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Minireview: Ghrelin and the Regulation of Energy Balance—A Hypothalamic Perspective

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The recently discovered hormone, ghrelin, has been recognized as an important regulator of GH secretion and energy homeostasis. Orexigenic and adipogenic ghrelin is produced by the stomach, intestine, placenta, pituitary, and possibly in the hypothalamus. The concentration of circulating ghrelin, principally derived from the stomach, is influenced by acute and chronic changes in nutritional state. To date, most studies focused on the role of ghrelin in GH secretion or its function in complementing leptin action to prevent energy deficits. The potential significance of ghrelin in the etiology of obesity and cachexia as well as in the regulation of growth processes is the subject of ongoing discussions. A large quantity of information based on clinical trials and experimental studies with ghrelin and previously available synthetic ghrelin receptor agonists (GH secretagogues) must now be integrated with a rapidly increasing amount of data on the central regulation of metabolism and appetite. In this overview, we summarize recent findings and strategies on the integration of ghrelin into neuroendocrine networks that regulate energy homeostasis. (*Endocrinology* 142: 4163–4169, 2001)

OBESITY AND RELATED disorders are among the leading causes of illness and mortality in the developed world (1). To better understand the pathophysiological mechanisms that underlie metabolic disorders, increasing attention has been paid to central regulatory elements in energy homeostasis, including food intake and energy expenditure (2–5). The past two decades have provided overwhelming evidence of the critical role that hypothalamic peptidergic systems play in the central regulation of appetite and metabolism (6, 7). The discovery of ghrelin (8–11) and its influence on appetite, fuel utilization, body weight, and body composition that is complementary to ghrelin's GH-releasing effect (12) adds yet another component to the complexity in the central regulation of energy balance.

Discovery of Ghrelin

Reverse pharmacology may be an appropriate term to describe the road to ghrelin's discovery. First, synthetic agonists with ghrelin-like activity [GH-releasing peptides (GHRPs) and GH secretagogues (GHSs)] were discovered by Bowers and co-workers in the late seventies (9, 13–15), followed by the cloning of ghrelin-GHS-receptor (GHS-R) in 1996 by Smith and co-workers (16–18). Subsequently, the elegant studies by Kojima and co-workers led to the identification of an acylated 28 residue peptide as an endogenous bioactive ligand for the GHS-R (8–11). It was called ghrelin, a term that contains "ghre-" as the etymological root for "growth" in many languages. "GH" and "relin," a suffix for releasing substances in generic names according to the USP Dictionary of USAN and International Drug Names, also represents an abbreviation for "growth-hormone-release," a characteristic effect of ghrelin (8, 11). The Ser^3-acylation that seems to be responsible for bioactivity of ghrelin is a modification that has been observed for the first time in mammalian physiology. There are no data to support the tempting speculation that the purpose of this modification is to increase ghrelin's lipophilic properties to facilitate transport across the blood brain barrier. However, the octanoyl side chain is essential for binding and activation of the GHS-R subtype-1a *in vitro* (19). Ghrelin might also bind to different GHS-R subtypes or receptor families where the octanoyl side chain is not needed. Detection and purification of the gastric enzyme responsible for the acylation of ghrelin may shed light on this fascinating question and will possibly even reveal the existence of other putative hormones carrying this modification.

Sources of Ghrelin

Ghrelin is predominantly produced by the stomach (8, 20–22), whereas substantially lower amounts are derived from bowel (21, 22), pituitary (23), kidney (24), placenta (25), and hypothalamus (8, 22). Although the majority of circulating ghrelin is produced in the stomach, other sources may increase ghrelin secretion in a compensatory manner. After gastrectomy, for example, plasma ghrelin level is surprisingly reduced only by 65% (26).

One of the most urgent and debatable hypotheses is whether ghrelin is produced in physiologically relevant amounts in the hypothalamus. This unresolved issue is the focus of several research groups. Data published or presented at recent meetings, in accordance with our own on-

Abbreviations: AGRP, Agouti-related protein; GHRP, GH-releasing peptide; GHS, GH-secretagogue; GHS-R, GHS receptor.

going investigations, have shown ghrelin to be present in several regions of the hypothalamus through the use of immunohistochemical detection methods (8, 22). Depending on the ghrelin epitope recognized by the antibody in use, ghrelin-positive cells have been identified in varying hypothalamic areas, whereas all of these antisera have been successfully used in RIAs. Detection of hypothalamic ghrelin mRNA by use of PCR has been accomplished (8); however, this issue needs to be further investigated by the detection and regional distribution of ghrelin mRNA by *in situ* hybridization. At this point, it is not clear if the detection of ghrelin in the hypothalamus using immunohistochemistry reflects ghrelin peptide that is produced by hypothalamic neurons or ghrelin peptide that is derived from the stomach. It seems logical that gastric ghrelin reflects an acute nutritional state. However, even minimal ghrelin expression in the hypothalamus (8, 12) or circulating placental ghrelin during pregnancy (25) may significantly influence food intake, nutrition partitioning, and fat utilization. Regardless of the source, in the end it is most likely the modulation of hypothalamic circuits by ghrelin that mediates changes in energy homeostasis.

Ghrelin and GH Secretion

Based on rodent experiments (27–29) and clinical studies (30–33), it is evident that ghrelin is indeed a potent GH-releasing agent. However, no significant correlation seems to exist between plasma ghrelin concentrations and circulating levels of GH or IGF-I (unpublished data, Tschöp *et al.*) even though both ghrelin and GH increase during fasting (34, 35). Very recent data indicate that most of the ghrelin-induced GH secretion is not only directly opposed by somatostatin action, but also involves mediation through GHRH (33, 36, 37). However, ghrelin also releases GH *in vitro* from primary rat pituitary cells (8, 12), and GHRP-2, a potent ghrelin receptor agonist, releases GH *in vivo* in patients with GHRH receptor mutations (38). This indicates the existence of GHRH-independent effects of ghrelin on GH secretion mediated by hypophyseal GHS-Rs, which were originally cloned from the pituitary (16). Alternatively, ghrelin may stimulate an unidentified hypothalamic agent (U-factor) that, in turn, stimulates GH release (39).

Ghrelin and Energy Balance

The first published evidence for the involvement of ghrelin in the regulation of appetite was provided by Ghigo and co-workers (30). They described that 3 out of 4 healthy volunteers spontaneously reported hunger following ghrelin administration as a "side effect" in a clinical study analyzing GH release (30). This hunger-inducing effect of ghrelin has now been confirmed in two more studies, where, again, 3 out of 7 (33) and 9 out of 11 individuals report hunger as the only sensation after ghrelin injection (40). A large number of animal studies added strength to the argument that ghrelin is involved in the regulation of energy balance. For example, exogenous ghrelin induces adiposity in rodents by stimulating an acute increase in food intake, as well as a reduction in fat utilization (12, 41–46). Adipogenic as well as orexigenic effects of ghrelin are independent from its ability to stimulate GH secretion (12, 46) and are most likely mediated by a specific central network of neurons that is also modulated by leptin (2–7, 9, 12, 41–46). Regulation of ghrelin secretion, as well as its biological effects, appear to be opposite those of leptin. However, from a teleological point of view, ghrelin and leptin might really be complementary players of one regulatory system that has developed to inform the central nervous system about the current status of acute and chronic energy balance (12, 38–49). In addition, a specific role for ghrelin might be to ensure the provision of calories that GH requires for growth and repair (41).

In humans, circulating ghrelin levels are decreased in chronic (obesity) (48) and acute (caloric intake) (26, 34, 47) states of positive energy balance, whereas plasma levels of ghrelin are increased by fasting (12, 34) and in cachectic patients with anorexia nervosa (26). Of course, it has yet to be proven that the rather modest changes in circulating ghrelin, in the 100 fmol range, have physiological relevance for hypothalamic receptor sites. One plausible explanation is that if ghrelin is indeed a hormone signaling the need to conserve energy (12), ghrelin secretion is triggered to counter further deficit of energy storage and to prevent starvation or cachexia. A very recent study shows a pre-meal rise of human plasma ghrelin, suggesting a possible role of ghrelin as a hunger signal triggering meal initiation (34). In rodents, fasting and hypoglycemia increase ghrelin levels, whereas intake of food, especially carbohydrates (dextrose), decreases ghrelin secretion (12, 41, 50). We speculate that this obvious connection between glucose levels, ghrelin secretion and GH secretion is likely to be involved in the physiological mechanism of diagnostic procedures such as oral glucose tolerance testing (for acromegaly) and insulin tolerance testing (for GH deficiency). Differential effects of ghrelin might be mediated by separate ghrelin (GHS-R) subtypes as recently suggested by Thorner and co-workers (51). Based on a series of elaborate studies using GHS-R antagonists ([D-Lys^3]GHRP-6 and BMS-265711, also an NPY-antagonist) and an NPY-Y1-R antagonist ([D-Trp32]NPY), they showed that the orexigenic effect of ghrelin can be dissociated from its GH releasing effects, suggesting distinct GHS-R-subtypes. Based on the observation of differential orexigenic effects of hexarelin and its analogs and GH secretagogue actions at the pituitary gland (52, 53), the existence of additional subtypes of the GHS-R (16–18) had previously been hypothesized. The putative adipogenic effects of ghrelin in humans remains to be shown because it is possible that ghrelin has different effects on energy balance in humans and rodents. In addition, ghrelin-induced adiposity could be only a transient effect and the therapeutic potential of ghrelin in cachectic humans might therefore turn out to be as disappointing as the efficacy of leptin for the therapy of human obesity (5, 54). Carefully conducted clinical studies focusing on body composition as well as long-term studies on ghrelin treatment in rodents are necessary to further address this question.

Ghrelin and Brain Centers of Energy Balance

Our current understanding of the involvement of different hypothalamic systems in metabolic regulation arises from early degeneration studies in rats. Destruction of distinct

hypothalamic regions, particularly the ventromedial nucleus but also the areas of the paraventricular and dorsomedial nuclei, induced hyperphagia (55–60). In contrast, discrete lesions placed in the lateral hypothalamus (61, 62) reduced food intake. During the last two decades, a substantial amount of research demonstrated that NPY, administered into the cerebral ventricles (63) or other specific hypothalamic sites (64), induced food intake. However, in addition to NPY, several other hypothalamic peptides were found to affect appetite and feeding behavior (for details see Refs. 2–7). Appetite- stimulating neuropeptides include melanin concentrating hormone, hypocretins/orexins (produced in a distinct subset of neurons of the lateral hypothalamus perifornical region) (65–68) and agouti-related protein (AGRP, coproduced with NPY in the same arcuate nucleus neurons) (69–71). Appetite-suppressing neuropeptides include the POMC derivate, α-MSH (6, 7, 72) that is produced in arcuate nucleus perikarya (73).

An important milestone to link the central regulation of metabolism with peripheral levels of energy storage was the discovery of the adipose hormone, leptin. Genetic mouse or rat mutants, including *db/db* and *ob/ob* mice and *fa/fa* rats become strikingly obese. Molecular analysis has shown that the primary genetic defect in these animals relates to either abolished leptin production (*ob/ob* mice) or impaired leptin receptors (*db/db* mice; *fa/fa* rats; leptin-R) (5, 74–77). Similar examples of obesity in humans have been found and are associated with a mutation of leptin or the leptin-receptor (78–80). Leptin is released by adipose tissue and has been suggested to be the key-signal reflecting adipose stores. Leptin receptors are found in the hypothalamus, particularly in the arcuate nucleus where leptin is thought to exert its primary feedback signaling (81–87). Recent experiments in rodents and primates have been attempting to tie together the diverse hypothalamic peptidergic systems with hormone receptors, including leptin receptors, to decipher the hypothalamic signaling modality underlying the regulation of daily energy homeostasis (81–92). A schematic illustration of some of these interactions and the way ghrelin signaling may be integrated into these circuits is shown on Fig. 1.

Peripheral ghrelin is mainly produced in the gastrointestinal tract (8, 10, 22–24). It reaches ghrelin-receptors in the anterior pituitary and potentially in the mediobasal and mediolateral hypothalamus through the general circulation to stimulate GH release and to regulate energy homeostasis (12). It remains to be determined whether circulating ghrelin can reach brain areas outside of the blood brain barrier only, such as the ventromedial arcuate nucleus (93), or it has the ability to target areas protected by the blood brain barrier. Areas protected by the blood brain barrier include most hypothalamic nuclei and the rest of the brain (93). Ghrelin-containing cells are also present in the mediobasal hypothalamus, where GHRH cells and the neuronal network that regulates energy balance are located (8, 22). Detailed phenotypes and macroscopic connectivity of different hypothalamic networks regulating metabolism have been described by numerous recent outstanding reviews (2–7). Among hypothalamic peptidergic circuits, particular significance is attributed to the arcuate nucleus opiate neurons that produce α-MSH, a main anorexigen and energy expenditure enhancer (72), and to its interrelationship with another group of arcuate nucleus neurons that produce both NPY and an endogenous antagonist of α-MSH, AGRP (69). The interaction between these two distinct populations of cells is currently considered as a *primum movens* in the regulation of energy homeostasis. However, there are other peptidergic circuits within the hypothalamus, including the lateral hypothalamic orexin/hypocretin- and melanin concentrating hormone-producing cells, that appear to respond to peripheral metabolic signals and alter food intake as well as energy expenditure (65–68). In light of the aforementioned excellent reviews (2–7), we will avoid an in-depth description of these peptidergic systems here but will attempt to emphasize a better appreciation of the neuronal doctrine for the integration of emerging experimental data on ghrelin.

In the brain, receptors for ghrelin were detected in multiple hypothalamic nuclei as well as in the hippocampus, substantia nigra, ventral tegmental area, and dorsal and median raphe nuclei (8, 94–98). In a series of experiments, Dickson and co-workers, first using synthetic GHS-R agonist, and then ghrelin, provided evidence that this novel metabolic hormone, in fact, interacts with the aforementioned hypothalamic peptidergic systems in the central regulation of metabolism (99–103). For example, they found that following central ghrelin administration, c-*fos*, an early proto-oncogen that reflects cellular activity, is induced in the medial arcuate nucleus where NPY/AGRP cells are located (103). It was also shown that Y1-receptor antagonists as well as melanocortin agonists and antisera to both NPY and AGRP may interfere with ghrelin's feeding-inducing effect (42, 43, 46). However, absence of NPY in genetically engineered NPY-ko mice does not diminish ghrelin-induced feeding or adiposity suggesting a key-role for AGRP in the mediation of ghrelin's effects on energy balance (12). The effect of ghrelin on metabolism seems to be the exact opposite to that of leptin (2–7, 9, 10, 12). In obesity, when plasma leptin levels are elevated, ghrelin plasma levels are decreased indicating physiological adaptations to the positive energy balance rather than an involvement in the etiology of obesity (48, 49). Of course, it is important to note that, while ghrelin is regulated acutely like a satiety factor, leptin levels are not regulated by meals, but rather by actual increase in adipose stores.

Ghrelin's Hypothalamic Signaling Requires Synapses

Figure 1 depicts a highly complex interaction between a variety of hypothalamic peptidergic systems, including the putative ghrelin network, in the central regulation of energy balance. It has to be noted, however, that this drawing is not all-inclusive and represents only the "tip of the iceberg." There are many more hypothalamic and extra-hypothalamic neurotransmitters and neuropeptides that act via the aforementioned circuits (for example coexistence of GABA with NPY; 91) or in separate pathways [for example, ciliary neurotrophic growth factor (CNTF), 104, 105], and are interconnected with the illustrated systems (for further review see Refs. 2, 6, 7). In addition, receptors for the different neuropeptides as well as for peripheral hormones that affect metabolism, including insulin, thyroid hormones, gonadal steroids and glucocorticoids, are also present in these re-

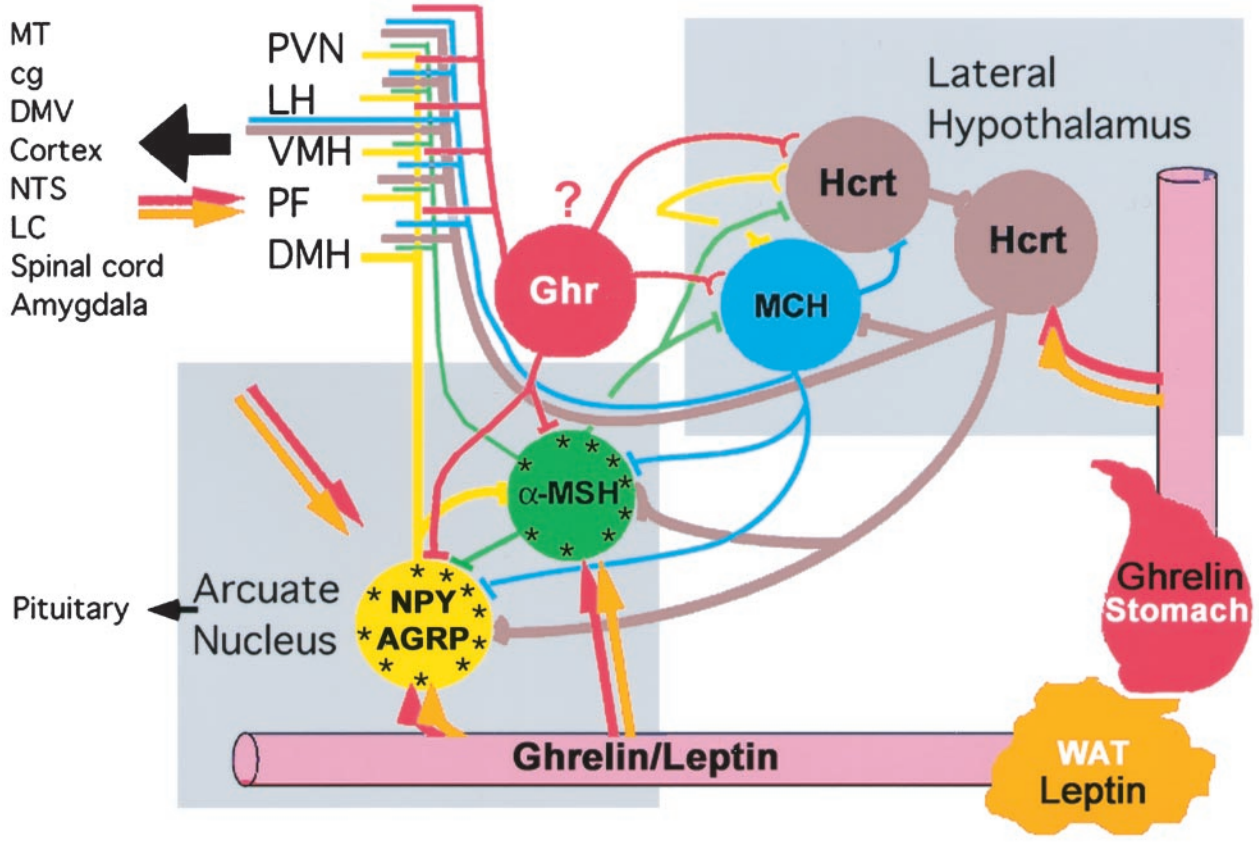

FIG. 1. Schematic representation of the interaction between key hypothalamic peptidergic systems in the central regulation of daily energy homeostasis and their relationship to peripheral and putative hypothalamic ghrelin. Ghrelin, a hunger signal, is released from the stomach into the circulation and may be produced (?) in a subset of hypothalamic neurons (*red*). Leptin, a satiety signal, is released from white adipose tissue (WAT) into the circulatory system. Ghrelin (*red arrows*) and leptin (*orange arrows*) directly target the hypothalamus and brain stem areas. While brain stem areas on this drawing are illustrated as efferent targets of hypothalamic circuits, critical pathways exists from the brain stem to the hypothalamus, as well, that can mediate ascending ghrelin and leptin signaling. AGRP is produced in NPY cells (*yellow*) and acts to block the inhibitory action of the POMC derivate, α-MSH (*green*), on feeding. Both AGRP/NPY and POMC cells are apparent targets of direct ghrelin action via GHS-R (*). The NPY neurons that receive lateral hypothalamic input, including HCRT (*brown*) and melanin concentrating hormone (MCH) (*blue*) innervation, project to a number of regions of the brain, particularly those implicated in feeding mechanisms, including the paraventricular nucleus (PVN), lateral hypothalamus, LH, ventromedial nucleus (VMH), perifornical region (PF), and dorsomedial nucleus (DMH). The same regions also receive direct lateral hypothalamic input as well as innervation from α-MSH cells. These regions, in turn, project (*large black arrow*) widely throughout the brain to loci including the medial thalamic nuclei (MT), central gray (cg), dorsal motor nucleus of the vagus (DMV), cortex, nucleus of the solitary tract (NTS), locus coeruleus (LC), spinal cord, and amygdala. Ghrelin-targeted arcuate nucleus neurons may also affect neuroendocrine cells that are responsible for the regulation of pituitary hormone secretions, including gonadotrophs (LH/FSH), TSH, ACTH, and GH. It is yet to be determined what role central *vs.* peripheral ghrelin plays in the regulation of this circuitry and at what sites and subcellular levels ghrelin signaling is interacting with that of leptin.

gions. During food deprivation when leptin levels rapidly decline (106) and NPY/AGRP production is elevated, but POMC neurons are suppressed (106–110), circulating ghrelin levels increase (12, 33, 41, 48) suggesting that leptin and ghrelin coregulate hypothalamic peptidergic systems in opposite ways. These observations further support the hypothesis that ghrelin, as a "hunger signal," is the counterpart of leptin aiming to prevent further energy deficit. However, considering the extreme complexity of hypothalamic interactions of different peptidergic circuits and peripheral hormone receptors, it is necessary to determine the hierarchy and direction of signaling flow within these systems to understand ghrelin's central effect on metabolic regulation. For that, a multidisciplinary approach is mandatory.

The hypothalamus is composed of a complicated set of regulatory neurons that in most cases cannot be identified by traditional means of cell segregation, *i.e.* location, soma size, or dendritic arbor. Therefore, to identify specific types of neurons, cytochemistry must be used. In addition, as in all other brain areas, the primary mode of communication between hypothalamic peptidergic circuits is via synapses. The only reliable way for assessing synapses is by the use of conventional electron microscopy and electrophysiology because proximity of different cells assessed by light microscopy is not a convincing indicator of neuronal interaction. Thus, determination of the qualitative and quantitative synaptological relationship between GHS-Rs, ghrelin-producing neurons, and other key hypothalamic peptidergic systems and their receptors will be an important step for gaining insight into the hypothalamic signaling modality of ghrelin. Of course, the anatomical experiments alone will not be sufficient to determine the actual involvement of the pre-

synaptic ghrelin system in the regulation of the postsynaptic circuit, but provides an invaluable map that is necessary for the correct interpretation of data gathered with other tools. In fact, anatomical studies need to be complemented by parallel electrophysiological analyses. An elegant example of such an approach is the recent work by Cowley and colleagues (111), in which leptin's effect was analyzed on genetically tagged arcuate nucleus α-MSH cells and the qualitative synaptology of these cells was simultaneously assessed. That approach not only eliminated the pitfalls of the individual experimental techniques but immediately provided a more comprehensive view on a given hypothalamic neuronal system (111).

The significance in determining the spatial relationship between different afferents using anatomical and electrophysiological tools may further be appreciated when one considers that a synapse is more potently able to affect postsynaptic cells when located proximally either on the cell body or postsynaptic dendrite than when it is located more distally. In addition, both previous electrophysiological (111–113) and morphological observations (92, 111) indicate that an extensive interaction exists between presynaptic terminals to affect hypothalamic cells both in the arcuate nucleus where NPY/AGRP and α-MSH cell bodies are located and in a model efferent target, the parvicellular paraventricular nucleus. The impact of ghrelin on arcuate and parvicellular paraventricular nucleus neurons will be readily dependent on their synaptic organization on the postsynaptic cells and their interaction with other systems presynaptically. One of the best examples to illustrate this synaptologic interaction is the relationship between the NPY/AGRP and α-MSH systems. Electrophysiological and anatomical observations pointed to both the arcuate and paraventricular nuclei as primary sites for the interplay between AGRP and α-MSH systems (111, 114). Because ghrelin's action appears to be mediated by the NPY/AGRP system, it is not unlikely that ghrelin will act in the arcuate nucleus as well as in the paraventricular nucleus to modulate the interaction between NPY/AGRP and α-MSH. It may be that peripheral and central ghrelin contribute equally to the regulation of both of these hypothalamic areas, but it is also conceivable that stomach-derived ghrelin affects the arcuate nucleus where the blood-brain barrier is less effective, whereas hypothalamic ghrelin is more involved in the modulation of hypothalamic sites within the blood-brain barrier, such as the paraventricular nucleus. An alternative and equally feasible pathway for ghrelin signaling from the stomach is via an ascending neural network through the vagus nerve and brain stem nuclei that ultimately reaches the hypothalamus (43).

When electrophysiological and anatomical techniques are combined with conventional physiological and molecular biological approaches, as well as with the very recently developed revolutionary tracing technique of DeFalco *et al.* [(115) which allows tracing of inputs of chemically identified subpopulations of neurons], it is reasonable to expect that not only a thorough understanding of ghrelin's action will be achieved at a faster pace, but great advances will be made toward the general understanding of the hypothalamic machinery in metabolism regulation.

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