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Ghrelin in the CNS: From hunger to a rewarding and memorable meal?

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Abstract

Ghrelin, the endogenous agonist of the growth hormone secretagogue receptor, has been shown to induce robust feeding responses in numerous experimental models. Although ghrelin comes from both peripheral and central sources, its hyperphagic properties, to a large extent, arise from activity at the brain level. The current review focuses on describing central mechanisms through which this peptide affects consumption. We address the issue of whether ghrelin serves just as a signal of energy needs of the organism or – as suggested by the most recent findings -also affects food intake via other feeding-related mechanisms, including reward and memory. Complexity of ghrelin's role in the regulation of ingestive behavior is discussed by characterizing its influence on consumption, reward and memory as well as by defining its function within the brain circuitry and interplay with other neuropeptides.

Keywords

ghrelin; food intake; reward; energy; brain

Ghrelin and its receptor: the beginnings

Bowers et al. discovered synthetic peptides that stimulated and amplified the release of growth hormone (GH) by acting at the hypothalamus and the pituitary (Bowers et al., 1980; Bowers et al., 1984). Their GH hypophysiotropic activity was distinct from the action of growth hormone releasing hormone (GHRH) and it was GHRH-independent. They were classified as growth hormone-releasing peptides (GHRPs) and growth hormone secetagogues (GHSs) (for review, see (Smith, 2005)).

The GHS binding sites are abundant in the central nevous system (CNS), especially in the hypothalamus and the pituitary (Sethumadhavan et al., 1991), yet the actual receptor remained undefined for years. In 1996, the G protein-coupled GHS receptor (GHS-R) was cloned from the hypothalami and pituitary glands (Howard et al., 1996). It was ultimately classified as type 1a or GHS-R1a; other types of receptors displaying affinity to GHSs have been found to date,

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including GHS-R1b, but their physiological importance remains unclear (Gnanapavan et al., 2002). The GHS-R is expressed widely in the brain and peripheral organs, including the stomach, intestine and pancreas (Howard et al., 1996; Kageyama et al., 2005; Papotti et al., 2000).

Identification of the GHS-R intensified search for its endogenous ligand, particularly because synthetic GHSs had been shown to affect a variety of processes other than GH release, including food intake (Locke et al., 1995). Kojima et al. reported the purification of a 28-amino acid peptide acting as a GHS-R agonist and dubbed it “ghrelin” (Kojima et al., 1999). They reported that the molecule contains an octanoyl group at a serine residue, and this feature was later associated with the ability of ghrelin to bind to the GHS-R1a and display functional activity (Bednarek et al., 2000). Presumably, unacetylated ghrelin, which has been found in the blood, may also possess physiological relevance although likely through a different receptor system (Hosoda et al., 2000).

Two reservoirs of ghrelin exist: in the gastrointestinal tract and in the CNS (Kojima et al., 1999; Lu et al., 2002; Mondal et al., 2005). The majority of ghrelin is synthesized by the stomach and released to the general circulation. Circulating ghrelin can probably reach central targets as it crosses the blood-brain barrier in a highly regulated process (Banks et al., 2002). One should note, however, that the blood-brain barrier passage has been shown primarily in the brain-to-blood direction. More recent data by Diano et al. demonstrate transport of ghrelin from blood to the hippocampus (Diano et al., 2006). Hence our knowledge of the ability of the ghrelin molecule to cross the barrier from the circulation into the brain is somewhat incomplete. Another pool of ghrelin is produced in one of the major hypothalamic sites involved in feeding regulation, the arcuate nucleus (ARC), primarily in its ventral portion (Lu et al., 2002; Mondal et al., 2005). In addition, this peptide is synthesized by a group of neurons adjacent to the third ventricle between four hypothalamic nuclei: dorsomedial (DMH), ventromedial (VMH), paraventricular (PVN), and ARC. These neurons project to multiple central regions releasing ghrelin directly within the brain circuitry (Cowley et al., 2003).

The discovery of ghrelin and its receptor was initially viewed as a milestone in understanding GH-related endocrine mechanisms. However, it soon became apparent that aside from ghrelin’s role as a GH secretagogue, it acts an orexigen. In fact, the endogenous GHS-R agonist administered peripherally and directly into the brain induces an acute and robust feeding response, only a bit lower in magnitude than the effect of neuropeptide Y (NPY) (Wren et al., 2001; Wren et al., 2000). Long-term injections of this peptide continue to stimulate consummatory behavior and promote weight gain by increasing caloric intake and by reducing energy expenditure. While ghrelin comes from both peripheral and central sources, its hyperphagic properties, to a large extent, arise from the activity at the brain level (e.g. (Kamegai et al., 2001; Luckman et al., 1999; Olszewski et al., 2003a; Olszewski et al., 2003b; Wren et al., 2002; Wren et al., 2000)). Hence, particular emphasis has been placed on defining central mechanisms involved in ghrelin-driven overconsumption, especially on characterizing the “place” of ghrelin and the GHS-R within the feeding-related circuitry. This functional interplay between ghrelin and other neural and neuroendocrine systems plays a decisive role in how the GHS-R ligand affects consumption. Therefore, the effect of this substance on the intake of foods that differ in caloric density, flavor, macronutrient composition, and palatability as well as the influence of various aspects of feeding on ghrelin profile reflect the interplay between ghrelin and other neuroactive components within the brain circuits.

Central sites of action

Peripheral and central injections of ghrelin induce voracious feeding under a variety of conditions in laboratory animals. Wren et al. injected freely fed rats intraperitoneally (IP) with

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ghrelin at the beginning of the light phase of the LD cycle, thus, at the time when the animals were sated following the night-time consummatory activity, which is particularly prominent at the onset and at the end of the dark period (Wren et al., 2000). These authors observed a 4- to 6-fold increase in the amount of ingested standard diet and the feeding response was seen as soon as within 1 hour post-injection. Interestingly, when the same animals were injected with ghrelin again just four hours later, they exhibited the second episode of elevated food intake, which was similar in magnitude to the initial one, whereas the effect of the first injection abated (Wren et al., 2000). Another study showed that rats receiving ghrelin 3 times daily still displayed elevated consumption upon each injection, and chronic IP administration of this peptide over several days lead to a gradual increase in overall 24-h food intake (Wren et al., 2001). It suggests that ghrelin's orexigenic properties remain unaffected even in the state of substantial satiation. It should be mentioned that a pronounced weight gain induced by ghrelin, aside from the influence on consumption levels, is partly associated with the reduction in fat utilization and energy expenditure as shown in experiments employing rats and mice (Tschop et al., 2000).

In a recent study, Hashimoto and colleagues observed that intravenous (IV) infusion of ghrelin in sated animals also induced hyperphagia and, noteworthy, this effect could be seen even in rats that had undergone 24 hour-long water deprivation prior to the pharmacologic treatment (Hashimoto et al., 2007). These investigators reported also that prandial drinking was unchanged by ghrelin during the injection-induced bout of feeding, even though water intake was significantly reduced when the feeding activity subsided. Hence, consummatory activity stimulated by ghrelin appears to be limited only to food intake.

Intracerebroventricular (ICV) administration of this peptide stimulates feeding and body weight gain to a similar degree as IP treatment (Wren et al., 2001; Wren et al., 2000). Third ventricular injection of the GHS-R agonist decreases latency to eat and increases meal frequency in the 3-h post-injection period (Faulconbridge et al., 2003). Moreover, as revealed via a side-by-side comparison, the magnitude of ghrelin-induced response is virtually identical with the one elicited by NPY (Wren et al., 2001; Wren et al., 2000). Conversely, a direct injection of anti-ghrelin immunoglobulin (IgG) suppressed food intake in animals subjected to short-term deprivation as well as in rats having ad libitum access to chow that were injected with the peptide at the beginning of the dark phase of the LD cycle (Nakazato et al., 2001). Nakazato et al. found that a chronic ICV infusion of 250 pmol of ghrelin per day for 12 days using an osmotic minipump in rats promoted a significant body weight gain and had a consistent feeding stimulatory effect on each day of peptide administration (Nakazato et al., 2001). These data strongly suggest that central GHS receptors may play a crucial function in mediating orexigenic properties of their natural agonist, especially considering the fact that ghrelin is probably capable of crossing the blood-brain barrier. In fact, Esler and colleagues tested the effectiveness of oral GHS-1Ra antagonists on consummatory behavior and body weight of mice (Esler et al., 2007). Although the reduction in feeding and body weight was apparent in antagonist-treated animals, these authors noted the difference in the efficacy and potency of the compounds, corresponding to the ability of these substances to penetrate to the CNS.

While it became apparent that the brain GHS receptors play a pivotal role in feeding regulation, a number of studies focused on identification of sites within the brain that mediate ghrelin-dependent hyperphagia. The analyses of the distribution of the ghrelin system components in the brain, i.e. the GHS-R, ghrelin-containing neurons and axon terminals, revealed their abundant presence throughout the feeding-related circuitry. In situ hybridization studies by Guan et al. showed that the receptor is expressed predominantly in the hypothalamus, with particularly high density levels in the ARC, PVN and VMH (Guan et al., 1997). A very thorough analysis performed by Zigman and coworkers compared GHS-R distribution in the rat versus mouse CNS (Zigman et al., 2006). They confirmed the earlier findings by Guan et

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al. pertaining to the hypothalamus and established that the highest level of expression exists in the sites classically viewed as involved in energy metabolism, including the ARC and PVN (Guan et al., 1997). They also determined that several brainstem areas are rich in the GHS-R, such as the nucleus of the solitary tract (NTS), area postrema (AP) and dorsal motor nucleus of the vagus (DMNV). This outcome indicated that ghrelin may influence the activity of wide brainstem-hypothalamic pathways and networks. In addition, the presence of the GHS-R in reward regions, including the ventral tegmental area (VTA) (Zigman et al., 2006), suggests a link between ghrelin and feeding for reasons other than hunger, namely, for palatability. Consistent with the presumed role of this peptide in consumption control in mammals, there were no striking discrepancies in the localization of the GHS-R between the mouse and rat brain, although the signal strength indeed differed in some sites (Zigman et al., 2006).

Ghrelin-containing axon terminals have been immunohistochemically detected in areas of the brain involved in feeding control, and the distribution of fibers roughly corresponds to the expression of the GHS-R. Particularly high density of these terminals within the hypothalamus can be found in the PVN, DMH, VMH, ARC and lateral hypothalamus (LH) (Cowley et al., 2003). Interestingly, in the PVN, the majority of terminals are localized within the parvocellular part of the nucleus, whose cells send projections to and receive input from, among others, brainstem sites, such as the NTS or DMNV (Buller et al., 2003; Sawchenko and Swanson, 1982). Ghrelin-positive fiber terminals have also been identified in extrahypothalamic sites, such as the nucleus accumbens (NAcc) and amygdala (Cowley et al., 2003), whereas the brainstem distribution of ghrelin-immunoreactive (IR) components remains to be characterized. The analysis of topography of the ghrelin system in the human brain yielded conclusions similar to those obtained in rodent studies in relation to the presence of fibers and terminals. However, ghrelin-IR cell bodies were not visualized in the ARC or any other area (Menyhert et al., 2006). Likely, this discrepancy can be attributed to the fact that animals used in the aforementioned anatomical studies had been treated with colchicine, axonal transport blocker, prior to being sacrificed.

It has been shown that peripheral and central injections of ghrelin at doses that stimulate consumption alter cellular activity in brain appetite centers. Lawrence and coworkers studied c-Fos immunoreativity in selected regions upon ICV delivery of ghrelin and a synthetic GHS-R agonist, GHRP-6 (Lawrence et al., 2002). The GHS-R stimulation caused an increase in the number of c-Fos-positive nuclear profiles in the majority of sites involved in feeding control, including the ARC, PVN, DMH, VMH and LH in the hypothalamus and the NTS and AP in the brainstem.

There is no clear consensus regarding the central distribution of immediate early gene products upon peripheral delivery of ghrelin and its analogs. Some authors suggested that changes can be observed only in very few sites. For example, Hewson et al. found that systemic administration of ghrelin and GHRP-6 elevated the number of neurons expressing c-Fos and Egr-1 proteins only in the ARC (Hewson and Dickson, 2000). The observed increase was even greater in fasted than in fed rats, which suggests that the central response induced by ghrelin is dependent upon the nutritional state and/or current consummatory activity of the animal. Ruter et al. found an increase in the density of Fos-positive cells in the ARC and PVN, but did not note any alterations in the brainstem (Ruter et al., 2003). Furthermore, Takayama and colleagues injected animals IV with ghrelin and determined that the treatment affected c-Fos profile in several feeding-related sites, including the ARC, CeA, NTS, DMNV and AP. The exact cause of these discrepancies is unknown, although one can speculate that, e.g., peripheral factors may modify significantly central actions of this peptide. In line with this hypothesis, it has been reported that deprivation and refeeding influence c-Fos patterns in the CNS structures following administration of synthetic and natural GHS-R agonists (Luckman et al., 1999; Tung et al., 2005). Also the internal milieu associated with hormones reflecting the nutritional/energy

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status of the organism, such as leptin, appears to alter responsiveness of ARC neurons to ghrelin and GHRP-6 (Hewson et al., 2002; Tung et al., 2001). Together, the c-Fos studies confirm that the ghrelin - regardless whether of peripheral or central origin - indeed affects neuronal activity at sites involved in food intake regulation, and that feeding-related brain circuitry integrates ghrelin signaling.

Generalized ICV injections of ghrelin provided substantial evidence indicating the importance of the central GHS-R in mediating ghrelin-dependent hyperphagia, however they did not define which populations of this receptor were in fact engaged in the process. Therefore, a series of studies was performed in which site-specific injections of the GHS-R ligand were followed by food intake measurements. Wren et al. conducted a survey comparison of orexigenic responses to ghrelin infused directly into several hypothalamic nuclei (Wren et al., 2001). These authors found an increase in a consummatory response following administration of ghrelin into the ARC, PVN, DMH, LH, anterior hypothalamic area (AHA), medial preoptic area (MPA) and supraoptic nucleus (SON). Although that study did not employ full dose response paradigms, as two doses were used throughout the experiment involving different sites, the lower 30-pmol dose of the compound stimulated consumption when injected only in the ARC and PVN, whereas the remaining sites required stimulation with 300 pmol ghrelin.

Subsequent feeding experiments confirmed that those hypothalamic regions are crucial relay stations for ghrelin's action (e.g. (Olszewski et al., 2003a; Olszewski et al., 2003b)). However, the ARC and PVN appear to serve as key elements of the network. In an interesting study, Tamura et al. injected ghrelin ICV in ARC-ablated rats. Animals whose ARC neurons had been destroyed did not exhibit an elevated consummatory activity in result of the injection, yet the stimulatory effect of the peptide on GH release was somewhat retained (Tamura et al., 2002). Intra-ARC delivery of anti-ghrelin IgG induced a short-lived (up to 6 hours) decrease in feeding, whereas an ICV injection produced a prolonged 24-h effect (Bagnasco et al., 2003). Hence ARC-derived circuitry sensitive to ghrelin appears to be involved in short-term regulation of consumption, whereas long-term feeding control may require the involvement of additional site(s). Finally, the prominent role of the ARC in mediating ghrelin's orexigenic properties has been established in both males and females. Currie found that ARC as well as PVN infusions of ghrelin induce a feeding response as robust as that observed due to ICV treatment in both male and female rats (Currie et al., 2005). These investigators also determined that ghrelin acting via these hypothalamic sites increased the respiratory quotient (RQ) due to the shift in energy substrate utilization. Olszewski et al. confirmed that ghrelin in the PVN stimulates feeding and, importantly, the PVN-derived pathways affected by the GHS-R ligand influence the activity of hypothalamic and brainstem regions involved in consumption control (Olszewski et al., 2003a).

Noteworthy, the brainstem populations of the GHS-R are involved in feeding regulation. Faulconbridge et al. compared the effect of third and fourth ventricular injection of ghrelin (Faulconbridge et al., 2003). They determined that the observed responses were similar regardless of the route of administration in terms of the amount of food eaten, shortened latency to meal and increased meal frequency within the 3-h study period. Robust consummatory activity was noted also following the infusion of ghrelin into the dorsal vagal complex (DVC), which consists of the NTS, AP, and DMNV. The 10-pmol dose necessary to elicit feeding via the DVC was three times lower than the minimum orexigenic dose reported for the ARC (Faulconbridge et al., 2003). Moreover, Gilg and Lutz showed that rats lesioned in the AP did not respond to repeated injections of ghrelin (Gilg and Lutz, 2006). Date et al. found that vagotomy and perivagal capsaicin application blocked ghrelin-induced hyperphagia and activity of ARC neurons (Date et al., 2002), although studies employing subdiaphragmatic vagal deafferentation and electrophysiological methods did not confirm the importance of vagal afferent signaling (Arnold et al., 2006). Furthermore, a disruption of hindbrain-

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hypothalamus pathways by performing midbrain transections rostral to the NTS has been found to prevent overfeeding driven by ghrelin (Date et al., 2006).

Finally, a limited number of studies have shown that ghrelin induces consumption when injected in sites which influence consumption stemming from reasons other than hunger, including the mesolimbic reward system, MPA, hippocampus and dorsal raphe nucleus (DRN), which suggests that it may have a much broader range of functional significance in feeding control than initially assumed (Carlini et al., 2004; Naleid et al., 2005; Szentirmai et al., 2007).

Altogether, the data signify the functional importance of ghrelin within a widespread network of brainstem and hypothalamic pathways, as well as outside these sites classically viewed as energy- and metabolism-related, such as in the reward system.

Ghrelin and feeding: from hunger to reward

Eating for calories versus flavor

While the relationship between the central ghrelin system and consumption has been shown beyond doubt, just as with any novel molecule linked with hyperphagia, the question arose as to whether consummatory responses evoked by this compound are related to hunger and energy needs or rather to search for palatability and gustatory reward.

A significant body of evidence gathered to date strongly suggests that ghrelin affects primarily mechanisms associated with energy-driven food intake. It has been shown that a current energy status of the organism affects ghrelin concentration in the blood: an increase in plasma ghrelin level occurs following a 48-h fast, whereas glucose infusion into the stomach induces an opposite effect (Tschop et al., 2000). A transient surge in ghrelin release precedes a meal in schedule-fed animals (Sugino et al., 2002). As mentioned above, the GHS-R and its ligand are plentiful within the brainstem-hypothalamic sites thought of as hunger control centers. Density of the GHS-R depends on the energy status of the animal. For example, GHS-R mRNA levels in the hypothalami of 48h-fasted rats are eight-fold higher than in fed rats (Kim et al., 2003). Enhanced receptor expression may contribute to the amplification of ghrelin action in a negative-energy balance state, especially considering the fact that ghrelin synthesis is increased simultaneously as well.

Generalized ICV as well as site-specific injection studies that employed “bland” diets, such as standard laboratory chow or powdered high-cornstarch mix, showed that ghrelin induces consumption of “unattractive” high-energy foods in the absence of more palatable tastants (e.g. (Olszewski et al., 2007b; Olszewski et al., 2003a; Olszewski et al., 2003b; Wren et al., 2001; Wren et al., 2000)). The orexigenic action persists regardless of the energy status of the organism, i.e., in both deprivation and overfeeding paradigms. It suggests that activation of the GHS-R by exogenous ghrelin may signal the necessity to replenish lacking calories. In line with the proposed involvement of the GHS system in consumption for energy, the intake of non-caloric tastants, such as saccharin, does not affect ghrelin mRNA levels in the brain. Neither does ICV administration of ghrelin change the consumption of the saccharin solution (Furudono et al., 2006).

Choice studies have provided a more accurate description of ghrelin’s influence on eating for flavor versus energy. Peptides classically implicated in reward consumption, such as opioids, induce a robust consummatory response of preferred foods. When two palatable diets are given simultaneously, such peptides increase the intake of a tastant toward which an individual animal displays greater preference (Olszewski et al., 2002; Welch et al., 1994). Studies on ghrelin have delivered somewhat conflicting data thus far. Shimbara and colleagues determined the

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initial preferences of rats toward isocaloric high-fat (HF) and high-carbohydrate (HC) diets and examined the intake of each diet as a result of ICV ghrelin treatment in fat- and carbohydrate-preferrers (Shimbara et al., 2004). Ghrelin preferentially elevated fat intake regardless of individual preferences of the rats used in the study. It should be mentioned, though, that both diets contained 10% of sucrose, which might have been a confounding element. On the other hand, injection of GHRP-2, a ghrelin analogue, in fat-preferring Osborne Mendel rats and in S5B/P1 (S5B) rats that favor low-fat foods stimulated consumption of the preferred macronutrient (Liu et al., 2004). The observed discrepancy may stem from the fact that the amount of ingested fat and/or carbohydrate, which obviously differs between animals displaying such disparate preference profiles, by itself has a prominent effect on ghrelin release to the general circulation and ghrelin mRNA levels in the stomach (Beck et al., 2002; Sanchez et al., 2004). Therefore, exogenous ghrelin may not act similarly in animals subjected to diets that differ in composition.

It is unlikely though that ghrelin does not stimulate carbohydrate intake at all. The majority of standardized laboratory chow diets used in a number of ghrelin studies were carbohydrate-based (e.g. (Olszewski et al., 2007b; Olszewski et al., 2003a; Olszewski et al., 2003b; Wren et al., 2001; Wren et al., 2000)). In addition, Bomberg et al. found that when calorie-dense yet “bland” chow and an “attractive” calorie-dilute sucrose solution are offered concurrently to ICV ghrelin-treated rats, these animals increase the intake of both ingestants. However, this stimulatory effect is much more pronounced with the less attractive tastant of a greater energy density (Bomberg et al., 2007). This outcome indicates that ghrelin is capable of increasing consumption of the ingestant containing only a carbohydrate, sucrose. Another important conclusion is that the GHS-R ligand preferentially stimulates the intake of foods that are calorically dense even though they may be subpar in their palatability to low-energy tastants. It is similar to the action of two other neuropeptides involved in the initiation of consumption to meet energy needs, namely NPY and Agouti-related protein (AgRP) and opposite to the effect induced by reward-related opioids (Giraudo et al., 1999; Olszewski et al., 2003c).

A genetic deletion of the GHS-R or ghrelin in mice prevents the development of the proper acquisition of a refeeding pattern in animals subjected to a daily scheduled food intake regimen, which indicates that ghrelin signaling is necessary either to generate an adequate consummatory response to obtain sufficient energy or to entrain feeding anticipation mechanisms (Abizaid et al., 2006). Importantly, this effect is visible when regular chow is presented as the sole source of calories. It should be noted, however, that a decreased feeding response in GHS-R-null mice can be also induced when a high-fat diet is offered ad libitum for a prolonged period of time. In diet-induced obesity paradigms, the lack of the GHS-R reduces but does not completely abolish body weight gain, but the effect is mitigated by the composition of a diet as well as by the gender of animals (Zigman et al., 2005).

It should be mentioned that ghrelin seems particularly effective in young and fast growing rats, which display very high energy needs, whereas older animals require repeated administration to enhance feeding (Gilg and Lutz, 2006). In addition, serum ghrelin profile in aging Brown Norway rats that display age-related anorexia does not change in response to 72-h fasting (Wolden-Hanson, 2006).

Ghrelin and other feeding neuroregulators

The hypothesis defining central ghrelin as a hunger-related molecule is in concert with the findings of “interplay” between the GHS-R agonist and other neuropeptides involved in feeding regulation. One of the most extensively studied is the relationship between ghrelin and two ARC peptides, NPY and AgRP, whose main role is to stimulate consumption to meet energy needs (Giraudo et al., 1999; Olszewski et al., 2003c). Some data suggest that ghrelin may merge the functional properties of both peptides, as it appears to affect both short- and long-term

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feeding, similarly to NPY and AgRP, respectively (Tang-Christensen et al., 2004). However, it should be noted that the evidence for longer-term effects is weaker. In the majority of studies, single injections of ghrelin typically have not produced increases in food intake longer than a few hours (e.g., see (Gaskin et al., 2003; Gilg and Lutz, 2006; Kalra et al., 2005; Olszewski et al., 2007b; Olszewski et al., 2003a; Olszewski et al., 2003b; Shrestha et al., 2004; Shrestha et al., 2006)).

Morphological studies revealed that NPY and ghrelin neurons form axo-somatic, axo-dendritic and even axo-axonic synapses (Guan et al., 2003). Presynaptic terminals of NPY neurons appear to be an important target of the GHS-R ligand (Cowley et al., 2003). Kohno and coworkers demonstrated that ghrelin directly interacts with NPY neurons in the ARC to induce Ca^{2+} signaling via PKA and N-type Ca^{2+} channel-dependent mechanisms (Kohno et al., 2003). Injection of ghrelin at an orexigenic dose in mice induced c-Fos IR in ARC NPY neurons. In fact, NPY-positive cells constituted approximately 90% of all activated neurons within this site, although it should be emphasized that AgRP and NPY exhibit a high degree of colocalization (Wang et al., 2002).

Molecular studies confirm the interplay between ghrelin and NPY/AgRP. Incubation of hypothalamic explants in the media enriched with the GHS-R agonist significantly increases NPY and AgRP gene expression (Goto et al., 2006). Interestingly, this effect persists only in the presence of glucocorticoids. In situ hybridization experiments showed that a single ICV injection of an orexigenic dose of ghrelin increases AgRP and NPY mRNA expression in the ARC of free feeding and deprived rats (Seoane et al., 2003). Chronic central infusion of the GHS-R agonist, which produces prolonged hyperphagia and body weight gain, also increases AgRP and NPY mRNA levels in that site (Kamegai et al., 2001). Kinzig and coworkers demonstrated that third-and fourth-ventricular injections of ghrelin cause equivalent increases in arcuate NPY mRNA expression (Kinzig et al., 2006). In an interesting study, Luquet et al. ablated NPY and AgRP neurons in mice postnatally (Luquet et al., 2007). Elimination of the two populations of neurons prevented ghrelin-dependent hyperphagia. Their lack also impaired a proper consummatory response in fasted animals presented in the refeeding period with calorie-dense chow, but did not have any effect when palatable diets were given. In concert with those findings, a 47% loss of AgRP neurons as a result of targeted neurotoxicity following bacterial artificial chromosome transgenesis resulted in a significant reduction of food intake and body weight in mice and made these animals insensitive to ghrelin (Bewick et al., 2005).

Pretreatment with the Y1 and Y5 receptor antagonist reduces or completely abolishes a consummatory response to ghrelin and synthetic GHSs (Bagnasco et al., 2003; Faulconbridge et al., 2005; Lawrence et al., 2002), and so does administration of alpha-melanocyte stimulating hormone (alpha-MSH) and its synthetic analogue, melanotan II (MTII) which compete with AgRP for the same melanocortin 3/4 (MC3/4) receptors (Olszewski et al., 2007a; Shrestha et al., 2004). Shrestha et al. performed a series of site-specific injection studies utilizing ghrelin, AgRP and MTII and concluded that ghrelin induction of feeding relies on the recruitment of AgRP –especially within the ARC-PVN pathway - as one of the obligatory mediators of its orexigenic effect (Shrestha et al., 2004; Shrestha et al., 2006). This notion is supported by the findings of Shaw et al. who determined that MC3 and MC4 receptor knock-out mice increase consumption when injected with the GHS-R agonist, but the magnitude of the feeding response is much lower than in wild-type controls (Shaw et al., 2005).

Finally, just as it has been observed with NPY and AgRP, ghrelin induces other appetitive behaviors related to the search and storage of high energy foods in Siberian hamsters, including foraging and food hoarding. This effect is unrelated to changes in locomotor activity in these animals (Keen-Rhinehart and Bartness, 2005). In fact, the Y1 receptor may be part of the pathway through which ghrelin affects foraging (Keen-Rhinehart and Bartness, 2007).

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Ghrelin affects activity of hunger-related neuropeptidergic systems outside the ARC as well. Lateral hypothalamic orexin neurons appear to mediate feeding stimulatory properties of the GHS-R agonist. Axonal terminals containing ghrelin make direct synaptic contacts with these cells in the LH (Toshinai et al., 2003). Injections of ghrelin in the LH and ICV at doses that stimulate feeding induce c-Fos-IR in orexin cells (Olszewski et al., 2003b; Toshinai et al., 2003). Conversely, blocking endogenous ghrelin with an appropriate IgG reduces activity of orexin-IR neurons. Orexin knock-out mice do not display hyperphagia upon administration of GHS-R ligands (Toshinai et al., 2003).

However, aside from its role in consumption control, orexin is well known for maintaining wakefulness. Importantly, ghrelin has been also found to alter sleep-wake cycle in rodents by promoting vigilance (Szentirmai et al., 2007). Hence, the apparent interaction between ghrelin and orexin systems raised the question as to whether the orexigenic action of ghrelin stems directly from its effect on strictly feeding-related mechanisms or whether to some extent, it is a result of altered arousal profile of animals. Obviously, from the behavioral perspective, it is extremely difficult if not impossible to dissociate behaviors to such level, i.e., food intake indeed requires alertness, thus, by itself it affects the sleep-wake pattern. Noteworthy, other peptides classically involved in consumption control, including NPY, have been linked to sleep-wake regulation processes, which does not diminish their importance in the regulation of mechanisms associated with consummatory behavior (Szentirmai et al., 2006). It is rather likely that the pleiotropic activity of neuropeptides facilitates integration of consumption- and wakefulness-related processes. In fact, this combined effect may be beneficial in the regulation of consummatory behavior as, e.g., increased alertness due to fasting-induced ghrelin surge presumably promotes a more effective search for the needed food.

Some data allow us to speculate that ghrelin may prepare the organism for consummatory behavior by influencing the activity of the hypothalamic-pituitary-adrenal (HPA) axis. Wren et al. reported that ghrelin induces release of both corticotropin-releasing hormone (CRH) and vasopressin from hypothalamic explants (Wren et al., 2002). Administration of ghrelin into the cerebral ventricles produces a similar effect and it also causes the release of corticosterone to the general circulation. Similar results were observed in vivo following injection of a synthetic GHS-R ligand, GHRP-6 (Thomas et al., 1997). In addition, Olszewski et al. found that ICV ghrelin activates magnocellular oxytocin neurons which send their projections to the posterior pituitary (Olszewski et al., 2007b). Altogether, these data support the hypothesis that ghrelin may be one of the crucial peptides ensuring HPA axial activity and corresponsing corticosterone peak, which precedes a meal especially under the fixed time presentation conditions (Honma et al., 1996).

Aside from interactions with orexigenic neuropeptides, ghrelin has been proposed to delay satiety by affecting activity of neurons synthesing peptides involved in termination of feeding. For example, ghrelin injection just prior to administration of peptide YY(3–36) and glucagon-like peptide-1 (GLP1) attenuated anorexigenic responses to those two peptides and increased the rate of gastric emptying (Chelikani et al., 2006).

The basic role of consummatory behavior is to obtain sufficient energy to maintain the proper functioning of the organism. Yet a drive to replenish lacking calories does not serve as a sole reason to initiate and maintain eating. In fact, the reward system encompassing a number of monoamines and peptides, such as dopamine and opioids, is involved in hedonic aspects of feeding. Hence, orexigenic reward-related neuropeptides stimulate the intake of palatable tastants regardless of the current energy status of the organism or the caloric load/density associated with a given diet. The level of the evoked consummatory response parallels the “attractiveness” of a tastant, so it is particularly high with high-fat and sweet diets (Levine et al., 2003).

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Ghrelin and the reward system

Although the majority of available data links the ghrelin system with energy-driven consumption, some evidence suggests a possibility that ghrelin may also be implicated in feeding for reward. The GHS-R ligand has been found to induce feeding when injected directly within the mesolimbic reward pathways. Naleid et al. found that administration of this peptide into the VTA caused a robust feeding response in ad libitum-fed rats (Naleid et al., 2005). This outcome was later confirmed by Abizaid and colleagues, who also showed that a single microinjection of the GHS-R antagonist, BIM28163, into this site attenuates feeding induced by deprivation and by peripheral administration of ghrelin (Abizaid et al., 2006). These authors determined that chronic intra-VTA infusion of BIM28163 decreased daily food intake in mice having free access to chow for 5 days. On the sixth day of the chronic antagonist treatment, the animals underwent 24-h deprivation, and upon refeeding, their consumption was still below control values. Stimulation of GHS-R in the NAcc, on the other hand, causes only a moderate hyperphagic response compared to the one observed following intra-VTA infusion of ghrelin (Naleid et al., 2005). Unfortunately, all those studies used standard, relatively “bland” diets, and this fact limits our understanding of the observed phenomena.

Orexigenic action of ghrelin in the VTA and NAcc pointed to a possibility of interplay with the reward-implicated monoamine, dopamine (DA). DA release within the NAcc supports motivation to eat during presentation of food stimuli (Volkow et al., 2002). DA neurons in the VTA mediate the reinforcement of natural rewards and addictive drugs (Yeomans et al., 1993). Moreover, the VTA-NAcc DA pathway modulates motivational salience, e.g. food searching (see (Kalivas and Volkow, 2005) for review). Abizaid and colleagues showed that ghrelin acting in the VTA triggers increased activity of DA neurons, synapse formation, and DA turnover in the nucleus accumbens (Abizaid et al., 2006). Third ventricular injection of ghrelin causes DA release within the NAcc (Jerlhag et al., 2006). An increase in the concentration of extracellular accumbal DA has been observed also upon direct intra-VTA administration of the GHS-R agonist (Jerlhag et al., 2007). Intra-VTA infusion of a selective GHS-R antagonist blocks the orexigenic effect of circulating ghrelin and reduces rebound consumption induced by fasting (Abizaid et al., 2006).

Obviously data are too scarce to provide definitive answers to the question whether the interaction between ghrelin and DA can be linked to reward or just motivation. However, one should remember that such distinction may prove to be very difficult, as the energy status of the organism, perception of foods as palatable and motivation to search for foods are intertwined (Levine et al., 2003). Future studies will likely reveal whether there exists a relationship between ghrelin and other peptides involved purely in feeding for pleasure. There is, e.g., a possibility that stimulation of the GHS-R increases opioid tone in the brain. Sibilia et al. found that ICV ghrelin-induced hyperalgesia is sensitive to central naltrexone treatment (Sibilia et al., 2006). Although the results of pain-related experiments cannot be considered as conclusive evidence explaining feeding phenomena, they clearly indicate the necessity to study this possible interaction also in relation to food intake control.

In sum, the ghrelin system is primarily involved in a number of processes that propel animals to acquire more energy and nutrients. It appears that other feeding-related mechanisms influenced by ghrelin, including reward, may be either completely independent from hunger-driven consumption or they may serve as additional enhancers of energy intake.

Ghrelin and memory retention

The ability to seek and find food is crucial for survival. After finding the food and ingesting it, it is important to remember where this food can be found or perhaps - more importantly - to be able to retain the successful approach that was used to find it. Effective learning and

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memory are thus likely to be crucial for survival during periods of food shortage. In 2002, the laboratory of Susana R. de Barioglio showed that ghrelin increased measures of anxiety in the open field and open arm plus maze test (Carlini et al., 2002). Moreover, ghrelin increased in a dose-dependent manner the latency time in the step-down test (inhibitory avoidance) after the ICV injection in rats. This was the first evidence that ghrelin increases memory retention. This prompted further studies where ghrelin was injected into other brain regions, such as the hippocampus, amygdala and dorsal raphe nucleus (DRN). The results suggested differential roles of the GHS-R ligand acting within those sites in the regulation of feeding, memory and anxiety-like behavioral responses (Carlini et al., 2004). Injection of ghrelin in the hippocampus and DRN increased food intake in relation to control rats, while injections into the amygdala did not affect food intake. Moreover, GHS-R stimulation in all these three regions clearly and dose-dependently increased memory retention.

Further studies showed that a selective serotonin reuptake inhibitor (SSRI), fluoxetine, given prior to ghrelin injection decreased expression of short- and long-term memory retention (Carlini et al., 2007a) suggesting that the effects of ghrelin on both feeding and memory retention could depend on the availability of 5-HT. Later studies showed that ICV injections of obestatin, a peptide hormone that is derived from the same polypeptide precursor (preprogrelin) as ghrelin, induces an increase in the percentage of open arm entries and in the percentage of time spent in open arms indicating an anxiolytic effect. Obestatin increased latency time in the step down test and the percentage time of novel object exploration, suggesting that this peptide influences learning and memory processes as well (Carlini et al., 2007b).

Our understanding of the role of ghrelin took a significant leap when the laboratory of Tamas Horvath showed that circulating ghrelin enters the hippocampus and binds to neurons of the hippocampal formation, where it promotes dendritic spine synapse formation and generation of long-term potentiation (LTP) (Diano et al., 2006). Ghrelin KO animals showed decreased numbers of spine synapses in the hippocampal brain region and impaired performance in behavioral memory testing, both of which were rapidly reversed by ghrelin administration, which was well in line with the earlier findings of de Barioglios' laboratory. These associations of memory and ghrelin prompted speculations that ghrelin could be important for us to remember where to find a meal (Moran and Gao, 2006) and the possible use of ghrelin agonists to enhance memory (Thomas, 2006). The neuroanatomical network integrating ghrelin into memory functions is, however, not well understood. It is likely that ghrelin exerts important actions not only within hippocampal sites but also in other telencephalic and basal forebrain sites, as speculated by Diano et al (Diano et al., 2006). Our understanding of the role of food intake molecules is still in its infancy, but it is likely that these findings on ghrelin will prompt further studies on the possible role of other food intake neuropeptides in memory functions.

Perspectives

Ghrelin serves as one of the most potent orexigens known to date and it affects feeding, to a large extent, via the central circuitry. Substantial evidence suggests that this peptide is primarily involved in hunger-driven consummatory behavior. However, it should be noted that ghrelin signaling does not just serve as a response to energy needs of the organism, but the mechanism of ghrelin-induced hyperphagia appears much more complex. Its influence on memory retention indicates that the GHS-R agonist may facilitate a successful search for food, thus, allowing the animal not only to initiate a meal, but also to find the source of energy based on previous experiences. In line with that, the observed interplay with the reward system may not necessarily reflect a presumed opioid-like "dessert phenomenon" of ghrelin. Perhaps it rather helps in the formation of positive associations with high-energy, untainted foods that later on - through ghrelin-dependent memory retrieval - can be searched for when calories are needed

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to maintain the proper energy balance. It is particularly likely in light of the findings indicating that the level of energy deprivation affects acceptance/attractiveness of presented diets (Levine et al., 2003), as this concept merges the issue of ghrelin's involvement in energy-driven consumption with memory and reward-related processes.

In sum, the orexigenic action of the GHS-R ligand stems from the simultaneous influence exerted over the plethora of neuropeptidergic pathways and neural mechanisms, hence, from its ability to mitigate numerous physiological and behavioral parameters related to feeding. Our view of ghrelin as an orexigen may soon need to be broadened from a relatively narrow perspective of a molecule that merely affects energy intake itself to a neural agent that prepares the organism for an effective and safe consummatory activity. This complexity of ghrelin's influence on consumption control through a variety of mechanisms requires further studies.

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