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REVIEW

"Sibling" battle or harmony: crosstalk between nesfatin-1 and ghrelin

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Abstract

Ghrelin was first identified as an endogenous ligand of the growth hormone secretagogue receptor (GHSR) in 1999, with the function of stimulating the release of growth hormone (GH), while nesfatin-1 was identified in 2006. Both peptides are secreted by the same kind of endocrine cells, X/A-like cells in the stomach. Compared with ghrelin, nesfatin-1 exerts opposite effects on energy metabolism, glucose metabolism, gastrointestinal functions and regulation of blood pressure, but exerts similar effects on anti-inflammation and neuroprotection. Up to now, nesfatin-1 remains as an orphan ligand because its receptor has not been identified. Several studies have shown the effects of nesfatin-1 are dependent on the receptor of ghrelin. We herein compare the effects of nesfatin-1 and ghrelin in several aspects and explore the possibility of their interactions.

Keywords NUCB2 · Brain-gut peptide · X/A-like cells · Growth hormone secretagogue receptor · Food intake · Diabetes mellitus

Introduction

Ghrelin and nesfatin-1 are both produced by the same kind of cells, X/A-like cells in the gastric fundus. Ghrelin, an orexigenic peptide, was first discovered to stimulate the release of growth hormone (GH) by Kojima in 1999 [1], while nesfatin-1 was identified as an anorexigenic peptide by Oh-I in 2006 [2]. Although nesfatin-1 and ghrelin exhibit opposite effects on some physiological functions, such as energy metabolism, glucose metabolism, gastrointestinal functions and regulation of blood pressure, they have similar effects in some aspects as for anti-inflammation and neuroprotection. The receptor for nesfatin-1 is still not identified; however, emerging evidences suggest that nesfatin-1 could exert its effects through the ghrelin receptor, growth hormone secretagogue receptor (GHSR) [3, 4]. Co-incubation or pre-incubation with nesfatin-1 or nesfatin-1-like peptide could block the stimulatory effects of ghrelin on GH mRNA and protein expression possibly through the same adenylate cyclase (AC)/protein kinase A (PKA)/cAMP-response element-binding protein (CREB) signaling pathway [3]. Our previous study also showed clearly that GHSR was essential for the effects of nesfatin-1 on regulating glucose metabolism [4]. Therefore, there might be several mechanisms underlying the effects of nesfatin-1, for example, direct effects on GHSR or modulation GHSR function by various pathways, which is of vital importance in the normal physiological functions. We herein compared the effects of nesfatin-1 and ghrelin in several aspects and gave some clues for their possible interactions.

Structure and distribution

Nesfatin-1 is derived from the 396-amino acid precursor, namely nucleobindin 2 (NUCB2) through the post-translational processing [2]. It gains almost all the attentions due to the absence of observed effects of the other two products of NUCB2, that is, nesfatin-2 and nesfatin-3 (Fig. 1). NUCB2/nesfatin-1 is detected in both peripheral tissues and the central nervous system (CNS). In the periphery, nesfatin-1 is mainly secreted by the gastric oxyntic mucosa, which is the main source for the majority of circulating nesfatin-1 [5]. It is also present in the liver, heart, testis, ovaries, uterus, adipose tissue and pancreas [5]. Nesfatin-1 could penetrate the blood–brain barrier (BBB) in a bidirectional pathway without saturation [6]. In the CNS, NUCB2/nesfatin-1 is

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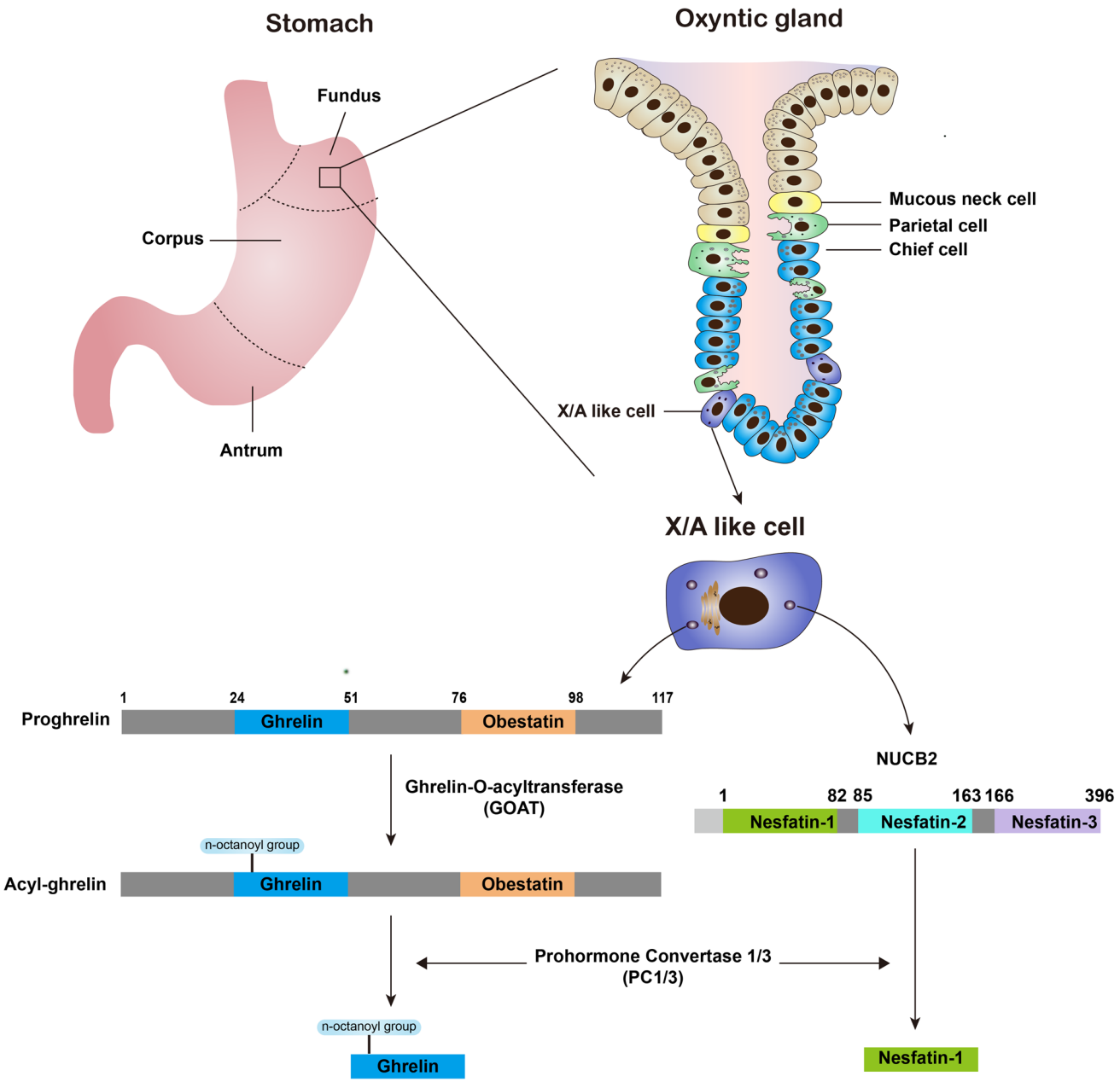

Fig. 1 The production of nesfatin-1 and ghrelin in gastric X/A-like cell. The structure of proghrelin contains both obestatin and ghrelin which is transformed to active peptide after acylation by GOAT. NUCB2 yields three kinds of peptides, nesfatin-1, nesfatin-2, and nesfatin-3. It is known majority of nesfatin-1 and ghrelin co-exist in X/A-like cells of oxyntic gland in stomach, where the PC 1/3 is responsible for the processing of both peptides

mainly found in the brain stem, midbrain, hypothalamus, central amygdaloid nucleus, cerebellum, etc. [7]. It is also expressed in the autonomic preganglionic neuronal group in the spinal cord [8].

Ghrelin is a 28-amino acid brain-gut peptide, derived from its precursor, proghrelin which also yield another peptide, obestatin, with post-translational modification [9]. Ghrelin exists in two forms: unacylated ghrelin and acylated ghrelin whose acylation is catalyzed by ghrelin O-acyltransferase (GOAT) (Fig. 1) [10]. In accordance with nesfatin-1, ghrelin is also mainly secreted by the gastric oxyntic mucosa, which is the main source for the majority of circulating ghrelin. It is also present in other sites of gastrointestinal tract, such as duodenum, jejunum, ileum and colon. Lower expression of ghrelin could be found in the pancreas, adipose tissue, kidneys, testes, placenta and hypophysis. Central source of ghrelin remains questionable and there appears no irrefutable evidence supporting that ghrelin is synthesized in the

CNS [11, 12]. Ghrelin signals might be sent to the CNS through the vagal afferent nerve and the blood circulation, but the accessibility of plasma ghrelin to the CNS is limited [13]. Circulating ghrelin might access neurons through fenestrated capillaries of the median eminence (ME) and the area postrema (AP) with incomplete BBB [11, 14]. A recent study reported that circulating ghrelin might cross the blood-cerebrospinal fluid (CSF) barrier mainly through GHSR-dependent pathway [15].

Nesfatin-1 and ghrelin co-exist in X/A-like cells of oxyntic gland in the stomach, where the prohormone convertase (PC) 1/3 is responsible for the processing of both peptides (Fig. 1) [16, 17]. The co-localization was also observed in the pancreatic islets of rats [18] and hypothalamus of goldfish [19]. Besides, the study in pejerrey showed that nesfatin-1 co-localized with ghrelin in enteroendocrine cells, absorptive cells and lamina propria cells of intestine [20]. By comparing with ghrelin, whether the central nesfatin-1 might also at least partly originate from the periphery, is worthy of further investigation.

Table 1 Distribution of nesfatin-1 and ghrelin receptor in the CNS in rats

Distribution in the CNS	Receptor of nesfatin-1 (unidentified)	Receptor of ghrelin (GHSR)
Cerebral cortex	+++	±
Hippocampus and septum	–	+++
Arcuate nucleus (ARC)	–	+++
Paraventricular nucleus (PVN)	+++	+
Ventromedial hypothalamus (VMH)	–	+++
Cerebellum	+++	–
Ventral tegmental area (VTA)	–	+++
Nucleus ambiguous	–	+++
Substantia nigra (SN)	–	+++
Nucleus of the solitary tract (NTS)	–	++
Area postrema (AP)	+++	+++
Dorsal motor nucleus (DMV)	+++	+

"+", weak; "++", medium; "+++", strong; "±", inconsistent visualization; "–", no report or not detectable

Receptors and interactions

Up to now, nesfatin-1 remains an orphan ligand because its own receptor has not been identified. The autoradiography showed that the receptors of nesfatin-1 were distributed in the gastrointestinal tract and endocrine tissues, such as the pituitary, pancreas, adrenal gland, testis and adipose tissue, and possibly in some viscera including heart, lung, liver, kidney, and skeletal muscle [21]. In the brain, nesfatin-1 receptors are distributed in the dorsal vagal complex (DVC) of brainstem, hypothalamic paraventricular nucleus (PVN), cerebellum, and cortex (Table 1) [21].

The unknown receptor of nesfatin-1 is presumed to be a kind of G-protein-coupled receptor (GPCR), which is involved in a variety of signaling pathways [22]. Nesfatin-1 could induce the phosphorylation of CREB through the mitogen-activated protein kinase (MAPK) and Ca^{2+} signaling pathway in neural cell lines [23]. In interleukin (IL)-1β-treated chondrocytes, nesfatin-1 could attenuate inflammation and matrix metalloproteinases expression through the inhibition of the NF-κB and MAPK signaling pathways [24]. Knockdown of hypothalamic nesfatin-1 reduced the phosphorylation of mammalian target of rapamycin (mTOR), as well as signal transducer and activator of transcription 3 (STAT3), and thus increased the expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6pase) to regulate glucose metabolism [25]. In trophoblast cells, nesfatin-1 promoted proliferation, migration, invasion and suppressed oxidative stress by activating phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTOR and AKT/glycogen synthase kinase 3β (GSK3β) signaling pathways [26]. In type 2 diabetes mellitus (T2DM) mice, nesfatin-1 stimulated free fatty acid utilization through AMP-activated protein kinase (AMPK)-acetyl-CoA carboxylase (ACC) signaling pathway in skeletal muscle cells (Fig. 2A) [27]. Meanwhile, nesfatin-1 promoted anorexia effects through receptors of other hormone, such as corticotropin-releasing 1/2 receptor ($CRF_{1/2}$-R) and histamine 1/3 receptors ($H_{1/3}$-R) [28]. The weight loss effects in obese rats were mediated by elevated nesfatin-1 levels via melanocortin receptors (MC-Rs)-extracellular signal-regulated kinase (ERK) signaling pathway [29]. In adult ventricular myocytes, nesfatin-1 modulated L-type Ca^{2+} channel through MC_4-R resulting in negative inotropic effects [30]. Central administration of oxytocin receptor (Oxy-R) blocker antagonized hypertension and anorexia induced by nesfatin-1 [31, 32], suggesting the involvement of oxytocin system in nesfatin-1's effects. It was also reported that cholecystokinin receptor (CCK-R) was involved in the effects of nesfatin-1, since CCK receptor selective antagonist reversed the anorectic effect and appetite-related factor expressions induced by nesfatin-1 (Fig. 2B) [33].

GHSR, the ghrelin receptor, was found as an orphan receptor in 1996, prior to the discovery of ghrelin. The gene of ghrelin receptor encodes two kinds of receptor, GHSR 1a and 1b, thereinto, GHSR1a is the effective receptor of ghrelin. GHSR1a is a kind of GPCR, which is distributed in both peripheral tissue and the CNS. GHSR1a mRNA exists in peripheral tissue including the pituitary gland, pancreas, adipose tissue, thyroid gland, spleen, kidney, adrenal gland

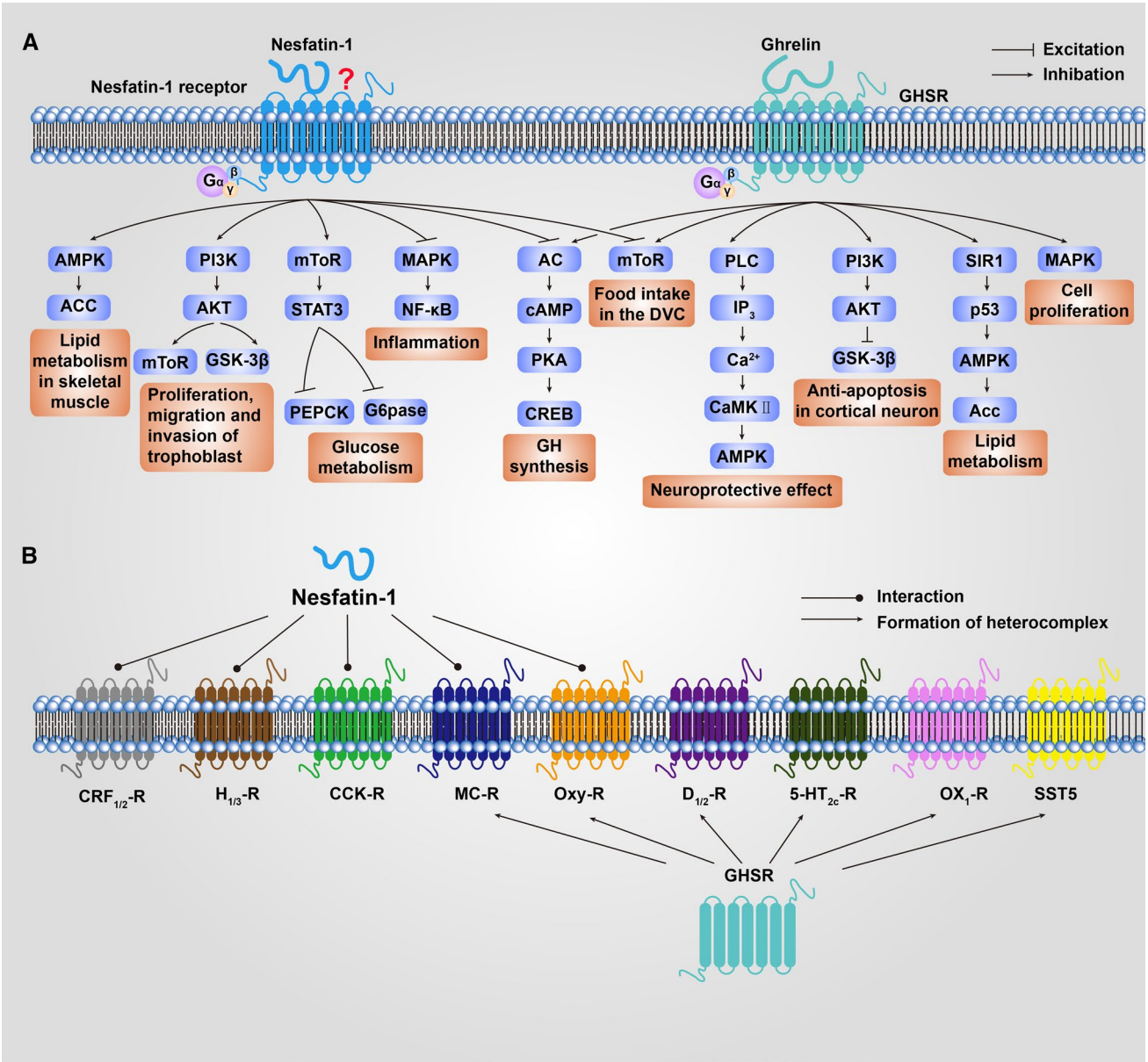

Fig. 2 The receptor signaling pathways of nesfatin-1 and ghrelin and interactions with other receptors. **A** The receptor of nesfatin-1 remains unknown and it is supposed to be a G-protein coupled receptor. Nesfatin-1 and ghrelin regulate food intake in the DVC and synthesis of GH through the same signaling pathway with completely opposite effects. **B** The actions of nesfatin-1 are dependent on $CRF_{1/2}$-R, $H_{1/3}$-R, CCK-R, MC-R and Oxy-R, while GHSR heterodimerize with MC-R, Oxy-R, $D_{1/2}$-R, 5-HT_{2C}-R and SST5. Both peptides are correlated with MC-R and Oxy-R

and cardiac muscles [34], and in the CNS including the hindbrain, midbrain, hypothalamus, and hippocampus (Table 1) [14, 35].

Various signaling pathways are involved in the effects of ghrelin, including PKA, Ca^{2+}, MAPK, mTOR, AMPK, and PI3K/AKT [36]. After binding to GHSR1a, ghrelin activated the subsequent G-protein, Gq or Gi/o, then simulated phospholipase C (PLC)-inositol 1,4,5-triphosphate (IP_3)-Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII)/AMPK or cAMP-PKA signaling pathway, respectively [37, 38]. It could prevent apoptosis in cortical neuronal cell through the PI3K/AKT-mediated inactivation of GSK-3β pathway [39]. It could inhibit lipogenesis and stimulated fatty acid oxidation via sirtuin1 (Sirt1)/p53/AMPK signaling pathways in the hypothalamus [40]. It promoted cell proliferation and decreased cell apoptosis via MAPK dependent pathway (Fig. 2A) [41]. The seeming paradox that wide distribution of GHSR1a is accompanied by the low expression of ghrelin in the CNS could be explained by the constitutive activity of GHSR and the

formation of heterocomplexes. Actually, even in the absence of ghrelin, GHSR1a could activate intracellular signal transduction and display a strikingly strong constitutive activity which accounted for approximately 50% of its maximal capacity [42]. Notably, GHSR1a heterodimerized with a variety of receptors, including dopamine receptor (D-R), MC_3-R, 5-serotonin 2c receptor (5-HT_{2c}-R), orexin 1 receptor (OX_1-R), Oxy-R, somatostatin receptor 5 (SST5). Bioluminescence resonance energy transfer (BRET) showed the existence of heterodimers formed by D_1-R and GHSR1a, which amplified the dopamine (DA) signaling in the presence of ghrelin [12, 43]. The similar effects were observed in the dimerization between D_2-R and GHSR1a in hypothalamic neurons [44]. The heteromerization influenced DA-mediated Gi protein activation by modulating the conformation of its α-subunit [45]. The inhibitory effects of D_2-R agonist on food intake were dependent on the formation of D_2-R and GHSR1a heteromers [46]. The receptors of ghrelin and melanocortin (an anorexigenic peptide) could form MC_3-R-GHSR1a dimers identified in the hypothalamic arcuate nucleus (ARC) to enhance melanocortin-induced intracellular cAMP accumulation and impair GHSR signaling of both agonist-independent and ghrelin-induced activity [47]. The formation of 5-HT_{2C}-R-GHSR1a heterodimers inhibited orexigenic signaling whereas 5-HT2c-R antagonist attenuated the formation of dimer and increased GHSR1a-induced food intake [48, 49]. Orexin is another kind of orexigenic peptide, whose receptor OX_1-R could form heterodimers with GHSR1a in HEK293 cells. Ghrelin, but not orexin, could activate Gα protein through GHSR1a/OX_1-R heterodimers [50]. Oxytocin is a pleiotropic peptide hormone with broad functions, and its receptor, Oxy-R, could also form heterocomplex with GHSR in cultured hypothalamus and hippocampus to impair the downstream Oxy-R signaling pathways [51]. The heteromers of GHSR1a and somatostatin receptor 5 (SST5) could regulate insulin secretion (Fig. 2B) [52].

In summary, nesfatin-1 receptor shares the overlapping distribution and similar intracellular signaling transduction with ghrelin receptor and interacts with analogous receptors (Fig. 2). Several studies simultaneously focused on the interaction of nesfatin-1 and GHSR. Ozturk et al. [53] observed that nesfatin-1 displayed anti-inflammatory and antioxidant effects on colitis via Oxy-Rs and ghrelin receptors. Kerbel et al. [20] demonstrated exogenous nesfatin-1 altered the central expression of ghrelin and GHSR1a in goldfish. Our previous study clearly stated that GHSRs were essential for nesfatin-1 to control glucose metabolism because GHSR antagonist and knockout of ghrelin receptor blocked the effect of peripheral nesfatin-1 on food intake and glucose metabolism [4]. For GH synthesis, nesfatin-1 and ghrelin exerted completely opposite effect via similar signaling pathway, cAMP/PKA/CREB signaling pathway [3]. Even co-incubation or pre-incubation with nesfatin-1 antagonized the stimulatory effects of ghrelin on GH secretion. Therefore, nesfatin-1 might act on GHSRs indirectly, nevertheless, there exits such a possibility that nesfatin-1 could act on GHSRs directly, which deserve further investigations.

The roles of nesfatin-1 and ghrelin in the central functions

Energy metabolism

Plasma nesfatin-1 levels were increased with elevated body mass index (BMI) [54], and a negative correlation between plasma ghrelin concentration and BMI was observed in humans [55]. Nesfatin-1 decreased energy intake and potentiated energy consumption, whereas ghrelin displayed opposite effects. The mechanisms underlying their effects on energy metabolism were discussed hereinafter from lower center to higher center (Fig. 3) [56].

In the medulla

In the medulla, the DVC, a central structure involved in the control of food intake, consists of three parts: the nucleus of the tractus solitarius (NTS), which receives and integrates afferent signals from viscera; the dorsal motor nucleus of the vagus (DMV), where the preganglionic vagal motor neurons are located; and the AP, which contains incomplete BBB and permits some circulating substances to contact with.

Peripheral nesfatin-1 might convey signals to NTS through the vagal afferent nerve, since nesfatin-1 could activate vagal afferent neurons by stimulating Ca^{2+} influx through N-type channels [57]. Nesfatin-1 is expressed in all the three parts of DVC [58] and other appetite suppressing hormones, such as leptin, glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK)-8 could inhibit food intake via activation of nesfatin-1-positive neurons in the brainstem [59]. Local administration of nesfatin-1 in the DVC inhibited food intake and body weight gain, as shown in our previous study [60]. Peripheral injection of nesfatin-1 midsegment only activated the proopiomelanocortin (POMC, the precursor of alpha-melanocyte-stimulating hormone (α-MSH)) positive neurons in the NTS but not in the ARC, indicating the important role of the NTS in feeding control [61]. In fact, oxytocinergic neurons originating from the PVN, which could be activated by nesfatin-1, enhanced the production of POMC from the NTS [62]. Besides, nesfatin-1 colocalizes with neuropeptide Y (NPY, an orexigenic peptide) and amphetamine-regulated transcript (CART, an anorexigenic peptide) in the NTS [58] and exogenous nesfatin-1 downregulated NPY expression, while CART expression remained unchanged [63].

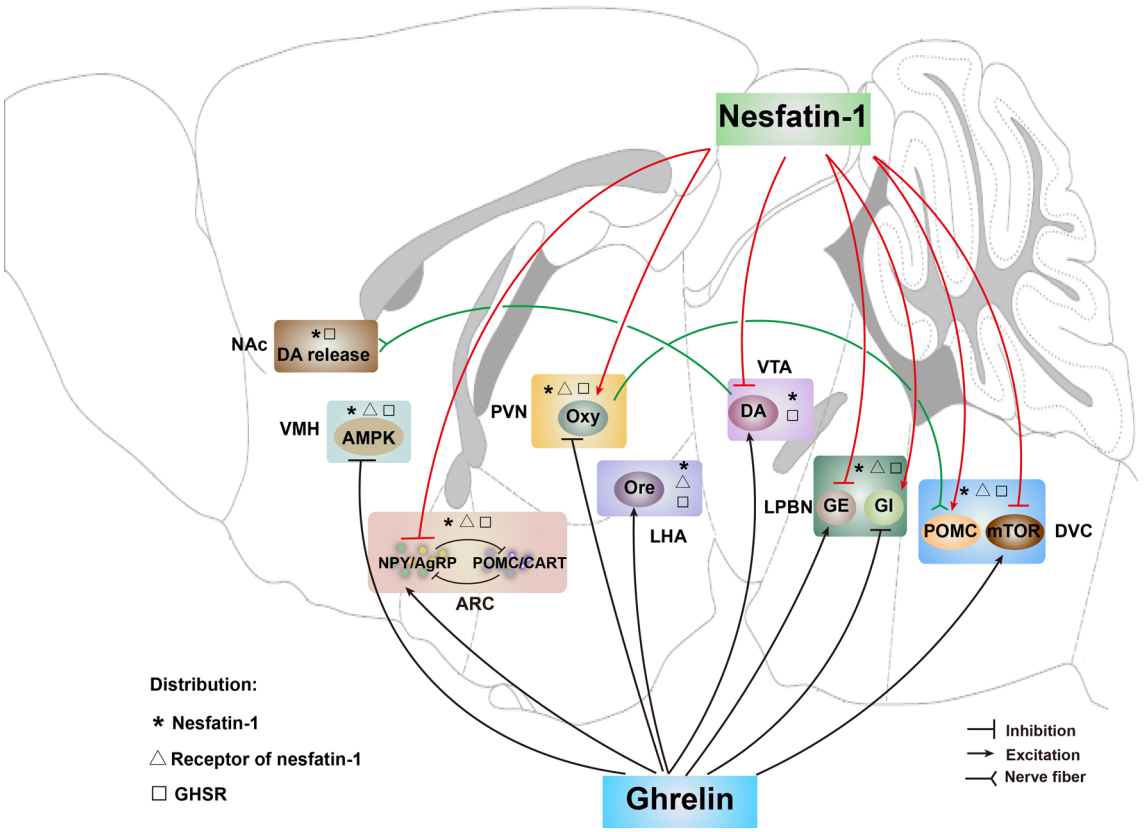

Fig. 3 The central mechanisms underlying the effects of nesfatin-1 and ghrelin on energy metabolism. In the hypothalamus, nesfatin-1 inhibits NYP/AgRP neurons and activates Oxy neurons in the PVN which terminates POMC neurons in the DVC. Ghrelin activates NYP/AgRP neurons in the ARC and Ore neurons in the LHA, while inhibits Oxy neurons in the PVN and AMPK activity in the VMH. In the midbrain, nesfatin-1 inhibits DAergic neurons, thereby decreasing DA release in the NAc, whereas ghrelin exerts completely opposite effects. In the LPBN of pons, nesfatin-1 inhibits and excites GE and GI neurons, respectively, while ghrelin reverses these effects. In the hindbrain, nesfatin-1 activates POMC neurons and inhibits mTOR activity in the DVC, while ghrelin activates mTOR activity

GHSRs are expressed in all the three parts of the DVC, where c-fos expression increased after intracerebroventricular (i.c.v) injection of ghrelin [64]. Hyperphagia could be induced by injection of ghrelin into either the third ventricle near the ARC or the fourth ventricle near the DVC [65]. However, the essential amount for direct induction of hyperphagia in the DVC [65] is much lower than that in ARC [66]. Despite this, merely selective expression of GHSR in the brainstem was insufficient to reserve peripheral ghrelin-induced feeding [67]. The vagus nerve also plays an important role in ghrelin orexigenic effect, since ghrelin failed to stimulate feeding after vagotomy [68] or impairment of gastric vagal afferent fibers [69]. In addition, antagonist of ghrelin receptor increased the ability of CCK to activate GLP-1 and prolactin-releasing peptide neurons in the hindbrain [70].

Nesfatin-1 and ghrelin shared a common signaling pathway in feeding control by DVC, with decreased food intake through inhibition of mTOR signaling by nesfatin-1 and increased food intake through activation of mTOR signaling by ghrelin [71].

In the pons

The parabrachial nucleus (PBN), a feeding-related center, is located in the pons of the brainstem and transmits gustatory and visceral information to higher centers, which attracts an ever-growing number of concerns [72]. Nesfatin-1 is expressed in the lateral parabrachial nucleus (LPBN) [58] and local injection of nesfatin-1 into the LPBN suppressed food intake and body weight gain, possibly due to the modulation of activities of glucose sensitive neurons, since nesfatin-1 inhibited most glucose-excitatory (GE) neurons and excited most glucose-inhibitory (GI) neurons, respectively [73]. However, ghrelin reversed all above effects [74]. In addition, injection of nesfatin-1 into the LPBN upregulated the expression of uncoupling protein 1 (UCP1) in brown adipose tissue to generate heat with its underlying mechanism

related to melanocortin system, as these effects could be blocked by $MC_{3/4}$-R antagonist [73]. GHSRs are abundantly expressed in the LPBN and functional silencing of GHSR-expressing cells prevented mice from body weight gain and fat accumulation under the condition of a high-fat, high-sugar diet [75]. Most GHSR-positive cells belong to glutamatergic population rather than calcitonin gene-related peptide (CGRP, an anorexigenic peptide) cell [75]. Whether the inhibitory effect of nesfatin-1 on food intake is through CGRP-positive neurons and the relationship between glucose sensitive neurons and CGRP-positive neurons are worthy of further studies.

In the midbrain

In the midbrain, DAnergic rewarding pathway from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is responsible for hedonic control of feeding. The opposite results are obtained in this pathway between nesfatin-1 and ghrelin.

Our data demonstrated that local administration of nesfatin-1 in the VTA reduced nocturnal food intake through inhibition of DAergic neurons in the VTA and the subsequent DA release in the NAc [76], which has been confirmed by Dore R et al. [77]. Ghrelin reversed all above effects [78, 79], moreover, blockage of D-Rs in the NAc attenuated the effect of ghrelin in the VTA [80]. Central injection of nesfatin-1 abolished the fasting-induced increase in the reward value of sucrose [81], while ghrelin potentiated the rewarding value of high-fat diet [82]. GHSRs are densely expressed in the VTA [35] and GHSR selective antagonist [78] or GHSR knockout blunted the orexigenic effect of ghrelin [83], but reexpression of GHSR1a in DAnergic neurons including those in the VTA partially restored food intake [83]. Ghrelin also interacts with opioid and endocannabinoids system to influence the reward-associated behaviors, since antagonist of opioid receptor or cannabinoid receptor partly blocked the ghrelin-induced food intake and motivated behavior or ghrelin-dependent DA release in the NAc [84], that might inspire to explore the correlation between nesfatin-1 and other transmitters in the midbrain.

In the hypothalamus

The hypothalamus is a classical central area regulating appetite and energy homeostasis. Two types of "first-order" neurons located in the ARC play important roles in the control of energy metabolism. The neurons coexpressing NPY and AgRP are responsible for orexigenic effects, whereas anorexigenic POMC and CART co-exist in the other group of neurons. Bilateral communications between the ARC and other hypothalamic nuclei such as the ventromedial hypothalamus (VMH), PVN and lateral hypothalamic area (LHA) compose the network to regulate energy homeostasis.

Central administration of nesfatin-1 suppressed food intake in rodents or goldfish [85]. The PVN seems to be the key acting site of nesfatin-1. In the PVN, NUCB2/nesfatin-1 expression was significantly decreased under starved conditions [2]. Third ventricle injection of nesfatin-1 upregulated c-fos expression in the PVN [62, 86]. Both oxytocin and melanocortin system are involved in the effects of nesfatin-1 in the PVN, since nesfatin-1 activated PVN oxytocinergic neurons which terminated at the POMC neurons in the NTS and antagonist of Oxy-R [62] or $MC_{3/4}$-R abolished the anorexigenic effects of central nesfatin-1. In addition, peripheral injection of CCK activated nesfatin-1-positive neurons in the PVN, indicating the interaction between nesfatin-1 and CCK system [87]. The NPY-positive neurons in the ARC seem to be another acting site for nesfatin-1. Application of nesfatin-1 decreased hypothalamic NPY mRNA levels [2] and hyperpolarized NPY-positive neurons [88], but did not influence the POMC/CART mRNA expression [2]. In addition, the CRF_2-R-dependent pathway is involved in the inhibitory effect of nesfatin-1 on food intake in forebrain [89].

The ARC serves as the most important central site for the effects of ghrelin on appetite. Ghrelin increased the spontaneous firing rate [90] and miniature frequency of excitatory synaptic current of NPY/AgRP neurons in vivo or in vitro [91]. Gene knockout and antagonist application confirmed the role of NPY/AgRP neurons, since the orexigenic effects of ghrelin could be eliminated in NPY/AgRP ablated mice [92] and antagonists of NPY/Y_1 receptor abolished ghrelin-induced feeding [93]. The dense expressions of GHSRs in NPY/AgRP neurons play an important role in orexigenic effect of ghrelin [94] because only deletion of GHSR in AgRP neurons attenuated diet-induced obesity [95]. In contrast to NPY/AgRP neurons, GHSRs are less expressed in POMC/CART neurons [96], suggesting that ghrelin might exert nearly no direct action on POMC/CART neurons. However, due to the reciprocal inhibitory projections between the two groups of first-order neurons, POMC/CART neurons were hyperpolarized after ghrelin application which stimulated the γ-aminobutyric acid (GABA) release from NPY/AgRP to POMC/CART neurons [97]. In addition, central injection of α-MSH inhibited ghrelin-stimulated hyperphagia, suggesting the crosstalk between ghrelin and melanocortin system [98]. The PVN seems to be another important site for ghrelin actions. Administration of ghrelin in the PVN alone is known to stimulate food intake and inhibit fat oxidation [99]. Fasting enhanced the density and strength of ARC nerve fibers projecting to PVN and the activation was impaired in mice lacking GHSR or pretreatment with blockage of GHSR signaling [100]. Inhibition of oxytocinergic neurons in the PVN contributed to orexigenic effects of ghrelin [3]. In the VMH, i.c.v. administration of

ghrelin decreased fatty acid synthase mRNA expression via inhibition of AMPK activity [101]. In the LHA, ghrelin depolarized orexin-positive neurons and enhanced the c-fos expression in these neurons [102]. Pretreatment of orexin receptor antagonist partially abolished central ghrelin appetite-stimulating effects [103]. The LHA even serves as a critical downstream site for hippocampus ghrelin-mediated hyperphagia [104]. In the hypothalamus, ghrelin not only promoted food intake but also decreased energy expenditure via inhibition of sympathetic projections to the brown adipose tissue [105]. Even after selective knockout of GHSR in hypothalamic AgRP neurons, mice fed with a high-fat diet exerted elevated energy expenditure and upregulated thermogenesis in both brown and white adipose tissue, suggesting GHSR inhibition in AgRP neurons could increase sympathetic activity [95].

From the above, it is known that nesfatin-1 inhibits NPY-positive neurons in the ARC, different from the activation by ghrelin. Moreover, mTOR signaling is involved in the ghrelin action on NPY/AgRP neurons, since inhibition of mTORC1 abolished ghrelin-induced upregulation of NPY and AgRP [40, 106], while majority mTOR-positive neurons in the ARC are also immunoreactive for nesfatin-1 [107]. One study observed the effects of nesfatin-1 on ghrelin, which showed that i.c.v. administration of nesfatin-1 suppressed preproghrelin, GHSR and NUCB2 mRNA expression in the forebrain of goldfish [51]. In contrast to the effects of nesfatin-1 on promoting activity of oxytocinergic neurons, GHSR forms heterocomplex with Oxy-R and impairs the oxytocinergic signaling [51]. In addition, nesfatin-1 promotes oxytocin release [62], whereas ghrelin inhibits oxytocin expression in the PVN [3]. In neonatal chicks, ghrelin inhibits food intake, opposite to the orexigenic effects of ghrelin in rodents [108, 109]. Coincidentally, both ghrelin and nesfatin-1 inhibit food intake through the CRF-R pathway in chicks [28, 108].

Anxiety, depression and stress

Anxiety

Nesfatin-1 exerts anxiogenic effect in rodents. Central infusion of nesfatin-1 [110] or its middle fragment [111] induced the anxiety-like behaviors in several maze tests, or in unfamiliar environment, while central blockage of endogenous nesfatin-1 with antibody attenuated anxiety-like behavior in male rats [112]. Peripheral continuous administration of nesfatin-1 also exhibited anxiogenic effect by downregulating expression of phosphorylated-ERK1/2 and the brain-derived neurotropic factor (BDNF) in the prefrontal cortex and hippocampus [113]. Human studies showed that alteration of nesfatin-1 levels in anxiety was inconsistent between genders. In male patients suffering from anxiety disorder, plasma nesfatin-1 levels were decreased [114], whereas in female obese patients, NUCB2/nesfatin-1 levels were upregulated [115]. However, improvement of anxiety did not alter NUCB2/nesfatin-1 levels [116].

Ghrelin seems to exert dual effects on anxiety. I.c.v. [117], amygdala, hippocampus [118] and hypothalamus [119] injections of ghrelin induced anxiety-like behaviors in the open field test, plus-maze test, or elevated plus maze, while antisense DNA for ghrelin exerted an anxiolytic effects in the elevated plus maze test, black and white test, or conditioned fear tests [120]. Conversely, Jensen et al. [121] reported overexpression of GHSRs in the amygdala or peripheral injection of ghrelin caused anxiolytic effect, which could be abolished in GHSR knockout mice. Acute energy restrictions stimulated endogenous ghrelin secretion and thus exerted anti-anxiety effects and the anxiolytic effects could be abolished by GHSR1a antagonist [122] and GHSR knockout [123], confirming the GHSR-dependent pattern.

Depression

Nesfatin-1 levels were profoundly increased in subjects suffering from depression [124] and in depressed patients with subclinical hypothyroidism [125]. NUCB2 mRNA contents in Edinger–Westphal (EW) nucleus were significantly higher in male depressed suicide victims than those in the control, but not in female ones [126]. The rats exhibited more despair after chronic nesfatin-1 application in forced swimming test and open field test, accompanied by hypothalamic–pituitary–adrenal (HPA) axis activation [127]. The higher nesfatin-1 levels in depressive disorder were correlated with elevated corticosterone, IL-6, and C-reactive protein (CRP) levels [128, 129]. Pretreatment with a traditional Chinese antidepressant medicine, Xiaoyaosan, downregulated nesfatin-1 levels in the PVN [130]. In contrast to above studies, Korucu et al. reported serum nesfatin-1 levels were decreased obviously in major depressive disorder with suicidal ideation [131]. In adolescent depression, nesfatin-1 levels were significantly lower than in the control group [132]. Hence, we performed a meta-analysis on 567 depressive patients and 447 control subjects and the result showed higher plasma levels of nesfatin-1 were associated with an increased risk of depression, indicating that nesfatin-1 might act as a potential novel biomarker for the diagnosis of depressive disorder [133].

Similar to the controversial effects of ghrelin on anxiety, the links between ghrelin and depression are also inconsistent. In humans [134–136] and rodents [137, 138], ghrelin levels were elevated in depressive status. Exogenous ghrelin generated antidepressant-like effect in tail-suspended mice and in the bilateral olfactory bulbectomy mice [139]. In humans, ghrelin exerted antidepressant effects in patients

with major depression [140]. However, in contrast to above results obtained from adult rodents, Jackson et al. [141] reported that acute i.c.v. administration of ghrelin displayed depressive-like effects in male juvenile rats by prolonging immobility time in forced swimming test. Similarly, Guo et al. [142] observed that GHSR knockout mice did not exert apparent depression after chronic social, but the control mice did, possibly due to the differential expressions of brain BDNF and IL-6.

One study simultaneously focusing on nesfatin-1 and ghrelin in depression showed that plasma levels of both peptides were increased in depressive patients and even associated with the severity of depression [129].

Stress

Restraint stress mobilized nesfatin-1-positive neurons in the hypothalamus, NTS [143], locus coeruleus (LC), rostral raphe pallidus (rRPa), and the non-preganglionic Edinger–Westphal nucleus (npEW) [144], most of which could also be activated by abdominal surgery, a kind of visceral stressor [145]. Only acute stress, instead of chronic stress, increased the plasma and hypothalamic nesfatin-1 levels in rats [146]. The elevated nesfatin-1 levels attributed to the activation all the three parts of HPA axis during stress. In the hypothalamus, nesfatin-1 activated the CRH-positive neurons by increasing cytosolic Ca^{2+} concentration [147]. Central administration of nesfatin-1 elevated ACTH and corticosterone levels in pituitary and adrenal gland, respectively [148]. In mouse corticotrophs, nesfatin-1 stimulated the synthesis of POMC which serves as the precursor of ACTH [149]. Glucocorticoid receptors were detected in nesfatin-1-positive neurons in the PVN [150], indicating a possible feedback control through adrenal signaling. Actually, nesfatin-1 expression is negatively regulated by adrenal steroids, as bilateral adrenalectomy triggered an increase in nesfatin-1/NUCB2 mRNA expression in the HPA axis [148]. Similar results were obtained in goldfish. NUCB2/nesfatin-1 was detected in all the three parts of the stress axis in goldfish [151] and restraint stress upregulated NUCB2/nesfatin-1 mRNA levels in hypothalamus and pituitary; moreover, nesfatin-1 application stimulated the release of cortisol and ACTH in vivo and in vitro [151].

When stress occurs, the changes in ghrelin levels seem to be controversial. Under acute stress, ghrelin levels were decreased by orderly activation of CRF_1-R, 5-$HT_{(1B)/(2C)}$-R and MC_4-R in mice [152]. However, Kristenssson et al. [153] reported that acute psychological stress led to elevated ghrelin levels. Chronic social defeat stress increased ghrelin levels in rats [137], while ghrelin mRNA levels of hypothalamus were significantly decreased by chronic restraint stress [154]. Nahata et al. [155] even found that plasma ghrelin concentration was not altered by restraint stress. In humans, one meta-analysis based on the data of 348 patients showed a short-term increase of ghrelin level following acute stress [156]. The altered ghrelin levels seem to influence the HPA axis, while the results also remain controversial. In rodents [157] and humans [158], ghrelin increased CRH, ACTH or corticosterone levels. However, Jensen M. et al. [121] reported no effect of ghrelin on plasma ACTH levels in mice. Alterations in HPA axis induced by stress also affect ghrelin secretion. Cortisol decreased serum ghrelin levels and its mRNA expression in the stomach in tilapia [159]. In patients with Cushing's syndrome, hypercortisol reduced plasma ghrelin levels, which were restored by surgical treatment [160].

Learning and memory

There are only a few studies that focus on the effects of nesfatin-1 on learning and memory and the results remain conflicting, while ghrelin exerts dual effects on learning and memory.

As demonstrated by Zhu Q et al. [161], nesfatin-1 did not affect learning and memory after infusion into the lateral amygdala or area CA1 of the dorsal hippocampus. Similarly, Ge et al. [113] verified that continuous intraperitoneal (i.p) injection of nesfatin-1 did not alter the performance of rats in the Morris water maze. In rat model with non-alcoholic fatty liver disease, increased plasma nesfatin-1 levels were found to be at least partly associated with impaired learning and memory [162]. Only one study showed that nesfatin-1 improved memory impairment induced by transient global cerebral ischemia/reperfusion (I/R), and the underlying mechanism might be related to its inhibitory effects on activation of microglial cells and caspase-3 [163].

GHSR mRNA is profoundly expressed in the CA1, CA2, and CA3 regions of hippocampus and the dentate gyrus in rat brain [35]. Memory could be improved in a dose-dependent manner through administration of ghrelin in the cerebroventricles, hippocampus, amygdala and dorsal raphe nucleus (DRN) [117, 118]. In ghrelin knockout mice, the exploration time of novel object decreased, which could be rescued by ghrelin re-supplement [164]. Blocking GHSR1a worsened memory encoding [165], while ghrelin receptor agonists ameliorated object recognition memory [166]. The mechanisms underlying memory enhancement of ghrelin partly lie in synaptic plasticity and hippocampal neurogenesis, since ghrelin increased the spine synapse density in CA1 region [164, 167] and promoted proliferation and differentiation of adult hippocampal progenitors [168]. In depressed mice, lower ghrelin levels caused decreased hippocampal BDNF levels and resulted in cognitive decline [169]. NMDA receptor is another site for ghrelin action. Ghrelin attenuated memory impairment induced by antagonist of NMDA receptor [170] and upregulated NMDA

receptor and MAPK1 expressions to improve memory [171]. In contrast, some studies documented the negative effects of ghrelin on memory. Spitznagel et al. demonstrated that cognitive function was negatively associated with ghrelin levels in non-demented elder adults [172]. In GHSR1a knockout mice, spatial memory was improved [173]. In addition, injection of ghrelin into lateral amygdala prevented acquisition of conditioned taste aversion [174]. A study done by Li N et al. might explain these contradictory results. They found that a selective increase of GHSR1a expression in hippocampus CA1 excitatory pyramidal neurons impaired hippocampus-dependent memory, whereas its upregulation in CA1 inhibitory interneurons improved hippocampus-dependent memory. [175].

Neuroprotection

Nesfatin-1 reduced the gene expression of NF-κB, levels of tumor necrosis factor alpha (TNF-α), IL-1β, IL-6 and activity of caspase-3 in traumatic brain of rat, which implies its anti-inflammatory and anti-apoptotic effects in the CNS [176]. In patients with traumatic brain injury (TBI), nesfatin-1 reflected severity of trauma and even served as a prognostic biomarker following TBI [177]. Nesfatin-1 protected PC12 cells against high glucose-induced cell injury by inhibition of apoptosis, autophagy and ROS production [178]. In chronic high-fat diet mice, obesity was associated with upregulated pro-inflammatory factors and NF-κB signaling components in hypothalamus, which was accompanied by decreased NUCB2/nesfatin-1 levels and diet and/or exercise interventions reversed above alterations [179].

Ghrelin ameliorated secondary brain injury induced by intracerebral hemorrhage through inhibition of nucleotide-binding oligomerization domain-like receptor pyrin domain-containing 3 (NLRP3) inflammasome and activation of nuclear factor-E2-related factor 2 (Nrf2)/anti-oxidative response element (ARE) signaling pathway [180]. Ghrelin restored cerebral microvascular integrity, reduced vascular leakage [181] and improved neural survival [182] by inhibition of apoptosis, inflammation or oxidative stress. In amyloid β induced AD mouse models, ghrelin ameliorated cognition, synaptic plasticity deficiency and neuroinflammation [183].

Our previous data have elucidated the neuroprotective effect of both nesfatin-1 and ghrelin on Parkinson's disease (PD). Nesfatin-1 protected nigral DAergic neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity via C-Raf-ERK 1/2 pathway in vivo and in vitro [184]. Moreover, nesfatin-1 prevented the neurotoxicity of rotenone in DAergic cells [185]. Reduced nesfatin-1 in the brain might induce degeneration of nigrostriatal DAnergic system, which was mediated via mitochondrial dysfunction-related apoptosis [186]. Paralleled with nesfatin-1, similar results were obtained with application of ghrelin which antagonized the impairment of 1-methyl-4-phenyl-pyridinium (MPP^+) [187], except that ghrelin increased [188] and nesfatin-1 decreased the firing rate of nigral DAergic neurons [189]. Stutz et al. found that the protective effect on DAergic neurons mediated by GHSR was independent of their electric activity, which could explain the similar protective effects and opposite actions on the firing of DAergic neuron between nesfatin-1 and ghrelin [190]. In PD models, ghrelin blocked the activation of microglia, inducible nitric oxide synthase, reduced the expression of TNF-α and IL-1β [191], improved motor symptoms and partly restored tyrosine hydroxylase in substantia nigra via improving autophagic flux dysfunction and recovery of lysosome functions [192]. In PD patients, total and active plasma ghrelin levels were decreased [193]. Deep brain stimulation of subthalamic nucleus, as a type of treatment of PD [191], increased active ghrelin levels and body weight gain [194].

The roles of nesfatin-1 and ghrelin in the peripheral functions

Gastrointestinal functions

Nesfatin-1-positive neurons in the NTS were activated by gastric distension (GD) and exogenous nesfatin-1 modulated mechanosensitivity of gastric vagal afferent fibers [195]. Nesfatin-1 affects not only gastrointestinal sensory input but also gastrointestinal motility. Central injections of nesfatin-1, such as lateral ventricle [196], ARC [197], PVN [198], VMH [199] and amygdala [200], inhibited gastric motility and the underlying mechanisms were linked to oxytocin and melanocortin systems. Besides, nesfatin-1 affects gastric secretion. Central infusion of nesfatin-1 reduced gastric acid secretion dependent upon the inhibition of H^+/K^+-ATPase expression in the gastric mucosa [201]. The elevated nesfatin-1 levels also contributed to the protection of gastric mucosa impaired by stress, partly through the inhibition of gastric acid secretion [202]. In addition, nesfatin-1 affected the secretion of other gastrointestinal hormones, including CCK and peptide YY in mice intestine [203].

Ghrelin exerts different effects from nesfatin-1 on gastrointestinal functions. Activities of GD sensitive neurons in the DVC [204], hippocampus [205], and lateral septum [206] were modulated by micro-injection of ghrelin. Both ghrelin and GHSRs were detected in vagus nerve and ghrelin inhibited the mechanosensitivity of vagal afferent [207], while ghrelin receptor inverse agonist increased the firing rate of vagal afferent fibers [208]. Ghrelin regulates gastrointestinal motility during both digestive and interdigestive period. In rodents and humans, administration of ghrelin promoted

gastric emptying [98, 209, 210], which might be mediated by GABAergic neurons located in the AP [211]. During interdigestive period, ghrelin promoted migrating motility complex (MMC) phase III-like movement [212] and the promotion extended to the small intestine [213]. Ghrelin stimulated gastric acid secretion through vagus nerve and the underlying mechanism involved in the histamine and gastrin [214].

Despite the same origin in gastrointestinal tract, nesfatin-1 mainly exerts inhibitory effects on gastrointestinal functions, opposite to the major stimulatory effect of ghrelin. The studies focusing on nesfatin-1 and ghrelin showed that fasting generated opposite impacts on both peptides in the stomach, as ghrelin expression was upregulated and NUCB2/nesfatin-1 production was suppressed [215]. Histone deacetylases5 (HDAC5)-mTORC1 signaling served as common pathway leading to reciprocal changes in ghrelin and NUCB2/nesfatin-1 secretions [215]. In dogs, NUCB2 mRNA was expressed in all digestive organs including digestive tract and digestive glands [216], while ghrelin gene was only detected in the stomach [217]. A research in dogs showed that plasma nesfatin-1 concentrations might mark the inversed kinetics for ghrelin during MMC, since plasma ghrelin hit its nadir just when plasma nesfatin-1 levels peaked in late phase I [218]. Peripheral administration of nesfatin-1 inhibited gastric MMC in the fasted dogs, but did not affect gastrointestinal motility in the fed ones [218].

Blood glucose and diabetes mellitus

Nesfatin-1 is co-distributed with insulin in pancreatic β-cells and promotes the secretion of insulin, indicating the correlation between nesfatin-1 and glucose metabolism. In vitro study showed that nesfatin-1 potentiated glucose-induced insulin production by activation of L-type Ca^{2+} channels [219] and inhibition of voltage-gated K^+ channels [220] in isolated β-cells of pancreatic islet. In pancreatic β-cell-specific NUCB2 knockout mice, blood glucose levels were elevated and insulin secretion was inhibited [221]. Notably, nesfatin-1 exhibited direct hypoglycemic effect in peripheral tissue, since it enhanced phosphorylation of AKT and glucose transporter 4 (GLUT4) expressions in skeletal muscle and adipose tissue [222], decreased the glucose intake in the gut and increased glucose uptake in liver and muscle [223]. Nesfatin-1 exerts hypoglycemic effects in physiological state, which makes nesfatin-1 a possible candidate for hypoglycemic hormones besides insulin. In T2DM rats, nesfatin-1 levels decreased in the blood, which could be reversed by hypoglycemic agents [224]. In T2DM mice, central injection of nesfatin-1 decreased plasma levels of free fatty acid and promoted fatty acid oxidation in skeletal muscle [225]. In T2DM patients, higher plasma nesfatin-1 concentrations in the early stages might compensate for hyperglycemia and nesfatin-1 concentrations were reduced in patients receiving antidiabetic therapy [226]. In addition, NUCB2 gene polymorphism might be associated with T2DM, as a consequence of lower CG and GG genotype of NUCB2 gene in T2DM patients [227].

In contrast to the hypoglycemic effects of nesfatin-1, ghrelin exerts hyperglycemic actions. In rodents and humans, exogenous ghrelin suppressed glucose-induced insulin secretion [228–230] and increased plasma glucose levels [231]. In pancreatic β-cells, ghrelin reduced insulin secretion by activating voltage-dependent K^+ channels and by weakening Ca^{2+} signaling [232]. In addition, ghrelin stimulated somatostatin secretion from pancreatic δ-cells, which in turn suppressed insulin secretion [233]. The formation of GHSR1a-SST5 heterocomplex also contributed to the inhibition of insulin secretion [52]. Moreover, ghrelin stimulated glucagon secretion from α-cells, which elevated plasma glucose levels [234]. In T2DM patient, plasma ghrelin levels were decreased and were associated with insulin resistance [235]. Due to hyperglycemic effects of ghrelin, GHSR1a receptor antagonist, PF-05190457, was explored to treat T2DM [208].

In short, nesfatin-1 stimulates insulin secretion and decreases plasma glucose levels, while ghrelin exerts opposite effects. In T2DM, plasma nesfatin-1 levels are elevated and ghrelin levels are decreased. Bertucci et al. observed the alteration of both peptides after exposure to glucose in goldfish, concluding that preproghrelin was upregulated and NUCB2/nesfatin-1 was downregulated in cultured intestine sections [236]. GHSRs are involved in insulin secretion, since GHSR knockout mice showed significantly lower blood glucose levels under caloric restriction [237] and the glucose-stimulated insulin secretion was inhibited both in vivo and in vitro after specific GHSR knockout in β cells [238]. The direct effect of ghrelin on insulin secretion is inhibitory, but after deleting its receptors on β cells, insulin secretion was still inhibited, indicating the possible involvement of other insulinostatic hormones. It could not be ruled out that nesfatin-1 might be one of the candidates. Our results demonstrated that the improvement of nesfatin-1 on glucose tolerance could be abolished by pretreatment of GHSR antagonist or GHSR knockout, which implies the dependence of nesfatin-1 on GHSR in the regulation of glucose metabolism [4]. Whether nesfatin-1 could regulate pancreatic insulin release through GHSRs is worthy of further investigation.

Reproduction

The reproduction is mainly regulated by the hypothalamic-pituitary–gonadal (HPG) axis, which originates from hypothalamus, also serving as a feeding control center. In fact, reproductive functions are closely correlated with energy metabolism. It is vital for reproduction that the feeding

related hormones are maintained in appropriate range. The effects of nesfatin-1 and ghrelin on the three respective levels of the HPG axis will be discussed, with regards to different species.

Protein or mRNA of NUCB2/nesfatin-1 was detected in all the three parts of the HPG axis indicating the link between nesfatin-1 and reproduction. The actions of nesfatin-1 on reproduction have been observed in different species. In adult male rats, i.c.v. injection of nesfatin-1 suppressed gene expression of gonadotropin-releasing hormone (GnRH) in hypothalamus, follicle-stimulating hormone β (FSHβ) and luteinizing hormone β (LHβ) in the pituitary gland, as well as genes for testosterone synthesis in the testis [239]. Most results obtained in male pubertal rats were similar to those of the adults except for the elevated genes for testosterone synthesis [239]. In female pubertal rats, elevated NUCB2/nesfatin-1 mRNA levels in hypothalamus promoted pubertal maturation, since the central infusion of nesfatin-1 created a slight increase in circulating gonadotropins and continuous infusion of antisense morpholino oligonucleotides of NUCB2 prevented vaginal opening and reduced ovarian weights and plasma LH concentration [240]. In both male and female goldfish, peripheral injection of nesfatin-1-like peptide suppressed hypothalamic GnRH mRNA expression, as well as pituitary LHβ and FSHβ. In gonads, nesfatin-1 downregulated LH receptor, FSH receptor and enzymes for sex hormone synthesis [241]. Accordingly, the circulating testosterone and estradiol were decreased [241]. Nesfatin-1-like peptide inhibited oocyte maturation in zebrafish [241]. In general, nesfatin-1 has an inhibitory effect on reproduction in vivo in adult rodents and fishes. However, some results obtained in vitro seem to be different. Synthetic nesfatin-1 increased GnRH and LHβ expressions in hypothalamic and pituitary cell lines [242] and nesfatin-1 enhanced the testicular testosterone secretion in Leydig cells [239]. In addition to fishes and rodents, there were several studies focusing on porcine. I.c.v. administration of nesfatin-1 stimulated LH secretion in prepubertal gilts [243]. Nesfatin-1 significantly promoted the meiotic maturation [244] and stimulated cell proliferation and progesterone production in granulosa cells from large follicles [245].

The effects of ghrelin on reproduction have been studied in different species, with different results. In rodents, in vivo results showed that ghrelin inhibited hypothalamic GnRH secretion [246, 247], pituitary LH or FSH expressions or secretion [248–250], ovarian and testicular steroid hormone secretion [250, 251]. In vitro results showed that ghrelin stimulated GnRH release in hypothalamic explant of the prepubertal [246], and enhanced basal LH and FSH secretions in pituitary tissue [247], but decreased testosterone secretion in testicular slices [252]. In female rats, the main effect of chronic subcutaneous injection of ghrelin on the ovaries was inhibitory, including the number of corpora lutea, the mean diameter of follicle, corpora lutea, luteal cell, theca layer, oocyte and zona pellucida, as well as the whole ovarian volume [253]. Ghrelin inhibited male puberty onset, as balano-preputial separation was delayed. However, in prepubertal female rats, daily subcutaneous administration of ghrelin failed to influence puberty onset and the sexual hormone secretions [254]. In humans, ghrelin decreased both LH and FSH levels in females [255], but only LH in males [256]. Ghrelin attenuated basal and hCG-stimulated progesterone release in human luteal cells [257]. A human study showed that high serum ghrelin levels were correlated with constitutional delay of growth and puberty in adolescence [258]. In goldfish, ghrelin stimulated LH release in vivo and in vitro [259], while inhibited germinal vesicle breakdown of zebrafish oocyte maturation in vitro [260]. The effects of ghrelin on porcine reproduction were mainly concentrated in experiments in vitro and the results were not completely consistent. Some studies showed that ghrelin promoted reproductive functions: ghrelin reinforced estradiol and progesterone secretions, increased ovarian cell proliferation and enhanced blastocyst formation [261–263]. However, ghrelin inhibited LH and FSH induced follicular steroid secretion [264], as well as the organization of microtubules and microfilaments, potentially being a contributing factor in the lowered maturation rate [265].

In general, the regulation of nesfatin-1 and ghrelin in reproductive axis are complicated, varying with different species, genders and stages of development. The main roles of nesfatin-1 and ghrelin in rodents are similar, with inhibitory effects in vivo and excitatory effects in vitro. Nesfatin-1 inhibits reproduction in fish and promotes reproduction in porcine, while the effects of ghrelin on reproduction are inconsistent in above two species.

Cardiovascular functions

The distribution of ghrelin and nesfatin-1 in the cardiovascular centers and in myocardium suggests their role in controlling cardiovascular functions. The effects of the two peptides on the heart and blood vessels will be discussed, respectively.

In myocardial I/R injury models, nesfatin-1 exerted cardioprotective effects through anti-inflammation, anti-apoptosis, anti-oxidative stress and anti-autophagy [266]. Nesfatin-1 decreased cardiac troponin-T and pro-inflammatory cytokines, increased the expressions of p-AKT/AKT and p-GSK-3β/GSK-3βa [267] and suppressed necroptosis via modulation of receptor interacting kinase 1 (RIPK1)-RIPK3-the mixed lineage kinase domain-like protein (MLKL) axis and RhoA/Rho-associated coiled-coil-containing protein kinase (ROCK)/RIP3 signaling pathway [268] as well as attenuation of endoplasmic reticulum stress via AKT/ERK pathway after I/R injury [269]. In addition, high levels of

nesfatin-1 were probably associated with left ventricle myocardial remodeling [270]. In blood vessels, nesfatin-1 could alleviate endothelial inflammation induced by free fatty acids via the growth factor independent-1 transcriptional repressor (GFI1)/NF-κB signaling pathway [271]. However, nesfatin-1 promoted vascular smooth muscle cells proliferation [272], migration and neointimal hyperplasia by increasing matrix metalloproteinase 2 (MMP2)/MMP-9 levels and decreasing peroxisome proliferator-activated receptor γ (PPARγ) gene expression [273], indicating the role of nesfatin-1 in the formation of atherosclerotic plaque. Compared with the control group, higher plasma levels of nesfatin-1 were found in patients with coronary artery stenosis [274]. In the regulation of blood pressure, central nesfatin-1 significantly increased mean arterial pressure (MAP) by acting on $MC_{3/4}$-Rs in the NTS and sympathetic nervous system, since the vasopressor effect could be abolished by pretreatment with the $MC_{3/4}$-R antagonist or the alpha-adrenergic antagonist. In addition, after intravenous infusion of nesfatin-1, the arterial vessels contracted, resulting in increased peripheral resistance in rats [275] and chronic infusion of nesfatin-1 induced severe hypertension [272].

Ghrelin plays a protective role in myocardial I/R injury. Ghrelin reversed the alteration of infarct size and the levels of creatine kinase (CK), lactate dehydrogenase (LDH), TNF-α, IL-6, malondialdehyde (MDA), caspase-3, caspase-9, iNOS, superoxide dismutase (SOD), and glutathione (GSH)-peroxidase (PX) after myocardial I/R injury via the high mobility group box 1 (HMGB1)/Toll-like receptor 4 (TLR4)/NF-κB pathway [276]. In addition to the anti-inflammatory and antioxidant effects, ghrelin resisted myocardial I/R injury by enhancing autophagy. After delivering ghrelin to the infarcted myocardium with an adeno-associated virus 9 vector, the ratio of LC3-II/LC3-I, formation of autophagosomes and genes expression involved in the autophagic pathways were elevated [277]. Chronic application of ghrelin improved ultrastructural changes after myocardial infarction, including increased numbers of intracellular organelles in endoplasmic reticulum, and decreased numbers of atrophic nuclei, phagocytes, irregular nuclear membrane and chromatin condensation [278]. Myocardial hypertrophy and myocardial fibrosis are important pathological features of myocardial remodeling. Karcz-Socha I et al. reported that circulating ghrelin levels were associated with left ventricular mass index [279]. After transverse aortic constriction, ghrelin knockout mice exhibited severer cardiac hypertrophy and inhibition of parasympathetic activities, while re-application of ghrelin attenuated cardiac hypertrophy by activating the cholinergic anti-inflammatory pathway [280]. Both in vivo and in vitro experiments confirmed that ghrelin inhibited angiotensin-induced myocardial fibrosis via PPAR-γ/transforming growth factor-β1 (TGF-β1) [281] or Nrf2/NADPH/ROS pathway [282]. Ghrelin protects not only the myocardium but also the vascular endothelium. Ghrelin ameliorated endothelial inflammation via AMPK/NF-κB signaling pathway [283], and inhibited atherosclerosis by preventing endoplasmic reticulum stress [284]. Ghrelin reduced intraplaque angiogenesis and lowered the thickness ratio of the intima to media by downregulating vascular endothelial growth factor (VEGF) and VEGF receptor 2 levels in an atherosclerotic rabbit model [285]. In the regulation of blood pressure, low circulating ghrelin levels are correlated with elevated blood pressure [286]. Exogenous administration of ghrelin decreased MAP in rabbits, rats and even humans [287]. The underlying mechanisms included vasorelaxation, inhibition of sympathetic activity, kidney diuresis, alleviation of oxidative stress and regulation of renin–angiotensin system [287–290].

Both nesfatin-1 and ghrelin exert similar protective effects on the cardiovascular system, but they have hypertensive and hypotensive effects, respectively, in the regulation of MAP.

Anti-inflammatory and anti-oxidative effects

Despite their previously stated opposite effects, nesfatin-1 and ghrelin exhibit similar anti-inflammation and antioxidation effects in various organs and tissues. (The cardiovascular system is not covered in this section, as discussed above.)

Serum nesfatin-1 levels were elevated in patients with Crohn's disease and ulcerative colitis [291], which indicates the anti-inflammatory effects of nesfatin-1. Actually, exogenous nesfatin-1 ameliorated necrotizing enterocolitis [292] and acetic acid-induced colitis [53]. Nesfatin-1 improved gastric ulcer by downregulating myeloperoxidase (MPO), MDA, chemiluminescence, IL-6, TNF-α and by upregulating GSH levels [293], in a COX-dependent manner [294]. Nesfatin-1 attenuated inflammation and oxidative stress in alveolar epithelial cell and in animal models of acute lung injury by inhibiting HMGB1, p38MAPK and NF-κB signaling pathways [295], while in NUCB2/nesfatin-1 knockout mice, acute lung injury caused by lipopolysaccharide was exacerbated [296]. In testicular torsion rat models, nesfatin-1 regulated pro-inflammatory/anti-inflammatory cytokine balance and reduced AKT/CREB expressions [297]. Nesfatin-1 alleviated osteoarthritis by suppressing the activation of NF-κB, MAPK, and the Bax/Bcl-2 signaling pathway [24].

Elevated plasma ghrelin levels have been observed in various inflammatory diseases, such as colitis [298], Crohn's disease [299], sepsis [300] and pancreatitis [301]. Ghrelin inhibited the expression of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α in human T lymphocytes and monocytes [302]. In sepsis-induced inflammation, ghrelin downregulated the pro-inflammatory cytokines mediated by the MAPK phosphatase-1 [303] and elevated the levels of antioxidants such as GSH, catalase (CAT) and SOD against the oxidative stress [304]. Ghrelin improved acute

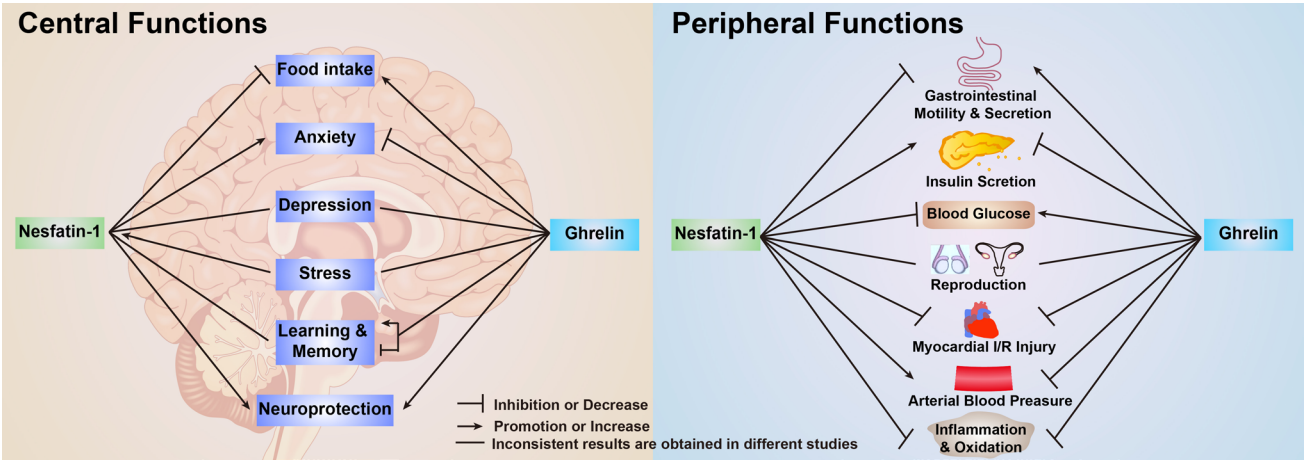

Fig. 4 The comparison of nesfatin-1 and ghrelin in central and peripheral functions. In the CNS, nesfatin-1 inhibits food intake and induces anxiety. Stress activates nesfatin-1-positive neurons. Ghrelin stimulates food intake and exerts dual effects on learning and memory. Both nesfatin-1 and ghrelin exhibit neuroprotective effects. In peripheral system, nesfatin-1 inhibits gastrointestinal motility and secretion, stimulates insulin secretion, decreases blood glucose levels, and increases arterial blood pressure, while ghrelin reverses all above effects. Both nesfatin-1 and ghrelin attenuates myocardial I/R injury and exerts anti-inflammation and antioxidation effects

pancreatitis via inhibiting NF-κB signaling pathway, thus reducing the release of inflammatory cytokines [305].

Both nesfatin-1 and ghrelin possess anti-inflammatory and anti-oxidative effects. One group simultaneously observed the protective effects of both peptides on gastric ulcer. The results showed that a much lower dose of nesfatin-1 (0.02 nmol/Kg) appeared to be slightly less effective than ghrelin (4 nmol/Kg) according to the loss of gastric surface epithelium, glands erosion, cell inflammation and bleeding scores [306]. In colitis [53] and acute pancreatitis models [307], nesfatin-1 could alleviate inflammations via ghrelin receptor, since GHSR antagonist attenuated this protective effect, suggesting the interaction between nesfatin-1 and GHSR.

Conclusion and perspectives

Generally, nesfatin-1 displays opposite effects compared to ghrelin in the regulation of energy metabolism, blood glucose levels, gastrointestinal functions, blood pressure, but exhibits similar effects in cardiovascular protection, anti-inflammation, antioxidation and neuroprotection via distinct or similar mechanisms (Fig. 4). The early identifications of ghrelin and GHSR have led to numerous related studies. Therefore, clinical applications of ghrelin could enlighten those of nesfatin-1. For example, it should help with exploring the roles of nesfatin-1 in the treatment of obesity, neurodegenerative diseases, diabetes, myocardial I/R injury, inflammation, oxidative stress-related diseases, etc. [308, 309]. The antagonist of nesfatin-1 receptor might be applied in the treatment of cachexia, anorexia, GH deficiency and gastroparesis [310]. Therefore, the discovery of nesfatin-1 receptor could potentially contribute to the treatment of certain diseases and further clarification of the mechanisms of nesfatin-1 actions. The overlapping receptor distribution and the interaction between nesfatin-1 and ghrelin indicate the potential crosstalk. The dependence of nesfatin-1 on GHSR provides some strategies for searching the receptor of this orphan ligand. It could not be ruled out that nesfatin-1 might serve as an endogenous ligand for other known receptors, that the unknown nesfatin-1 receptor might form heterocomplex with other known receptors, or that nesfatin-1 even serves as an allosteric modulator for other GPCRs. Experimental techniques such as Biacore, proximity ligation assay (PLA), co-immunoprecipitation and BRET, may be conducive to exploring the interactions between nesfatin-1 and other receptors.

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Data availability Enquiries about data availability should be directed to the authors.

Declarations

Conflict of interest We have no conflicts of interest to declare.

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