the central orchestration of food intake[113] and neuroanatomical evidence indicated that ghrelin neurons in the arcuate nucleus are connected with neurons containing the orexigenic peptides,[114] agouti-related peptide (AgRP) and neuropeptide Y (NPY).[112,115] Peripheral injection of ghrelin selectively activates NPY neurons in mice[116] and similarly, intracerebroventricular injection of ghrelin activates NPY/AgRP positive arcuate neurons and upregulates the expression of NPY and AgRP mRNA.[117] The importance of NPY and AgRP signaling for the mediation of ghrelin's orexigenic effects was highlighted pharmacologically using anti-NPY and anti-AgRP antibodies.[118] Experiments in genetically modified mice showed that animals lacking NPY and AgRP do not respond to a peripheral ghrelin injection, whereas mice lacking either NPY or AgRP still increase food intake upon ghrelin administration[119] indicating a compensatory action of these two peptides. In addition to the stimulation of these NPY/AgRP-related orexigenic pathways, ghrelin inhibits the activity of proopiomelanocortin containing neurons resulting in reduced anorexigenic melanocyte stimulating hormone and cocaine- and amphetamine-regulated transcript signaling.[112]

Along with the underlying neural network involved in ghrelin orexigenic action, recent studies underpinned the subcellular signaling mechanisms in theses neurons. Ghrelin increases mitochondrial respiration in NPY arcuate neurons, an effect shown to depend on uncoupling protein 2 (UCP2) by the use of UCP2 knockout mice.[120] In addition, ghrelin-induced activation of NPY neurons and associated increase of food intake was also reduced in mice lacking UCP2[120] indicating a crucial involvement of this signaling pathway in ghrelin's orexigenic action. Fasting stimulates phosphorylation of hypothalamic AMP activated protein kinase (AMPK) resulting in decreased hypothalamic levels of malonyl-CoA and increased carnitine palmitoyltransferase 1 activity, effects mimicked by brain injection of ghrelin.[121] Conversely, blockade of AMPK signaling blunted the feeding stimulatory action of ghrelin.[121] This ghrelin-induced activation of AMPK was still observed in mice lacking UCP2,[120] whereas blockade of AMPK signaling reduced food intake in wild type but not UCP2 knockout mice, indicating that UCP2 is a downstream mediator of AMPK.[120] Lastly, chronic injection of ghrelin results in greater body weight gain in UCP2 knockout mice compared to UCP2 expressing wild type littermates which was associated with decreased fat oxidation,[122] a finding indicating the importance of UCP2 for the restriction of fat storage.

In contrast to the vast amount of data on ghrelin, the effects of desacyl ghrelin on food intake are less well characterized.[123] Initial studies reported a capsaicin insensitive reduction of food intake following intraperitoneal injection of desacyl ghrelin in rats[124] and intracerebroventricular or intraperitoneal injection in mice.[125] However, other studies did not report this anorexigenic effect following peripheral injection of desacyl ghrelin in fasted rats[126] or mice.[127] One study even reported a stimulation of food intake following intracerebroventricular injection of desacyl ghrelin at low dose in rats,[128] an effect that could reflect acylation of the peptide. In addition, another study showed that intraperitoneal injection of desacyl ghrelin, although not altering food intake when injected alone, abolished the ghrelin-induced stimulation of food intake following simultaneous intraperitoneal injection in rats.[126] This leads to the hypothesis of an interaction between these two peptide forms which could play a role in the regulation of food intake. Additionally, mice over-expressing both ghrelin and desacyl ghrelin show a decrease of food intake.[129]

In addition to the regulation of food intake, desacyl ghrelin has also been implicated in the regulation of long-term body homeostasis. Mice over-expressing desacyl ghrelin show reduced body size accompanied by a decreased body weight[130] associated with reduced perirenal and epididymal fat depots[131] compared to wild type littermates, whereas food intake was not altered[130] suggesting that desacyl ghrelin may act as a negative regulator of fat storage. However, one has to keep in mind that these mice displayed supraphysiological (10-50 fold increased) circulating desacyl ghrelin levels[130] that could greatly influence the results. However, in vitro desacyl ghrelin stimulates intracellular lipid ac-

cumulation in human adipocytes derived from obese subjects[132] which may contribute to the fat storage under conditions of obesity. These divergent results could be due to in vivo versus in vitro conditions or reflect species differences to be further characterized.

Obestatin

The initial study describing obestatin suggested a physiological anorexigenic role for obestatin and proposed a counter-regulatory mechanism opposing ghrelin's action.[8] However, only very few subsequent studies were able to partially reproduce these data,[133-136] whereas the vast majority of those studies conducted were unable to demonstrate an inhibitory effect of obestatin on food intake or body weight.[75,77,78,136-148] Thus, obestatin is not considered a physiological regulator of feeding or body weight homeostasis and therefore it was proposed to be renamed ghrelin-associated peptide.[149]

Nucleobindin2/Nesfatin-1

The landmark paper of Oh-I and colleagues[26] described a food intake inhibitory action following third ventricular injection of full length NUCB2 and nesfatin-1. In addition, repeated administration reduces body weight gain associated with a decrease in fat mass. The nesfatin-1 injection into the lateral or third brain ventricle-induced anorexigenic action with a delayed onset has been consistently reproduced in consecutive studies in rodents[150-155] and also in goldfish.[88] Conversely, blocking endogenous NUCB2/nesfatin-1 signaling using an anti-NUCB2 antisense oligonucleotide or anti-nesfatin-1 antibody injected into the third brain ventricle increased food intake in rats[26] indicating a physiological inhibitory effect of NUCB2/nesfatin-1 on food intake. In addition to the forebrain action, nesfatin-1 injected into the hindbrain at the level of the cisterna magna or the fourth ventricle also decreased dark phase food intake within the first hour post injection[153] pointing towards several responsive brain sites which may involve different downstream signaling. This is also supported by the fact that blockade of the anorexigenic corticotropin releasing factor receptor 2 (CRF_2) signaling system[156,157] using the CRF_2 antagonist, astressin$_2$-B injected into the lateral brain ventricle abolished the nesfatin-1-induced decrease of dark phase feeding, while the hindbrain nesfatin-1-induced anorexigenic effect was not altered.[153] Moreover, the melanocortin 3/4 receptor antagonist, SHU9119[26,158] and an oxytocin antagonist[150,154] abolished the forebrain nesfatin-1-induced decrease of food intake while leptin signaling is not involved.[26,150,159] Taken together, nesfatin-1 mediates its anorexigenic action via downstream CRF_2, melanocortin 3/4 and oxytocin signaling. In addition, also the blockade of NPY signaling may contribute to the anorexigenic effect based on in vitro data showing that nesfatin-1 hyperpolarizes isolated arcuate NPY neurons.[160] Interestingly, nesfatin-1 selectively inhibits dark phase food intake under ad libitum feeding conditions,[26,150,153,158] whereas during the light phase and after fasting inconsistent results were reported.[153,158] These data may support a specific interaction of nesfatin-1 with other neuronal circuitries uniquely recruited during the physiological dark phase of eating[161-163] which has to be investigated in future studies. A recent study characterized the feeding microstructure following intracerebroventricular injection of nesfatin-1 in mice showing that the peptide decreases dark phase feeding by inducing satiation (indicated by reduction of meal size) and satiety (indicated by decreased meal frequency associated with prolonged inter-meal intervals).[152]

While good progress has been made in identifying the central food intake reducing actions of nesfatin-1, the peripheral role is less well established. One study reported that full length nesfatin-1 and the 30 amino acid mid segment, nesfatin-1$_{24-53}$ reduce the dark phase feeding following intraperitoneal injection of a higher dose in ad libitum fed mice.[164] This is likely mediated by the vagus nerve since capsaicin pretreatment abolishes this effect[164] and nesfatin-1 activates the Ca^{2+} influx in primary cultured nodose ganglion neurons in vitro.[165] However, another study did not observe the anorexigenic effect of peripherally injected nesfatin-1 in mice and[152] as well as in rats,[153] whereas in goldfish only at a high dose, nesfatin-1 also reduces food intake.[88] Based on these data, nesfatin-1 exerts its anorexigenic action more potently when injected into the brain than peripherally and its peripheral effects still await thorough characterization. Whether different receptor subtypes are involved will also have to be established.

Summary and Perspectives

During the past decade the gastric endocrine-brain axis was the object of growing interest in the context of its role in food intake regulation. While the gastric endocrine X/A-like cell was thought to be restricted to the stimulation of food intake due to the synthesis and release of the orexigenic peptide, ghrelin, new developments, namely the better characterization of desacyl ghrelin along with the identification of the anorexigenic hormone, nesfatin-1 in X/A-like cells provided a paradigm shift promoting

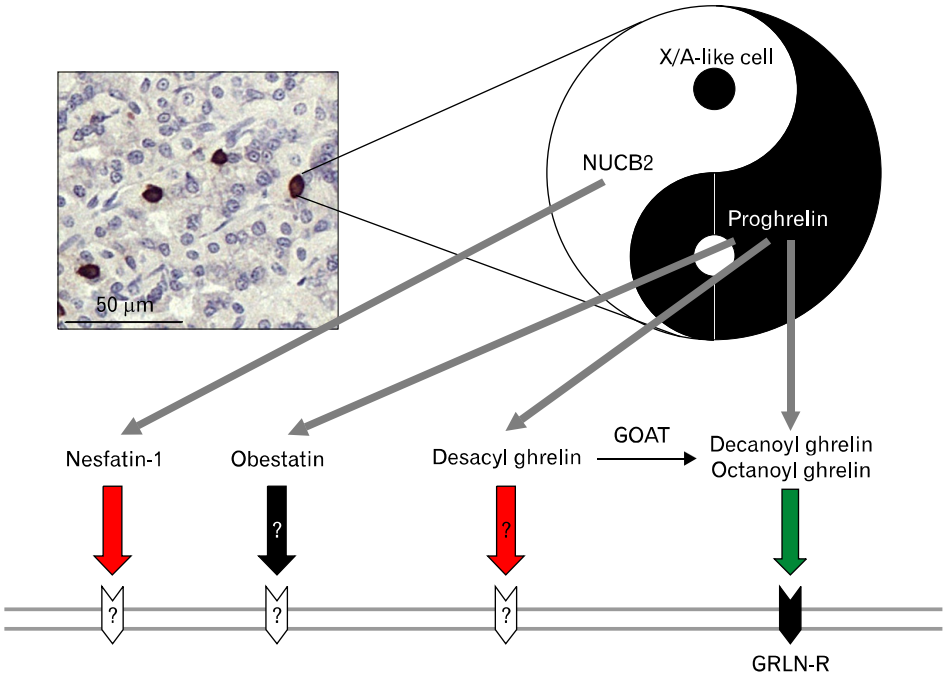

Figure. Peptide products of the gastric X/A-like cell and their effects on food intake. Red arrows indicate inhibition, the green arrow stimulation of food intake and the black a lack of effect on feeding. The question marks indicate lack of knowledge on effect and mediation. The insert shows rat gastric mucosa stained with an anti-ghrelin antibody. GOAT, ghrelin-O-acyltransferase; GRLN-R, ghrelin receptor; NUCB2, nucleobindin2.

this cell type as a dual regulator of food intake (Figure). In addition to the effects on feeding, all these hormones are involved in long-term body homeostasis with a stimulatory role of ghrelin and an inhibitory effect described for desacyl ghrelin and nesfatin-1. Although our knowledge greatly increased over the past years, several important questions remain to be answered. Those encompass the identification of yet unknown receptors using desacyl ghrelin and nesfatin-1 as their endogenous selective ligands and related signaling pathways. In addition, mechanisms regulating the activity of the ghrelin acylating enzyme, GOAT remain to be characterized and the role of peripheral nesfatin-1 has to be better defined. New tools including the isolation of ghrelin cells using mouse models (eg, those expressing green fluorescent protein bound to the ghrelin promoter) will help to advance the field and answer those questions.

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