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Ghrelin action on GnRH neurons and pituitary gonadotropes might be mediated by GnIH-GPR147 system

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Abstract: Acylated ghrelin (AG) effect on GnRH secretion is mediated, at least in part, by GH secreta-gogue receptor (GHS-R) which is present in the GnRH neurons. As the acylation is mandatory for binding to GHS-R, unacylated isoform of ghrelin (UAG) action on gonadotropin secretion is likely to be mediated by other receptors or mediators that have not been identified yet. UAG, therefore, may act partially via a GHS-R-independent mechanism and inhibitory impact of UAG on GnRH neurons may be executed via modulation of other neuronal networks. Ghrelin and gonadotropin inhibitory hormone (GnIH), two agonistic peptides, have been known as important regulators of reproductive events. Potential impact of ghrelin on the activity of GnIH neurons is not exactly known. Both GnIH and ghrelin are potent stimulators of food intake and inhibitors of gonadotropin release. By binding G-protein coupled GnIH receptor (GnIH-R), GPR147, which is located in the human gonadotropes and GnRh neurons, GnIH exerts an inhibitory effect on both GnRH neurons and the gonadotropes. The GnIH-GPR147 system receives information regarding the status of energy reservoir of body from circulating peptides and then transfers them to the kisspeptin-GnIH-GnRH network. Due to wide distribution of this network in brain GnIH neurons may project on ghrelin neurons in the arcuate nucleus and contribute to the regulation of UAG's central effects or vice versa. Together, the unidentified ghrelin receptor in the hypothalamus and hypophysis may be GnIH-R. Therefore, it is reasonable that ghrelin may act on both hypothalamus and hypophysis via GnIH-GPR147 system to block gonadotropin synthesis and secretion.

Keywords: ghrelin; GnIH-GPR147 system; GnRH neurons; pituitary gonadotropes.

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Insight into the well-defined link between the adipose mass and kisspeptin-GnIH-GnRH neuronal network

There is a well-defined link between the adipose mass and reproductive functions. Energy storage reservoir of female is adipose tissue which is essential for the initiation and the maintenance of reproductive functions [1]. Indeed, an increase in adipose mass initiates the development of physical signs of puberty [2], whereas depletion of adipose mass delays the initiation of puberty [3]. Similarly, female fertility is negatively affected by perturbation in the adipose mass [4].

Determination of leptin and ghrelin raised an interest in the mechanisms of food intake and adipose tissue accumulation and their impacts on the GnRH secretion. Activation of GnRH pulse generator in the hypothalamus is the first event which is responsible for the initiation of pubertal development. GnRH pulse generator is regulated by multiple peripheral and central peptides such as ghrelin, leptin, kisspeptin and gonadotropin inhibitory hormone (GnIH). Peripheral peptides inform

the kisppetin-GnIH-GnRH neuronal networks about the status of energy reservoir. Kisspeptin and GnIH are counteracting neuropeptides. While kisspeptin exhibits a stimulatory effect on gonadotropes, GnIH inhibits FSH and LH secretion [5, 6]. In contrast to kisspeptin, GnIH and ghrelin are agonistic in their impacts on both food intake and GnRH secretion. Both peptides increase food intake but decrese gonadotropin secretion [7–9]. On the other hand, impact of kisspeptin administration on feeding is variable. It exerted an anorexigenic impact in fasted mice [10] but it did not cause any change in feeding behaviors in rats [11, 12]. Due to wide distribution of kisspeptin-GnIH-GnRH network in various brain areas, GnIH neurons may project on ghrelin-storing neurons in the hypothalamus and hypophysis contributing to the regulation of ghrelin's central effects or vice versa.

Reasons that led us to the hypothesis

The impact of peripheral peptide signals on hypothalamus and pitiutary gland are quite complex and primarily conducted through their own receptors. On the other hand, GnRH neurons do not express leptin receptors, however, leptin continues to stimulate GnRH neurons. Therefore, it could be suggested that existence of individual receptor in GnRH neurons may not be crucial for the biological activity of a peptide [13, 14]. To overcome this situation some peptides use mediator molecules. For example, insulin-like growth factor 1, NPY, pro-opiomelanocortin, and kisspeptin coordinate the effect of leptin on GnRH neurons [15].

In regard to the central regulation of gonadotropin secretion and food intake, the arcuate nucleus has a pivotal role. Ghrelin is secreted from gastrointestinal tract and exerts its effect on the arcuate nucleus [16]. It is a ligand for the GH secreta-gogue receptor (GHS-R) [17]. Due to wide distribution of GHS-R in the brain, ghrelin can be involved in the regulation of hypophyseal and gonadal functions [7]. Interestingly, while orexigenic peptides supress reproductive function anorexigenic peptides exhibits an activatory effect. Accumulated data reported that ghrelin could partially be involved in the suppression of spontaneous LH release [18, 19].

GnIH is a newly explored neuropeptide in the brain of Japanese quail as an inhibitory substance for FSH and LH release. GnIH shows inhibitory effect on both gonadotropin synthesis and secretion [6]. Its action on gonadotropin-releasing hormone (GnRH) storing neurons and gonadotrope cells is intervened by the GnIH receptor (GnIH-R), GPR147 [20].

While the critical role of ghrelin upon hypothalamic and pituitary regulatory mechanisms of reproductive events has been well established, the way how ghrelin exhibits its some central effect on GnRH neurons and gonadotropes has not been fully known yet. It is well known that GnIH and ghrelin are agonistic in their impact on both food intake and gonadotropin secretion. Both ghrelin and GnIH suppress gonadotropin release and gonadal steroid secretion but they increase feeding by binding their receptors GHS-R and GPR147, respectively. Acylated ghrelin (AG) impact on GnRH release is mediated, at least in part, by GHS-R which is found in the GnRH neurons [21]. In addition to direct effect on GnRH neurons, ghrelin has an indirect inhibitory effect on GnRH neurons. As the acylation is mandatory for binding to the GHS-R, unacylated isoform of ghrelin (UAG) effect on gonadotropin secretion is likely to be mediated by other receptors or mediators that have not been identified yet [22]. UAG, therefore, may act partially via a GHS-R-independent mechanism. Therefore, the inhibitory effect of UAG administration on GnRH neurons may be executed via modulation of other neuronal networks.

Functional similarity between GnIH and ghrelin led us to establish a hypothesis regarding the exertion of central effects of UAG. In this short review, in view of the accumulated literature data we will try to explain our hypothesis stated below under two titles.

1. Unacylated ghrelin may exert some of the central effects through the additional, not yet proved receptor including the GnIH-GPR147 system (Figure 1).
2. GHS-R independent action of UAG on GnRH neurons and pituitary gonadotropes may be coordinated by GnIH.

Unacylated ghrelin may exert some of the central effects through the additional, not yet proved receptor including the GnIH-GPR147 system

Ghrelin, its isoforms, identified and unidentified receptors

Ghrelin is a well-recognized regulator of food intake and reproductive events signaling to the brain from the periphery. Central action of this circulating orexigen is

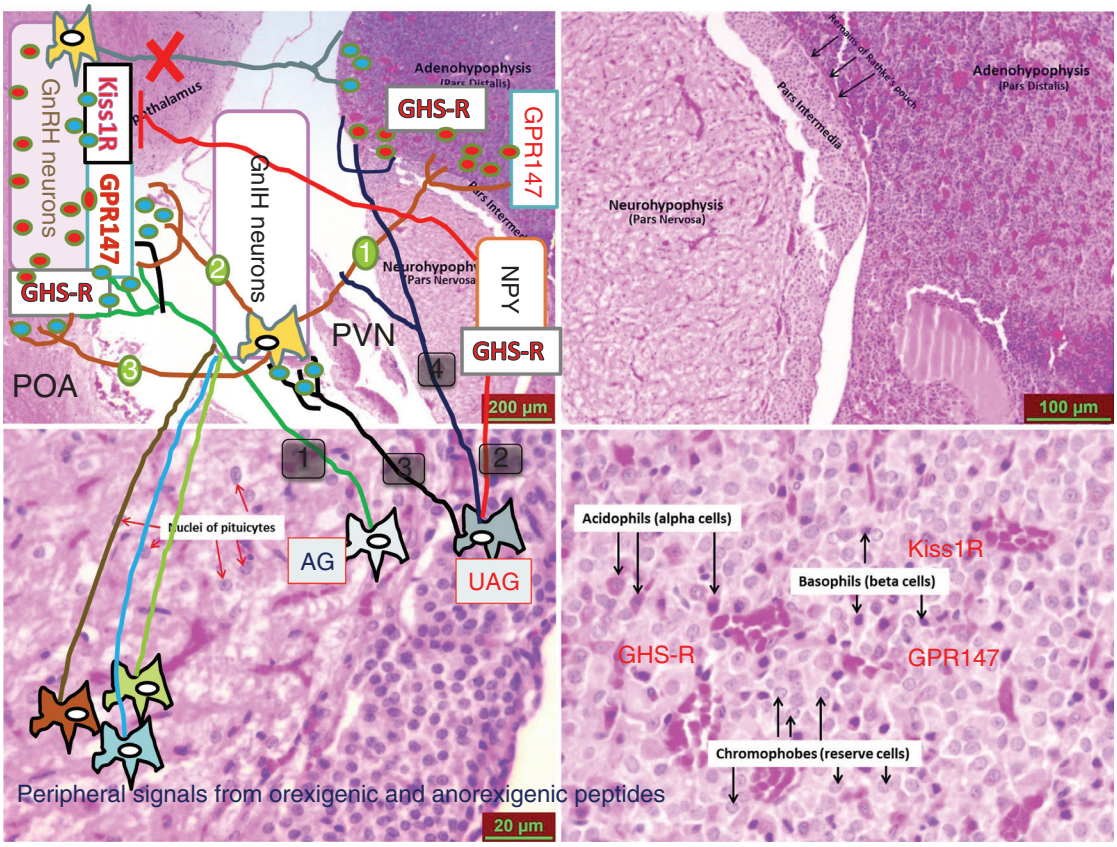

Figure 1: Schematic representation of possible inhibitory pathways responsible for inhibitory action of both acylated ghrelin (AG) and unacylated isoform of ghrelin (UAG) on hypothalamic neurons and gonadotropes. Background of figure consists of H&E stained rat hypothalamus and hypophysis. Impact of AG on GnRH secretion is mediated, at least in part, by ghrelin receptors (GHS-R) which exist in GnRH neurons (1; green line). As the acylation is mandatory for binding to the GHS-R, effect of unacylated isoform of ghrelin on gonadotropin secretion is likely to be mediated by other receptors or mediators that have not been identified yet. Ghrelin produces a significant decline in LH secretion and Kiss1 expression in hypothalamus. Due to kisspeptin neurons do not express ghrelin receptors inhibitory effect of ghrelin on kisspeptin neurons may be mediated by NPY neurons that express proper receptor for ghrelin (2; red line). GHS-R-independent action of UAG in hypothalamus and gonadotropes may also be mediated by both GnIH neurons (3; black dashed line, green circle 1, 2, and 3) and GnIH-R (GPR147). Furtehermore, inhibitory effect of UAG on gonadotropes may be mediated by either GHS-R or GPR147(4; blue dashed line). POA, Preoptic area; PVN, paraventricular nucleus; Kiss1R, kisspeptin receptor. Adapted from references within the text.

to stimulate feeding and suppress LH release [23, 24]. GHS-R1a and GHS-R1b are subtypes of ghrelin receptors. Functionally active form of GHS-R is GHS-R1a. In contrast, GHS-R1b is deprived of the high ligand-binding affinity [25]. This biological inactivation prevents binding to a ligand or transducing a signal [17]. It is well known that acylation of ghrelin at Ser3 is compulsory for the binding of ghrelin to GHS-R1a [26]. GHSRs are widely distributed in the hypothalamus and pituitary gonadotropes [21, 27]. Hence, a great number of studies investigating the impact of ghrelin on LH release have used the acylated ghrelin (AG). But, circulating levels of unacylated isoform of ghrelin (UAG) and its half-life surpass those of AG. On the other hand, UAG is considered to be inert until the Martini's study [22]. They reported that infusion of both acylated and UAG to adult male rats lead to a significant decline in circulating gonadotropin levels. They concluded that because the acylation was necessary for binding to GHS-R, UAG action on gonadotropin release was probably mediated by other receptor subtypes or other mediators that had not been identified yet. UAG effect was, at least in part, conducted through a GHS-R1a-independent mechanism [22]. Similarly, GHS-R1-independent action of ghrelin in brain has also been reported by Halem et al. [28]. In view of the above facts, orexigenic peptide ghrelin may exert its effect through an additional, not yet proved receptor. As a conclusion, GHS-R1-independent action of unacylated ghrelin in hypothalamus and gonadotropes may be mediated by both GnIH neurons and GnIH-R (GPR147).

GnIH-GPR147 system

In 2000, Tsutsui et al. have explored a new neuropeptide which hinders gonadotropin secretion in hypothalamus of Japanese quail and named it GnIH [6]. It accomodates reproductive events by inhibiting LH and FSH synthesis and secretion in avian species. GnIH-storing neurons are scattered throughout many brain areas such as diencephalic and mesencephalic regions as well as in the median eminence of many bird species [29]. Accordingly, GnIH neurons are located in the paraventricular nucleus (PVN) direct to GnRH-storing neurons in the preoptic area (POA). Close proximity of GnIH-storing neurons to GnRH-storing neurons in the POA [29] could be suggested that GnIH might have an impact on GnRH release. By inhibiting adenylate cyclase, GnIH inhibits GnRH-induced cAMP signaling in mouse gonadotrope cell line, LβT2 [30]. Moreover, GnIH inhibits GnRH-stimulated PKA dependent extracellular signal-regulated kinase (ERK) activation [30].

GnIH exerts its effect through putative G-protein coupled GnIH receptor (GnIH-R), GPR147. GnIH-R is revealed in various brain regions such as GnRH neurons and gonadotropes in the pituitary gland [20, 31]. Yin et al. [20] described GPR147 in quail diencephalon including hypothalamus. GnIH neurons projected to both GnRH-I and -II storing neurons which were displayed to express GnIH-R [32]. GnIH effect on male and female reproductive function is similar. Central or peripheral administration of GnIH reduces LH secretion in many female and male species [33]. As GnIH-R is also expressed in gonadotrope cells of the anterior hypophysis, GnIH might act directly on gonadotropes to reduce gonadotropin secretion [34, 35]. In addition, GnIH might block LH and FSH secretion by inhibiting the action of GnRH-storing neurons directly acting on the gonadotropin secreting cells. Accordingly, Krigsfeld et al. have reported that GnIH administration blocks the GnRH-dependent FSH and LH release in pituitary gonadotrope cells [34]. Together, inhibitory action of GnIH on gonadotropin synthesis and secretion may result from both direct action on gonadotropes or indirect action on GnRH1 neurons [35]. Moreover, GnIH-R is located together with LHβ mRNA and FSHβ mRNA-storing cells intervening the suppressor impact of GnIH on gonadotropin release [36]. By binding its receptor, GnIH blocks both LH and FSH synthesis and secretion by hindering action of GnRH-storing neurons and also inhibits gonadotrope cells unmediated. In good agreement with that intraperitoneal administration of the GRP147 antagonist, RF9, led to a slight increase in LH release [37].

Evidence supporting indirect ghrelin actions

Inhibition of LH pulse frequencies following ghrelin administration suggest that ghrelin exerts its effect on GnRH neurons. Unlike the leptin, ghrelin effect on GnRH secretion is mediated, at least in part, by ghrelin receptors which present in the GnRH neurons [21]. In addition to direct impact on GnRH neurons, ghrelin has an indirect inhibitory effect on GnRH neurons. Supportive evidence for indirect effect of ghrelin on GnRH secreting neurons comes from two studies. Intravenous injection of ghrelin produces a significant decline in both LH secretion and Kiss1 expression in hypothalamus [38]. As mouse kisspeptin neurons do not express ghrelin receptors [39] how does ghrelin inhibit kiss1 expression? It is possible that the inhibitory effect of ghrelin on kiss1R expression may be mediated by NPY neurons that express proper receptor for ghrelin [40]. Likewise, Forbes et al. [41] reported that kisspeptin mRNA expression was blocked by ghrelin. Accordingly, both ghrelin injection and food restriction induced hyperghrelinemia lead to a decline in Kiss1 mRNA levels [41, 42]. Moreover, inhibitory effect of ghrelin on GnRH neurons was blocked by a NPY Y5R antagonist [43]. These data suggest that both Kiss1 and NPY expressing neurons may contribute to the inhibitory impact of ghrelin on GnRH neurons. Hence, ghrelin-induced reduction in hypothalamic Kiss1 mRNA expression may be one of the critical factors in the blockage of FSH and LH release (Figure 1).

By using acylated ghrelin, Farkas et al. [21] have investigated whether GnRH secreting neurons contain GHS-R or not. Significant increase in free Ca^{2+} levels inside the GT1-7 neurons following ghrelin administration and determination of GHS-R mRNA expression in GnRH neurons proved the presence of GHS-R in those neurons. This well designed study provided evidence for direct action of acylated ghrelin on GnRH neurons for the first-time. But they did not investigate the possible role of UAG administration on GnRH neurons. However, Martini et al. [22] showed that both AG and UAG reduced circulating levels of LH and FSH. They concluded that UAG may act partially via a GHS-R-independent mechanism. Therefore, inhibitory effect of UAG administration on GnRH neurons might be mediated through modulation of kisspeptin and NPY neurons. When the results of Farkas and Martini's studies and also others were considered together, it would not be wrong to suggest that ghrelin not only has a direct effect on GnRH neurons but also has an indirect effect. GHS-R, GPR147, Y, and Kiss1R are receptors of the ghrelin, GnIH, NPY, and kisspeptin, respectively. Due to wide distrubiton of these neurons and their cognate receptors ghrelin, GnIH, NPY, and kisspeptin, neurons might form a network

and receive input from each other. So the inhibitory effect of ghrelin on GnRH neurons and gonadotropes could be mediated by GnIH. It is more likely that GnIH-R (GPR147) might be activated indirectly by unacylated ghrelin.

GHS-R independent action of UAG on GnRH neurons and pituitary gonadotropes may be coordinated by GnIH

GnIH and ghrelin effect on gonadotropin secretion may change according to study design and studied species

Ghrelin effect on gonadotropin secretion is dependent on experimental models. Although ghrelin predominantly exerts an inhibitory effect on GnRH neurons [7] the nature of this effect varies in some species including human. Ghrelin administration inhibits LH pulse frequency in rat [44], sheep [23], and rhesus monkeys [45]. Kluge et al. have reported that ghrelin inhibits LH secretion in humans [18]. Ghrelin also decreases LH responsiveness to GnRH [46]. Moreover, timing of puberty in male rats is partially delayed after repeated ghrelin administration [47]. Interestingly, increased LH secretion is detected in rat pituitary tissue after ghrelin administration [47]. Very surprisingly, intravenous administration of a single dose of ghrelin does not lead to any influence on LH secretion in four human participants [48].

Similar to ghrelin studies, there is some diversity about inhibitory effect of GnIH in gonadotropin secretion. Depending on the study method and animal species different results can be obtained. This neuropeptide blocks both LH and FSH release from the quail hypophysial tissue [6]. Likewise, central GnIH administration blocks the gonadatropin secretion in white-crowned sparrows [49, 50]. Bentley et al. [29] further noted that administration of GnIH inhibited GnRH-dependent LH secretion in sparrows. Similar to female, application of GnIH into the third ventricle of male rats blocked reproductive events [33]. Chronic treatment with GnIH of mature male quail leads to decline in plasma LH levels. Moreover mRNA expressions of FSH-beta and LH-beta are reduced [51].

Incompatible with above findings, administration of GnIH via intra venous route did not lead to any impact on basal LH levels, but GnRH-dependent LH release was slightly inhibited [52]. Similar results were reported by Murakami et al. [53]. They showed that GnIH exhibits small effect on LH release in cultured rat hypophysis. They have further reported that central injections of GnIH do not exhibit any impact on LH concentrations. As a consequence, the unstable effect of both GnIH and ghrelin on gonadotropin secretion among studies and species led us to think that these two peptides show functional similarity.

GnIH neurons may project ghrelin containing GnRH neurons

GnIH neurons are in close relation with GnRH neurons in many species including humans [31]. Accordingly, both GnRH1 and GnRH2 neurons express GnIH-R mRNA. While GnRH1 stimulates FSH and LH secretion from the hypophysis [31]. GnRH2 [54] is reponsible for regulation of reproductive events both in avian species and mammals. Therefore, it could be suggested that functions of GnRH-storing neurons were regulated by GnIH-soring neurons [32]. GnIH-storing neurons also exhibit many other regulatory functions in many brain regions [35]. They send fibers not only to GnRH neurons but also many other neurons including kisspeptin and NPY neurons [55]. Different from GnIH, kisspeptin exhibits a stimulatory impact on GnRH-storing neurons [5]. Accordingly, Irwig et al. have shown that administration of Kiss1 centrally activates the release of LH and FSH [56]. Kisspeptin-induced stimulation of gonadaotropin release occurs via the hypothalamic GnRH neurons. Both Kiss 1 and GnIH mRNA expressing neurons have also been shown in hypothalamus [56]. Similar to GnIH neurons, ghrelin containing hypothalamic neurons send many efferent fibers to neurons storing neuropeptide Y (NPY) and agouti related protein which may induce the secretion of these orexigenic peptides [57, 58]. Stimulation of preovulatory LH surge in rats is required NPY activation [59]. Therefore, NPY may contribute to the inhibitory effect of both GnIH and ghrelin on gonadotropin secretion. As a result, it would not be wrong to tell that ghrelin containing hypothalamic neurons and GnIH-storing neurons work together for modulating the reproductive events.

Impact of GnIH and ghrelin on ovarian function

Gonads of rat and humans contain transcripts for ghrelin and its cognate receptor [26, 60]. Moreover, human and rodent placenta expresses ghrelin [61]. Both oocyte and surrounding somatic cells of mammalian and birds contain both GnIH and GnIH-R [62, 63]. Likewise,

functional GHS-R1a was found in the ovary of many species including humans [64, 65]. Unlike the central effect, GnIH and ghrelin are antagonistic in their impacts on ovarian functions. Both GnIH and ghrelin exert their effect on the ovary and follicles via their own receptors. GnIH administration stimulates testicular apoptosis and blocks normal testicular development in male quail [51]. In contrast, ghrelin acts as a survival factor by regulating anti-apoptotic effects in porcine ovarian cells [66]. GnIH administration inhibits steroid synthesis and germ cell maturation [62, 63]. On the contrary, expression of follicular GHS-R1a is associated with follicle maturation [64]. Taken together, GnIH and ghrelin may be the peptides which collectively inhibit the gonadotropin secretion. Likewise, these two peptides might regulate food intake. However, further studies are needed to elucidate this close relation between GnIH and ghrelin peptides.

Discussion

Hypothalamic neuropeptide GnIH inhibits gonadotropin release and centrally regulates many reproductive functions. Moreover, wide distribution of both GnIH neurons and their receptors in diencephalic and mesencephalic regions suggested that many behavioral and physiological functions were regulated by this neuropeptide [29, 32]. Ghrelin and GnIH are agonistic in their impacts on both food intake and GnRH secretion. GnIH neurons collect information regarding the energy reservoir status of body from orexigenic and anorexigenic neurons and then transmit them to GnRH neurons. Both GnIH and ghrelin are potent stimulators of food intake and inhibitors of gonadotropin release [8, 9, 67].

It has been reported that kisspeptin-GnIH-GnRH system is affected by many peripheral and central peptides such as leptin, proopiomelanocortin, orexin, and NPY [55]. Therefore, the central impact of ghrelin might be regulated by this neuronal network. Herein, we summarized compelling evidence which suggested a possible involvement of ghrelin and GnIH in the metabolic regulation of reproductive events. GnIH receptors are located in the hypothalamus and hypophysis where they obtain information regarding the energy status of our body. Ghrelin containing neurons may send an information about the energy reservoir status of body to kisspeptin-GnIH–GnRH neurons to reduce gonadotropin synthesis and release. As a result, by affecting kisspeptin-GnIH-GnRH system, unacylated ghrelin may modulate reproductive functions.

Taken together, the unidentified ghrelin receptor in the brain may be GnIH-R. Therefore, it is logical to think that ghrelin acts on hypothalamus by way of GnIH-R to block gonadotropin release as well as at the hypophysis. In addition to central action of ghrelin and GnIH, direct regulation of gonadal activity by ghrelin and GnIH is possible. In order to understand the possible mechanisms of ghrelin isoforms on GnRH neurons and gonadotropes, a study using female rats treated with GRP147 antagonist, RF9, is required. Whether GnIH-GPR147 neuronal system is modulated by changes in circulating ghrelin levels warrants further investigation.

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