Distribution and Biological Functions of the Novel Anorexic Neuropeptide nesfatin-1 (Post-print)

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Abstract

Nesfatin-1 is a newly discovered anorexigenic neuropeptide that is primarily located in the hypothalamus and brainstem in the central nervous system, and mainly distributed in the stomach, intestine, pancreatic islets, and gonads in the periphery. Its expression is regulated by nutritional, physiological, and pathological factors, as well as other feeding hormones. Nesfatin-1 cooperates with multiple hormones in central neurons and peripheral cells to participate in energy metabolism processes such as feeding and glucose homeostasis, activates the hypothalamic-pituitary-adrenal (HPA) axis to participate in stress responses, and can also act on the HPA axis to influence the initiation of developmental periods. This review focuses on the distribution of nesfatin-1 in the central and peripheral systems, factors affecting its expression, and its biological functions.

Full Text

Distribution and Biological Functions of the Novel Anorexigenic Neuropeptide Nesfatin-1

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Abstract

Nesfatin-1 is a recently discovered anorexigenic neuropeptide that is predominantly expressed in the hypothalamus and brainstem centrally, and in the

stomach, intestine, pancreatic islets, and gonads peripherally. Its expression is regulated by nutritional status, physiological and pathological conditions, and other feeding-related hormones. Nesfatin-1 colocalizes with multiple hormones in central neurons and peripheral cells to participate in energy metabolism processes such as feeding regulation and glucose homeostasis. It also activates the hypothalamic-pituitary-adrenal (HPA) axis to mediate stress responses and influences the initiation of puberty. This review focuses on the central and peripheral distribution of nesfatin-1, factors affecting its expression, and its biological functions.

Keywords: nesfatin-1; distribution; expression; anorexia; biological function

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The peripheral and central nervous systems regulate animal feeding behavior and maintain energy balance through complex neuronal networks and hormonal systems. Numerous hormones have been identified as participants in feeding and energy homeostasis, including leptin, ghrelin, orexin, insulin, agouti-related peptide, adiponectin, and cocaine- and amphetamine-regulated transcript peptide. In 2006, nesfatin-1 was identified as a novel hypothalamic and brainstem peptide involved in regulating feeding behavior. Intracerebroventricular (icv) injection of nesfatin-1 suppresses food intake in animals, and chronic administration leads to loss of body fat and weight [1]. The discovery of nesfatin-1 has attracted extensive attention and research interest. Current research on nesfatin-1 primarily focuses on its distribution in the body, factors influencing its expression, biological functions, and underlying mechanisms. Therefore, this review provides a comprehensive overview of recent research progress on nesfatin-1 in these four aspects.

1. Nesfatin-1 Structure, Distribution and Expression

Nesfatin-1 is one of three products derived from nucleobindin-2 (NUCB2), an 82-amino acid (AA) neuropeptide with a molecular mass of 9.8 kDa that possesses the anorexigenic activity of NUCB2 [2]. The middle fragment of nesfatin-1, spanning amino acid residues 24 to 53, is designated as M30. The M30 amino acid sequence is highly conserved across species, being identical in rats and mice, and differing by only two residues from the human sequence (Fig. 1 [Figure 1: see original paper]). M30 inhibits food intake in a dose-dependent manner with a half-maximal inhibitory concentration (IC50) identical to that of nesfatin-1 (0.36 nmol/g body weight) [3].

1.2 Distribution and Colocalization with Other Hormones

Nesfatin-1 is widely distributed throughout the central nervous system, including the hypothalamic paraventricular nucleus (PVN), arcuate nucleus (Arc), supraoptic nucleus (SON), lateral hypothalamic area (LHA), zona incerta [1], dorsomedial nucleus, ventromedial nucleus [4], brainstem nuclei such as the Edinger-Westphal nucleus, dorsal motor nucleus of the vagus (DMNV), raphe

pallidus (Rpa), nucleus of the solitary tract (NTS) [4], and locus coeruleus (LC), anterior and intermediate lobes of the pituitary [5], ventrolateral medulla (VLM), cerebellar Purkinje cells [6], spinal sympathetic and parasympathetic preganglionic neurons, and the pyramidal cell layer of the hippocampus [7]. Immunoreactivity for nesfatin-1 is most intense in the hypothalamus among central nervous system structures [6].

Dual-label immunofluorescence staining has revealed that central nesfatin-1 colocalizes with vasopressin, oxytocin (OXT), thyrotropin-releasing hormone (TRH), and corticotropin-releasing hormone (CRH) in the PVN and SON [4-5]; with tyrosine hydroxylase (TH) in the Arc and NTS; and with serotonin (5-HT) in the Rpa. Neuropeptide-Y (NPY) neurons are closely apposed to nesfatin-1 neurons in the Arc and LHA [4]. Nesfatin-1 also colocalizes with gonadotropin-releasing hormone (GnRH) in the anterior and posterior lateral tuberal nuclei of the goldfish hypothalamus [8].

In the periphery, NUCB2 is widely expressed in goldfish tissues including liver, adipose tissue, ovary, testis, muscle, rectum, foregut, and gills, with hepatic expression substantially higher than other tissues [2]. In mice, NUCB2/nesfatin-1 is broadly distributed in peripheral tissues including spleen, thymus, heart, testis, ovary, and uterus. Unlike in goldfish, nesfatin-1 expression is lowest in liver and muscle of mice, suggesting potential functional differences between rodents and aquatic animals. Dual-label immunofluorescence demonstrates that nesfatin-1 colocalizes with ghrelin in X/A-like endocrine cells of gastric oxyntic glands [9] and in endocrine cells of the goldfish foregut [10]. NUCB2 colocalizes with insulin in pancreatic β-cells [11] and with incretin hormones including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) in mouse intestinal epithelial cells [12].

1.3 Factors Affecting Expression

1.3.1 Nutritional Status Fasting significantly reduces the number of activated nesfatin-1 neurons in the rat hypothalamic PVN and SON, as well as plasma nesfatin-1 levels, while refeeding after fasting markedly increases activated nesfatin-1 neurons in these nuclei, indicating that nesfatin-1 is subject to negative feedback regulation by nutritional status [13]. Interestingly, both short-term and long-term fasting in goldfish decrease hypothalamic NUCB2 expression while simultaneously increasing hepatic NUCB2 expression, suggesting that NUCB2/nesfatin-1 may play a specialized role in energy metabolism regulation in the liver of aquatic animals during food deprivation [2].

Nesfatin-1 colocalizes with the mammalian target of rapamycin (mTOR) in Arc neurons centrally [14] and with the mTOR downstream target pS6K1 in gastric X/A-like endocrine cells peripherally [15]. As a cellular energy sensor [16], mTOR may regulate nesfatin-1 expression by sensing changes in nutritional status.

1.3.2 Physiological and Pathological States Plasma nesfatin-1 levels after fasting are significantly higher in older individuals compared to younger ones [17]. Patients with restrictive-type anorexia nervosa exhibit significantly lower plasma nesfatin-1 levels than healthy controls [18]. Individuals with type 2 diabetes have significantly lower plasma nesfatin-1 levels than both healthy subjects and type 1 diabetes patients [17]. The emetic toxin deoxynivalenol activates nesfatin-1 neurons in the porcine VLM, dorsal vagal complex, PVN, Arc, and SON [19]. In high-fat diet-induced obese mice, adipose tissue nesfatin-1 protein expression and circulating nesfatin-1 levels are significantly elevated [15].

1.3.3 Regulation by Other Feeding-Related Hormones The anorexigenic hormone cholecystokinin dose-dependently activates nesfatin-1 neurons in the rat PVN and NTS [13,20]. Elevated intracellular calcium concentration ([Ca2+]i) serves as a marker of neuronal activity [21]. High glucose (10 mmol/L) and insulin (10-13 mol/L) significantly increase [Ca2+]i in PVN nesfatin-1 neurons, thereby activating these neurons [22]. Intracerebroventricular injection of 50 pmol leptin significantly enhances PVN NUCB2 mRNA expression [23].

The serotonin 5-HT receptor agonist m-chlorophenylpiperazine, which suppresses appetite, significantly increases hypothalamic NUCB2 mRNA expression [24]. Conversely, intracerebroventricular injection of the orexigenic hormone ghrelin at 1 ng/g suppresses forebrain NUCB2 mRNA expression [10].

2. Biological Functions of Nesfatin-1

2.1.1 Central and Peripheral Effects on Food Intake

Short-term intracerebroventricular injection of nesfatin-1 dose-dependently reduces food intake in rats and mice [1,13,25]. Administration of nesfatin-1 antibody Ab24 immunoglobulin G (IgG) significantly increases food intake [1]; chronic infusion of nesfatin-1 into the third ventricle markedly decreases both food intake and body weight gain, whereas continuous icv injection of NUCB2 antisense morpholino oligonucleotides to suppress endogenous hypothalamic NUCB2 significantly increases food intake and body weight gain. Intraperitoneal (ip) injection of nesfatin-1 significantly reduces food intake in goldfish [2]. In mice, subcutaneous (sc) injection of both nesfatin-1 and M30 suppresses food intake, and consecutive ip injections of nesfatin-1 for 6 days persistently reduces body weight gain [3].

2.1.2 Mechanisms of Action

Regarding the transmission of peripheral nesfatin-1 signals, it is noteworthy that even when ip and sc doses in mice reach 23 times the central injection dose, they fail to suppress food intake [25], whereas in goldfish, the ip dose required to inhibit feeding is 100 times the icv dose [2]. These findings suggest that the anorexigenic action of nesfatin-1 is primarily centrally mediated [25].

How then does peripheral nesfatin-1 exert its appetite-suppressing effects? First, the blood-brain barrier (BBB) serves as a restrictive permeability barrier for nesfatin-1 transport between brain and blood. Studies by Pan et al. [26] and Price et al. [27] have shown that nesfatin-1 crosses the BBB via unsaturated facilitated diffusion to reach brain parenchyma [27], with predominant binding to the hypothalamus [26]. The rate of nesfatin-1 clearance from brain to blood is limited by cerebrospinal fluid reabsorption [27].

Second, peripheral nesfatin-1 induces Ca2+ influx through N-type calcium channels in afferent vagal neurons, exciting the vagus nerve and transmitting signals to the NTS via nicotinic cholinergic receptor neurons [3,28-29]. Catecholaminergic NTS neurons may then project to the PVN to activate nesfatin-1 neurons [20].

Nesfatin-1 influences the excitability of feeding-related neurons. Price et al. [30] first demonstrated that the majority of PVN neurons respond to nesfatin-1 stimulation with either hyperpolarization or depolarization. Price et al. [31] further found that up to 82% of NPY neurons in the Arc undergo hyperpolarization in response to nesfatin-1. How then does nesfatin-1 affect neuronal membrane potential?

On one hand, Price et al. [31] used the ATP-sensitive potassium channel (KATP) antagonist glibenclamide to reverse nesfatin-1-induced hyperpolarization of NPY neurons back to baseline levels, suggesting that nesfatin-1 may activate KATP channels to hyperpolarize NPY neurons in the Arc, thereby suppressing the orexigenic NPY system.

On the other hand, the G protein receptor inhibitor pertussis toxin, Ca2+-free solution, protein kinase A (PKA) inhibitor KT 5720, and L-type and P/Q-type Ca2+ channel blockers verapamil and ω-conotoxin MVIIC all suppress nesfatin-1-induced increases in hypothalamic neuronal [Ca2+]i. This suggests that hypothalamic nesfatin-1 may interact with G protein-coupled receptors to open L-type and P/Q-type Ca2+ channels or activate PKA signaling pathways, leading to Ca2+ influx and neuronal excitation [4].

Mediation by leptin-independent hormones: Intracerebroventricular injection of nesfatin-1 significantly reduces food intake in leptin receptor-deficient obese Zucker rats, and nesfatin-1 antibody Ab24 IgG does not affect leptin-induced reduction in food intake. However, pretreatment with the melanocortin MC3/4 receptor antagonist SHU9119 abolishes the anorexigenic effect of nesfatin-1, indicating that nesfatin-1 signaling may be mediated by the melanocortin system independently of leptin [1].

Nesfatin-1 in the PVN may cause Ca2+ influx through L-type Ca2+ channels to activate oxytocinergic neurons, which project to the NTS to mediate anorexigenic effects via oxytocin receptors [32]. The CRH1/CRH2 antagonist astressin-B or the CRH2 antagonist astressin2-B abolishes the anorexigenic effect of icv-injected nesfatin-1 [13]. Nesfatin-1 in the PVN can also directly act on CRH and TRH neurons, releasing CRH and TRH that bind to correspond-

ing receptors on histamine neurons to promote histamine secretion and suppress feeding [33].

Reduced gastric motility: Central injection of nesfatin-1 decreases gastric motility [13,34]. Intracerebroventricular administration of nesfatin-1 inhibits gastric emptying independently of CRH [13].

Analysis of potential mechanisms underlying nesfatin-1-induced inhibition of gastric emptying in the forebrain reveals that nesfatin-1 neurons project among the PVN, Arc, and basomedial amygdala (BMA) of the forebrain limbic system. Endogenous nesfatin-1 partially reduces gastric motility by influencing the firing activity of gastric distension-sensitive neurons via a melanocortin pathway [35-36].

Additionally, injection of nesfatin-1 into the fourth ventricle or cisterna magna also suppresses food intake independently of CRH [13]. Further studies have shown that hindbrain injection of nesfatin-1 may cause Ca2+ influx through T-type Ca2+ channels in DMNV neurons, exciting efferent vagal nerves and inhibiting gastric acid secretion [34].

In summary, nesfatin-1 exerts its appetite-suppressing effects primarily through six pathways (Fig. 2 [Figure 2: see original paper]): (1) Peripheral nesfatin-1 crosses the BBB into the hypothalamus via facilitated diffusion through the bloodstream; (2) Peripheral nesfatin-1 anorexigenic signals are transmitted to the hypothalamus via vagal afferent nerves through the NTS; (3) Hypothalamic nesfatin-1 directly acts on neurons to hyperpolarize NPY neurons in the Arc through KATP channels, thereby suppressing orexigenic NPY neurons; (4) Nesfatin-1 opens L-type and P/Q-type Ca2+ channels to cause Ca2+ influx and activate hypothalamic neurons, mediating its anorexigenic effects through OXT, CRH, TRH, histamine, and melanocortin systems; (5) In the forebrain, nesfatin-1 influences the excitability of gastric distension-sensitive neurons via a melanocortin pathway to reduce gastric motility; and (6) In the hindbrain, nesfatin-1 causes Ca2+ influx through T-type Ca2+ channels to activate DMNV neurons, exciting efferent vagal nerves and inhibiting gastric acid secretion.

2.2 Effects on Glucose Homeostasis and Insulin

In type 1 diabetic mice, intravenous injection of nesfatin-1 reduces blood glucose levels in an insulin-dependent manner [38]. Nesfatin-1 can stimulate secretion of the incretin hormones GLP-1 and GIP [12] and directly act on pancreatic β-endocrine cells to promote insulin secretion by causing Ca2+ influx through L-type calcium channels [39].

In insulin-resistant obese mice, central nesfatin-1 improves insulin sensitivity by activating the insulin receptor (INSR)/insulin receptor substrate-1 (IRS-1)/AMP-activated protein kinase (AMPK) pathway and the mTOR/protein kinase B (Akt) pathway. This suppresses expression of hepatic gluconeogenic rate-limiting enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxyki-

nase, reduces hepatic glucose production, and enhances muscle glucose uptake [40-41].

2.3 Involvement in Stress Response

Central nesfatin-1 expression responds to stress induction. Both acute restraint stress and water avoidance stress significantly increase activated nesfatin-1 neurons in the PVN, SON, LC, NTS, RPa, and VLM [7,42-43]. Central nesfatin-1 can induce activation of tryptophan hydroxylase (TPH, the rate-limiting enzyme in 5-HT synthesis), tyrosine hydroxylase (TH, the rate-limiting enzyme in catecholamine synthesis), and CRH neurons in the PVN, thereby increasing plasma adrenocorticotropic hormone and corticosterone levels to activate the hypothalamic-pituitary-adrenal (HPA) axis and mediate stress responses [42-43].

Peripheral administration of nesfatin-1 can mitigate stress-induced damage. Intraperitoneal injection of nesfatin-1 reduces caspase-3-mediated neuronal apoptosis, suppresses nuclear factor kappa B (NF- B)-dependent inflammatory responses, and alleviates subarachnoid hemorrhage-induced neural injury and oxidative brain damage [44]. During the acute phase of ischemia/reperfusion injury, peripheral nesfatin-1 injection decreases plasma endothelial nitric oxide synthase (eNOS) levels and oxidative stress indices [45].

2.4 Effects on Reproduction

The effects of nesfatin-1 on reproduction primarily manifest in the initiation of puberty. In rodents, nesfatin-1 can even maximize luteinizing hormone (LH) secretion under adverse metabolic conditions [46]. However, in goldfish, nesfatin-1 significantly reduces expression of cGnRH-II and sGnRH in the hypothalamus, LH-β and FSH-β in the pituitary, and decreases serum LH levels. It also markedly reduces both basal and maturation-inducing hormone-induced germinal vesicle breakdown rates in cultured zebrafish primary follicles, thereby decreasing oocyte maturation [8].

These findings indicate that, unlike in rodents, nesfatin-1 exerts inhibitory effects on reproduction in fish, though the underlying reasons require further investigation.

Conclusion

Nesfatin-1 is widely distributed throughout the body and primarily participates in energy metabolism regulation. Its biological functions involve appetite suppression, blood glucose reduction, increased insulin sensitivity, and stress responsiveness, while its effects on reproduction mainly involve participation in puberty onset. Its mechanisms of action include both neuronal excitation and inhibition. However, research on nesfatin-1 remains in its early stages. The precise mechanisms by which it selectively acts on cell membranes to open ion

channels for neuronal excitation or inhibition remain unclear, and the nesfatin-1 receptor has yet to be identified, which substantially limits mechanistic studies. The discovery of nesfatin-1 has further enriched the neurohormonal network regulating energy homeostasis. Elucidating its mechanisms of action could provide theoretical foundations for clinical treatment of obesity and diabetes, as well as for applications in animal production to regulate feeding, stress, and reproductive processes.

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Note: Figure translations are in progress. See original paper for figures.