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REVIEW

Multi-functional peptide hormone NUCB2/nesfatin-1

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Abstract The recently discovered nesfatin-1 is regulated by hunger and satiety. The precursor protein NUCB2 is proteolytically cleaved into three resulting fragments: nesfatin-1, nesfatin-2, and nesfatin-3. The middle segment of nesfatin-1 (M30) is responsible for limiting food intake, while the exact physiological role of nesfatin-2 and nesfatin-3 are not currently known yet. This hormone plays role/roles on diabetic hyperphagia, epilepsy, mood, stress, sleeping, anxiety, hyperpolarization, depolarization, and reproductive functions. This review will address nesfatin, focusing on its discovery and designation, biochemical structure, scientific evidence of its anorexigenic character, the results of the human and animal studies until the present day, its main biochemical and physiological effects, and its possible clinical applications.

Keywords NUCB2/nesfatin-1 · Nesfatin-1 · NUCB2 · Satiety

Abbreviations

aa	Amino acid
ACTH	Adrenocorticotropin
AGA	Appropriate gestational age
AgRP	Agouti-related peptides
ANS	Autonomic nervous system
ARC	Arcuate nucleus
as-MON	Antisense morpholino oligonucleotide
BMI	Body mass index
CART	Cocaine- and amphetamine-regulated transcript
CCK	Cholecystokinin
CF	Cystic fibrosis
CRF	Corticotropin-releasing factor
DMH	Dorsomedial hypothalamus
DON	Deoxynivalenol
ERK ½	Extracellular signal-regulating kinase 1/2
FM	Fat mass
GDM	Gestational diabetes mellitus
HOMA-IR	Homeostatic model assessment-insulin resistance
ICV	Intracerebroventricular
IHC	Immunohistochemistry
IRS	Insulin receptor substrate
LGA	Large gestational age
LHA	Lateral hypothalamic area
LPS	Lipopolysaccharides
MCH	Melanin-concentrating hormone
MetS	Metabolic syndrome
mNTS	Medial nucleus of the solitary tract
NAFLD	Non-alcoholic fatty liver disease
NEFA	Nuclear EF-hand acidic
NPR-A	Natriuretic peptide receptor A
NPY	Neuropeptide Y
NPY/AGRP	Neuropeptide Y/Agouti-Related Peptide
NST	Nucleus of the solitary tract
NUCB2/NEFA	Nucleobindin 2
OGTT	Oral glucose tolerance test
OT	Oxytocin
PC	Prohormone convertase
PCOS	Polycystic ovary syndrome
PCR	Polymerase chain reaction
pGC	Guanylate cyclase
PKA	Protein kinase A

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PKG	Protein kinase G
PND28	Postnatal day 28
PND7	Postnatal day 7
PND0	Postnatal day 0
POMC	Pro-opiomelanocortin
SON	Supraoptic nucleus
PSVT	Paroxysmal supraventricular tachycardia
PVN	Paraventricular nucleus
SAH	Subarachnoid hemorrhage
SGA	Small gestational age
SNP	Sodium nitroprusside
SNPs	Single-nucleotide polymorphisms
SNS	Sympathetic nervous system
VMN	Ventromedial hypothalamic nucleus
VIP	Vasoactive intestinal peptide
VMH	Ventral medial hypothalamus
ZI	Zona incerta
α-MSH	α-Melanocyte stimulating hormone

Introduction

The recently discovered nesfatin-1 is regulated by hunger and satiety [1], and its expressions are *ubiquitous* in many organs and tissues [1, reviewed 2]. Nesfatin-1 level in biological fluids shows a decrease [3–11] or increase [11–20] or no changes [21, 22] in some pathological conditions. Although it has many biochemical and physiological functions, the concerned hormone is mainly involved in the reduction of food intake and a related decrease in body weight [1, reviewed 2]. Based on polymorphism data, it was also reported that heritable defects of NUCB2 gene could be involved in the etiopathology of human obesity [23–25]. Therefore, this collection attempts to review and examine the past and present studies of NUCB2 and nesfatin-1.

Nesfatins

Recently, quite astonishing studies have been carried out to reveal the biological transport pathways and signals of the molecules involved in the maintenance of food intake and energy balance. In this context, Dr. Mori and his team announced to the world in 2006 that there was another hormone [1], in addition to leptin which was discovered in 1994 [26], which gave a sense of satiety and that they designated this hormone influencing the lipids [1, reviewed 2]. Nesfatin-1 is one of the three products, which has an anorexigenic activity. Nesfatin-1, which is composed of 82 amino acids, has a molecular weight of 9.8 kDa [1, reviewed 27], and the half-life of nucleobindin2 (NUCB2) messenger ribonucleic acid (mRNA) was approximately 6 h [28]. Other nesfatins originating from the NUCB2/nuclear EF-hand acidic (NEFA) [nesfatin-2 and nesfatin-3] do not have any anorexigenic activity [27]. Therefore, almost all studies conducted at present have focused on nesfatin-1.

NUCB2/nesfatin-1 is composed of a 24-aa signal peptide, and 396 amino acid (aa). Then, NUCB2/NEFA is post-translationally divided into three segments by the prohormone convertase (PC), which are the *N*-terminal segment NUCB2/nesfatin-1 (1-86), two C-terminal peptide nesfatin-2 (85-163), and nesfatin-3 (166-396) [1, reviewed 27]. This shows that NUCB2/nesfatin-1 is a precursor producing several active peptides through differential proteolytic processing (Fig. 1).

The parts that are indicated in shades are considered the segments where the peptide is severed for the formation of nesfatin-1, 2, and 3. The structure of nesfatin-1 is also tripartite, the segment starting from the *N*-terminal end and going up to 23 amino acids is called N23, the middle segment covering the amino acids from 23 to 53 is called M30, and the segment from the 53rd to 82nd amino acids toward the carboxy terminus is called C29 [reviewed, 29] (Fig. 1). These three segments are demonstrated in Fig. 1 in a representational manner. The dose-dependent inhibition of food intake by nesfatin-1 is attributed to the mid-segment, whose aa sequencing is similar to that of alpha-MSH and Agouti-related peptides (AgRP) [1, reviewed 29–31].

Discovery and designation

Classically, if a tissue or gland has endocrinological functions, it is expected to conform to the following rules [32]: (a) When this tissue is removed from the body, the absence of its incretion should create a hormone-specific problem. (b) When the removed tissue is implanted elsewhere in the body, the observed problem should disappear. However, the implantation of tissues that function in relation to the nervous system may not produce the expected result as its connection to the nerve has been severed (hypophysis gland, for instance). (c) When the hormone is injected into the body, the problems that are observed in the tissue should be eliminated. However, molecular techniques and genomic data which clinical chemistry and biochemistry are currently trying to integrate rapidly enable the detection of a lot of hormones. In this context, Oh-i et al. [1] stimulated the PPAR-y ligand in SQ-5 cells derived from lung carcinoma expressing leptin and leptin receptors using troglitazone. Thus, they showed that the part of DNA whose expression has increased due to troglitazone stimulation corresponds to the gene coding NUCB2/NEFA. After this finding was obtained, it was observed that NUCB2 was expressed in key hypothalamic nuclei involved in body weight control and that the

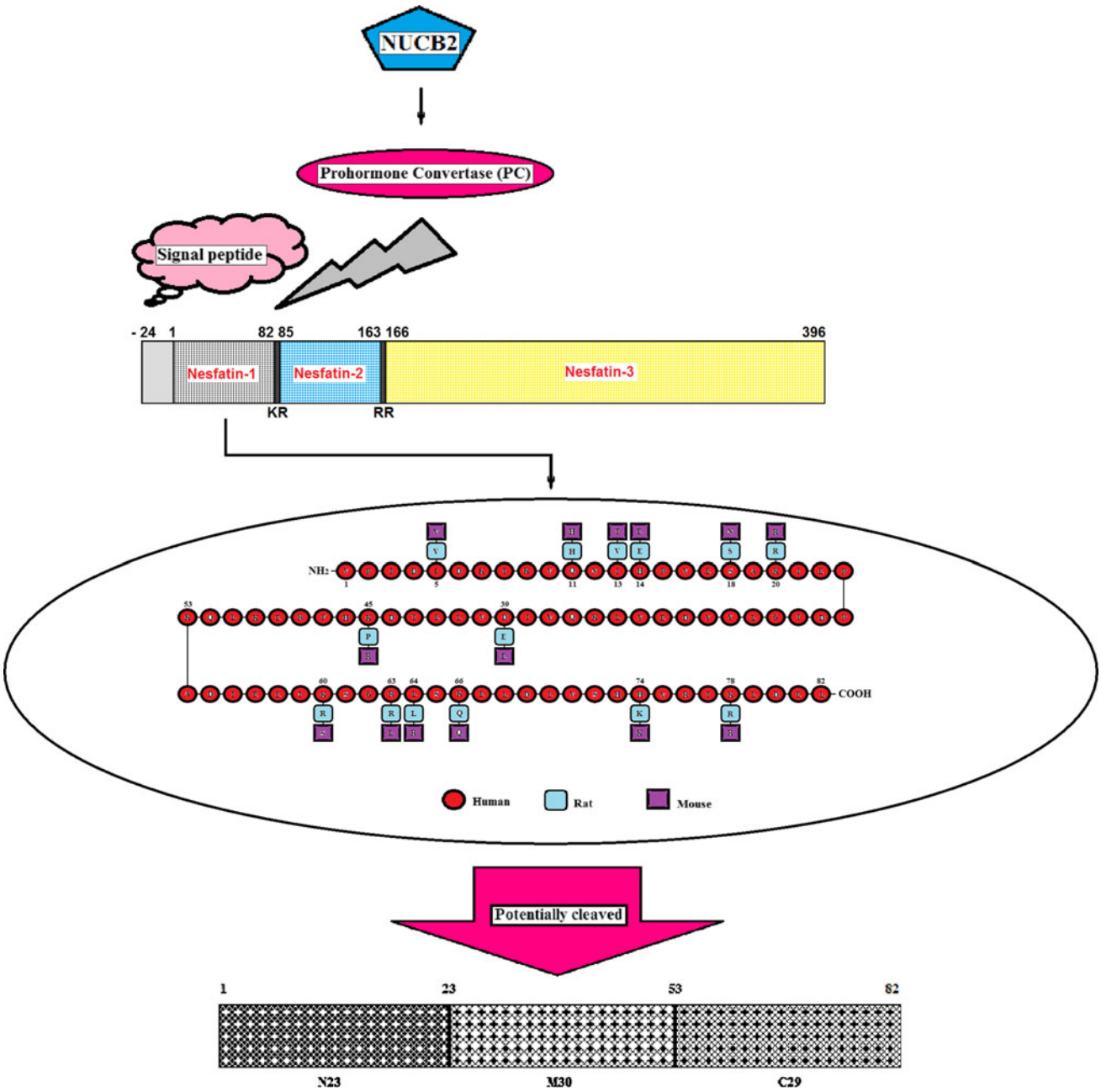

Fig. 1 Derivation of nesfatin-1 from NUCB2 and its division into three different segments and the amino acid sequencing of NUCB2/nesfatin-1 in rat, mice, and human species. Sources [27, 29, 93, 94] were used when drawing this figure. The rat amino acid sequence of NUCB2 shows 87.4 % homology with humans and 95 % homology to mice

intracerebroventricular (ICV) injection of the recombinant NUCB2 protein inhibited food intake, while the ICV injection of nesfatin-1 Ab24-derived IgG increased food intake [1, 33]. Additionally, based on the fact that the concerned peptide affected the feeling of satiety and affected lipid amounts by causing a decrease in subcutaneous and mesenteric fat after 24 h of fasting, this peptide coded by NUCB2/NEFA was called NUCB2/nesfatin-1 [1]. The feeling of satiety caused by ICV administration of nesfatin-1 is replaced by a feeling of hunger upon administration of astressin$_2$-B which is a CRF2 receptor antagonist [34].

Evidence that it is anorexigenic

Immediately after Oh-i et al. [1] presented the evidence that nesfatin-1 is a new satiety molecule, research laboratories studying orexigenic (appetite enhancing) and anorexigenic (causing loss of appetite) molecules focused on this topic.

For instance, reduction of the hypothalamic NUCB2/nesfatin-1 content by the central infusion of an antisense morpholino oligonucleotide (as-MON) brought about an increase in food intake and body weight, while ICV injection of nesfatin-1 inhibited food intake in a dose-dependent manner in adult rats [1, 35]. Also, Gantulga et al. exhibited that paraventricular nucleus (PVN) nesfatin-1 neurons are activated by meal-evoked metabolic factors and just suggest a role in satiety. Thus, long-term nesfatin-1 administration brings about weight loss [36]. Nesfatin-2 and nesfatin-3, on the other hand, did not elicit any anorectic response [1, reviewed 2, 29].

Another critical point is that although nesfatin-1 is shown to act independent of the leptin signal, it was identified as a satiety molecule that is associated with melanocortin signaling in the hypothalamus [37, reviewed 38]. That is because nesfatin-1 was capable of inhibiting food intake in rodents which had the inactivated mutations of the leptin receptor and in cases of leptin resistance like diet-induced obesity [37, 38, reviewed 29, 39]. Another point that supports the idea that the action of nesfatin-1 is independent from leptin is the fact that nesfatin-1 is not involved in the activation of pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons nesfatin-1 in the arcuate nucleus (ARC), which is among the primary mechanisms by which leptin inhibits food intake [37, reviewed 29, 39]. However, the anorectic hormone cholecystokinin (CCK) was shown to activate NUCB2/nesfatin-1 neurons in PVN and nucleus of the solitary tract (NST), which suggests that the concerned neuron populations mediate the satiety-inducing effects of this peptide [40, reviewed 41].

In addition to all these functional tests, neuroanatomic data also demonstrate that nesfatin-1 is involved in the central pathways controlling food intake. Thus, preliminary immunohistochemistry (IHC) analyses showed the presence of the NUCB2/nesfatin-1 protein in some hypothalamic nuclei like the ARC, PVN, supraoptic nucleus (SON), lateral hypothalamic area (LHA), and zona incerta (ZI), as well as the brain stem areas like the NTS which plays critical roles in the regulation of eating [42]. mRNA and/or immunoreactivity of NUCB2/nesfatin-1 was also reported in hypothalamic and extra-hypothalamic areas [42, 43].

Co-expression analyses of NUCB2/nesfatin-1 in certain hypothalamic nuclei have helped the identification of the possible neuroendocrine mechanisms and anorectic effects of nesfatin-1. For instance, NUCB2/nesfatin-1 was found to be markedly co-localized with oxytocin (OT), and vasopressin, though to a lesser extent, in the PVN and SON in rats, and immunoneutralization of endogenous nesfatin-1 reduced OT release in PVN [30, 43]. The ultimate effector for the nesfatin-OT circuit might be the POMC neuron population localized in the NTS of the brain stem, as (i) NUCB2/nesfatin-1 and OT were shown to specifically activate POMC neurons in the NTS and (ii) an antagonist of melanocortin-3/4 receptor inhibited the anorectic effects of nesfatin-1 [1]. Additionally, the blockage of corticotropin-releasing factor receptor 2 (CRF2) was shown to counteract the food intake-inhibiting effects of ICV nesfatin-1 injections [34, 44]. Besides, NUCB2/nesfatin-1 was found to be highly co-expressed with the orexigenic peptide melanin-concentrating hormone (MCH) in the neurons occupying the lateral part of the tuberal hypothalamic region in rats [45, 46]. Electrophysiological studies revealed that a possible mechanism by which nesfatin-1 exercised its anorectic effects might be its inhibition of neuropeptide Y (NPY) neurons in ARC [42, reviewed 29, 38, 39].

Nesfatin-1 can pass through the blood–brain barrier without reaching saturation [47]. Therefore, the endogenously produced nesfatin-1 can easily reach the brain, where it inhibits food intake. It is speculated that nesfatin-1 exercises these inhibitory effects by modulating the excitability of glucose-sensitive neurons, such as those in PVN, LHA, and ventromedial hypothalamic nucleus (VMN) [48]. It is believed that X/A cells (ghrelin cells) of the stomach act together in line with the Yin and Yang principle to control hunger and satiety [49]. These data suggest that nesfatin-1 controls energy homeostasis and consequently gives a sense of satiety. Moreover, the relation between nesfatin-1 and energetic homeostasis can also be illustrated by the activation of nesfatin-1 during glucoprivation induced by insulin or glucose antimetabolites 2-deoxyglucose (2-DG) or 5-thio-glucose administration in rats [50].

An overall evaluation of these experimental data proves that nesfatin-1 is an appetite-inhibiting molecule; this claim was confirmed by experiments which included rats in which the central injection of nesfatin-1 reduced food intake in the dark phase and mice in which recombinant human, rat, and mouse nesfatin-1 proteins acutely inhibited food intake [1].

How nesfatin-1 inhibits appetite and the possible relations that are obtained between the mediating molecules and organs in the process are presented in Fig. 2.

As partially described in the Fig. 2, nesfatin-1 is believed to exercise its appetite-inhibiting effect in the following three ways [51–53]: (1) NUCB2/nesfatin-1 synthesized from the tissues is transported to ARC and other parts of the brain in the blood circulation after passing through the blood–brain barrier by active transport and affects appetite. (2) Peripherally synthesized nesfatin-1 stimulates vagal afferent nerve endings, which in turn stimulate the hypothalamus through nucleus solitarius which has a vagal connection. (3) Since NUCB2/nesfatin-1

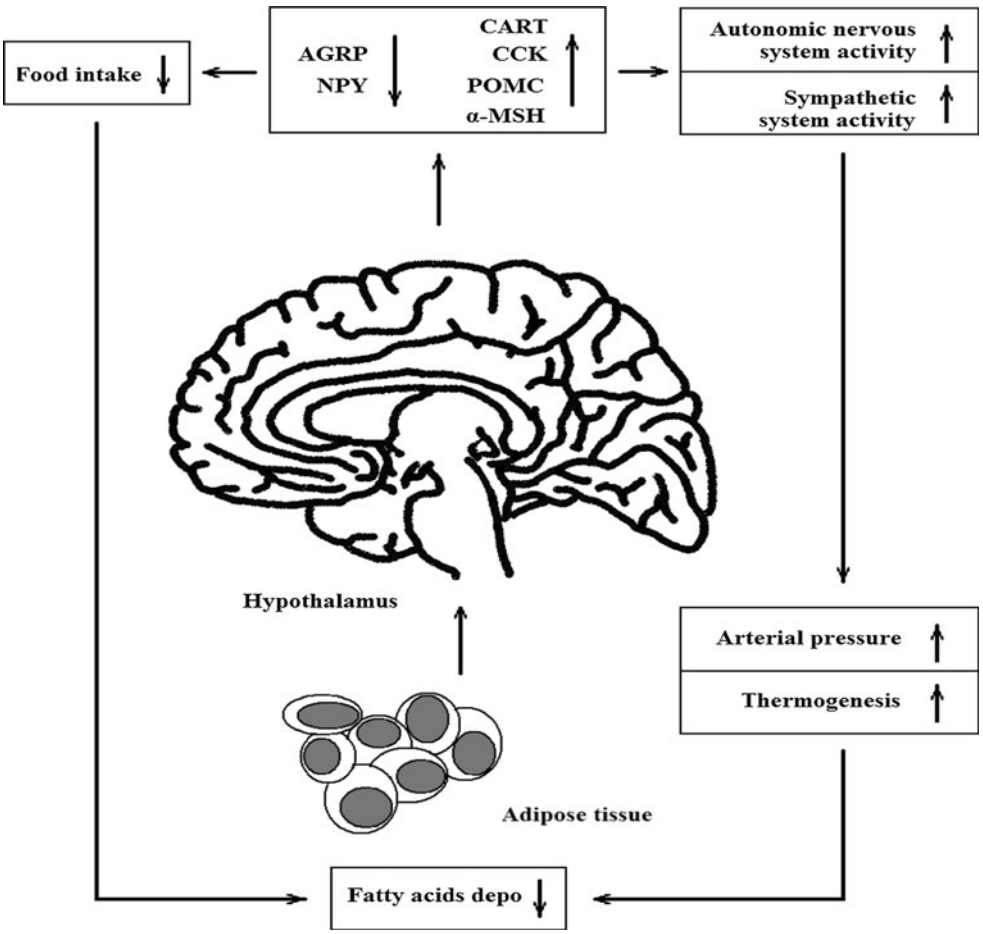

Fig. 2 Summary of some major effects (including food restriction) of NUCB2/nesfatin-1. Adiposity signals influence central circuits in the hypothalamus. Cocaine- and amphetamine-regulated transcript (*CART*), α-melanocyte stimulating hormone (*α-MSH*), cholecystokinin (*CCK*), and pro-opiomelanocortin (*POMC*) promote negative energy balance. Agouti-related peptide (*AgRP*) and neuropeptide Y (*NPY*) neurons promote positive energy balance. Thus, nesfatin-1 may inhibit appetite by directly modulating energy balance and causes decrease in depository of fatty acids, and may also have a crosstalk with autonomic nervous system (ANS) and sympathetic nervous system (SNS)

is locally synthesized in the hypothalamus, it may inhibit appetite by directly inhibiting the Neuropeptide Y/Agouti-Related Peptide (NPY/AGRP) and other cells in ARC.

Animal studies

The presence of NUCB2/nesfatin-1 was first detected in brain tissues of rats [1]. Also, NUCB2/nesfatin-1 has recently been found in the hypothalamic nuclei and brainstem of pigs [54] and mice [55]. It has been shown that nesfatin-1 is expressed in almost every tissue that has been examined (Fig. 3). However, It was should noted that even though the manufacturers of nesfatin-1 antibody have stated that their antibody reacts with nesfatin-1 specifically, those kits have not been well accepted among experts. This is largely because the reagents may cross-react with the nesfatin-1 antibody and also nesfatin-1antibody used cross-reacts with NUCB2. For ease of reading, it was decided in this part of the review to classify methods that show the presence of nesfatin-1 in animal and human tissues and to present all the data including the details within this classification (Fig. 3).

Expression analyses in the purified small endocrine cells of the gastric mucosa demonstrated that the NUCB2 mRNA levels at this site were higher than those in the brain [56]. NUCB2/nesfatin-1 expression in the middle segment of the gastric mucosa of rats was shown to have a common localization with the orexigenic hormone ghrelin in X/A-like cells (PD1 cells in humans) [56]. Likewise, a small number of cells on the floor of the gastric glands in rats were shown to co-express NUCB2/nesfatin-1 and somatostatin [56]. Somatostatin CART protein product 9.5 and vasoactive intestinal peptide (VIP) are co-localized with NUCB2/nesfatin-1 in the digestive systems of dogs [57]. The function of NUCB2/nesfatin-1 found in the LI cells of the dog digestive system is not known yet. Similar to its co-localization with ghrelin in the stomach, NUCB2/nesfatin-1 is also co-localized with several other hormones in some other tissues [49, 58–61]. For instance, it is jointly localized with oxytocin and vasopressin in the PVN and SON neurons. The neurons transporting NUCB2/nesfatin-1 in PVN also contain 24 % oxytocin, 18 % vasopressin, 13 % corticotropin-releasing hormone (CRH), and 12 % thyrotropin-releasing hormone (TRH) [43, reviewed 29, 38, 41].

Insulin-expressing beta cells of rats and mice also express NUCB2/nesfatin-1. Also, alpha cell islets of rats and mice synthesize NUCB2/nesfatin-1 in addition to glucagons [62, 63]. In the ARC area, CART and tyrosine hydroxylase are

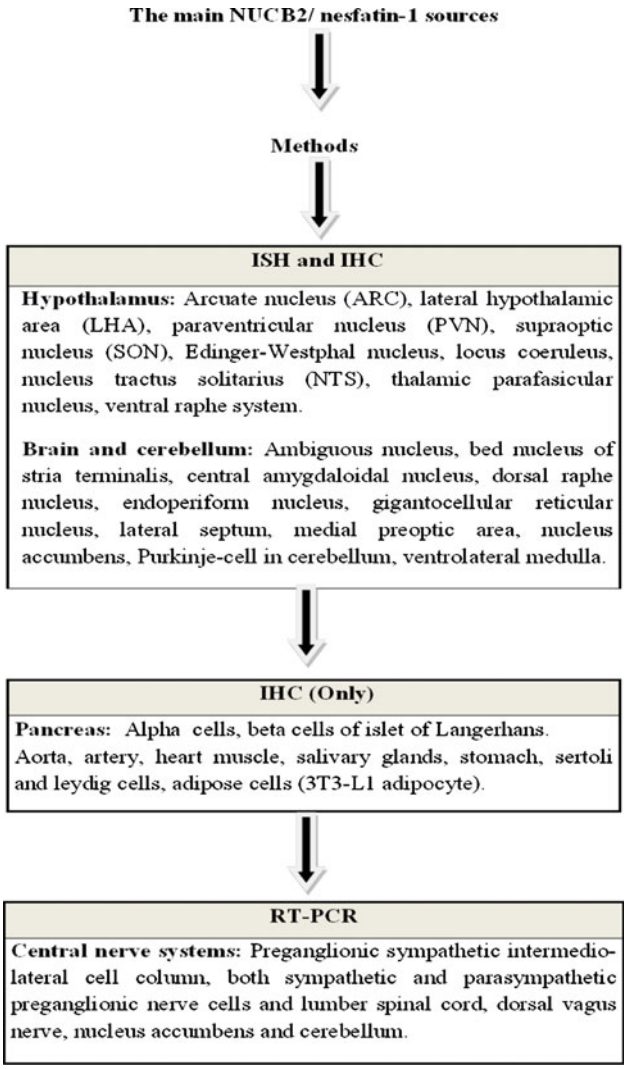

Fig. 3 Main source of NUCB2/nesfatin-1 immunoreactivity in organs and tissues. *ISH* in situ *hybridization*, *RT-PCR* real time-polymerase chain reaction. It is drawn with references to sources [1, 37, 54–56, 58, 95 and reviewed 38, 41, 42]

synthesized with nesfatin, which is co-localized with MCH in the LHA area [45, 64]. The site which is 35 % NUCB2/nesfatin-1-positive in the SON area also contains oxytocin and 28 % vasopressin [43]. Since intra-nasal routes provide easy access to the brain, mice were administered nasal NUCB2/nesfatin-1 in one study, which reported inhibited food intake for 6 h following the administration. This result suggests that if nesfatin-1 is to be used in the treatment of obesity, nasal pathway may be more appropriate for its administration [reviewed 29]. Additionally, subcutaneous administration of nesfatin-1 also inhibits food intake and has a longer lasting effect than intraperitoneal administration [33]. It was also shown in a study that deoxynivalenol (DON) stimulates the main structures involved in food intake in pigs [54] and mice [55], and suggest that catecholaminergic and NUCB2/nesfatin-1 neurons may be contributing in the anorexigenic effects of the mycotoxin. All these experimental data indicate that rather than being just a simple anorectic signal in the hypothalamus, nesfatin-1 functions as an indispensable regulator of energy homeostasis in various tissues and the closely related neuroendocrine functions [54]. Besides, the effects of nesfatin-1 on food intake were investigated by administering 25 or 100 pmol nesfatin-1 to mice and a marked decrease was established in the body weight of the animals at the end of experiment [65].

Nesfatin-1 inhibits nocturnal feeding behaviors and gastrointestinal motility in mice [66]. It also suppresses the gastric acid release in a dose-dependent manner [67]. The central injection of nesfatin-1 reduces gastric emptying in rats [34]. The evidence that nesfatin-1 causes the activation of efferent vagal neurons comes from the finding of a 16-fold increase in the c-Fos-positive neurons of the dorsal motor nucleus of the vagus nerve in nesfatin-administered rats, in comparison to the control rats [67]. The complicated balance between energy intake (food consumption) and consumption maintains the body weight. This energy balance is controlled by the hypothalamus in the brain [68]. Among the sites where NUCB2/nesfatin-1 is expressed are hypothalamic regions responsible for eating behaviors [42].

Lesions in the LHA were reported to cause hypophagia [69], and weight loss, while lesions in the VMH were reported to lead to hyperphagic obesity. Therefore, they assumed that the eating center is in the LHA and the satiety center is in the VMH [70]. Whereas the obesity induced by the lesions of this structure appears to be a consequence of this deregulation more than a role of the VMH in direct regulation of satiety [71], since the VMH plays an inhibitory role in glucagon secretion [72]. Expression of the satiety hormone NUCB2/nesfatin-1 was also found to be unchanged in the pancreas, liver, skeletal muscle, and brown adipose tissue of the rats with VMH lesions [73]. It was reported that 5-day administration of carbachol did not affect nesfatin expression. VMH lesions modulate the white adipose tissue by increasing or balancing the NUCB2/nesfatin-1 expression of the autonomous nervous system [73].

Besides being high on day 21 of the embryonic period, on day 1 of the postnatal life, and postnatal days 13, 20, and 27 of the neonatal rats, serum nesfatin-1 continues to increase with growth, which shows that the nesfatin synthesis by the gastrointestinal system and pancreas increases in relation to organ development [74]. NUCB2/nesfatin-1 is co-localized with the insulin released from the beta cells of the pancreas in all developmental stages. The percentage of co-localization varies with age. This shows that NUCB2/nesfatin-1 is age- and tissue-specific, and is critical in the growth part of the rat development physiology [74].

Nesfatin-1 molecule produces cytophysiological effects resulting from the inhibitor hyperpolarization of G protein-coupled receptor and its NPY/AgRP neurons in the ARC

and melanocortin signal in the paraventricular nuclei [75]. Ozsavci et. al. [76] explored the effects of intraperitoneal nesfatin administration on the oxidative stress profile and its passage through the blood–brain barrier in rats with subarachnoid hemorrhage (SAH). The authors who reported that nesfatin administration reduced tumor necrosis factor-alpha (TNF-alpha), 1beta, interleukin-6 (IL-6), and protein caspase 3 levels concluded that nesfatin-1 could serve a neuroprotective function by playing an anti-apoptotic and anti-inflammatory role in the central nervous system [76]. Also, lipopolysaccharides (LPS) injection or toxin administrations resulted in increase in the number of c-Fos+/nesfatin-1 + neurons in the PVN, SON, and NTS, and to a lesser extent in the ARC. Thus, it was argued that centrally released nesfatin-1 may contribute to the neural mechanisms leading to endotoxemic anorexia [60].

Nesfatin-1 amounts in the serum of the rats with metabolic syndrome (MetS) were observed to decrease significantly in comparison to those in the control group (with the exception of nesfatin-1 amount of MetS female rats). Amounts of nesfatin-1 in the reproductive organ tissues of the animals with MetS were found to have increased in comparison to those in the control group [77].

Apart from its anorexigenic effect, nesfatin-1 also impacts water intake. Rats that were injected with nesfatin-1 consumed less water. Therefore, nesfatin-1 may play a role in fluid and electrolyte homeostasis [78].

Human studies

Human nesfatin-1 studies started with Aydin and his co-workers [13]. They first analyzed nesfatin-1 amounts using Enzyme-linked immunosorbent assay (ELISA) in the saliva and serum of epilepsy patients, which were reduced with the use of anti-epileptic medication, but were still higher than the amounts in normal subjects [13]. In addition, the same authors then examined time-dependent nesfatin-1 change after epilepsy attacks in another study; finding that nesfatin elevated after the first attack, but fell in a time-dependent manner [14]. After first human study, Li and his co-workers also reported that nesfatin-1 was decreased in human subjects with type-2 diabetes and assumed that the observed decrement might be associated with diabetic hyperphagia [12]. Besides the above-mentioned two pioneering clinical reports, some other human subject studies will be also given in detail here. Serum/plasma nesfatin-1 concentration was measured using ELISA throughout all these studies below unless stated otherwise.

Nesfatin-1 concentrations in the blood and milk of mothers with gestational diabetes mellitus (GDM) were found to be decreased [7]. This peptide, whose presence was identified in babies, was reported to contribute to the growth, energy regulation, and maturation of the gastrointestinal system in infants [7]. Nesfatin-1 alterations in response to 75 g oral glucose tolerance test (OGTT) in 43 healthy males whose mean age was 24.5 ± 0.6 and mean body mass index (BMI) was 21.1 ± 0.3 kg/m^2 were studied and a marked negative correlation was established between nesfatin-1 concentrations on one hand, and BMI, body fat percentage, body fat weight, and blood glucose on the other [3]. It was also shown in a study that the maternal blood nesfatin-1 values of the GDM patients (5.5 ng/mL) and the blood nesfatin-1 values of the control group (8.1 ng/mL), and the comparison between cord blood nesfatin-1 values of the women with GDM (5.4 ng/mL) and those of the control group (6.2 ng/mL), both exhibited a decrease in nesfatin-1 values in GDM [8]. Nesfatin-1 levels in the sera of recently diagnosed type-2 diabetes patients and individual with impaired glucose tolerance were also found to be elevated, and thus it was argued that observed alterations to the concentration of nesfatin-1 in subjects with diabetes could suggest that these peptides have significant roles in the control of glucose metabolism [79]. Su et al. [80] previously reported that nesfatin-1 has anti-hyperglycemic properties but these are likely to be insulin-dependent.

Nesfatin-1 levels in serum were significantly lower in patients with polycystic ovary syndrome (PCOS) (0.88 ± 0.36 ng/mL) than controls (2.22 ± 1.14 ng/mL). It was argued that the decrement in nesfatin-1 concentrations might be associated with PCOS. Additionally, there was a negative correlation between nesfatin-1 levels and the BMI, glucose, and homeostatic model assessment insulin resistance (HOMA-IR) index of the PCOS patients [6].

It was also reported that nesfatin-1 levels were found to be significantly lower in women with restricting-type-thyrotropin-releasing anorexia nervosa (6.23 ± 0.70 ng/mL) compared with healthy subjects (8.91 ± 0.85 ng/mL). The restricting-type anorexia nervosa may experience long-term hunger and lack of food intake, which would result in lower nesfatin-1 levels [9].

Serum nesfatin-1 concentrations were also recorded to decrease significantly in non-alcoholic fatty liver disease (NAFLD) (0.26 ng/mL) compared with the control levels (0.38 ng/mL). Therefore, it was claimed that the decreased nesfatin-1 concentrations would be involved in the etiopathology of the disease [4].

Serum nesfatin-1 concentrations were dropped in lung cancer patients in comparison to the healthy subjects. Fat mass loss may drop serum nesfatin-1 levels in lung cancer patients with weight loss [10]. Nesfatin-1 levels were also significantly higher in a paroxysmal supraventricular tachycardia (PSVT) group than the control group, and positively correlated with heart rate [16]. Nesfatin-1 concentrations also dropped in large gestational age (LGA)

compared with appropriate gestational age (AGA) fetuses. Fetal nesfatin-1 concentrations were higher in cases of GDM, and cord blood nesfatin-1 concentrations were lower in cases of vaginal delivery [11]. Individual group differences also exhibited that nesfatin-1 concentrations were higher in small gestational age (SGA) than AGA infants. Nesfatin-1 concentrations in SGA infants were higher on postnatal day 0 (PND0) than in AGA infants, and lower on postnatal day 7 (PND7) and postnatal day 28 (PND28) [15, reviewed 2].

In another study, plasma nesfatin-1 concentrations remained unchanged after acute strenuous interval exercise and circuit exercise performed by male boxers whose mean age was 20.71 ± 2.6 years, mean height was 176.6 ± 2.8 cm, mean weight was 67.2 ± 3.3 kg, and mean BMI was 21.56 ± 1.42 kg/m^2 [21]. As known, nesfatin-1 has anti-hyperglycemic properties [80]. So, strenuous interval exercise seems to be partially lowering blood glucose, this may explain why their levels remained unchanged [21].

Although its etiology is not known for sure, major depressive disorder has been associated with hormonal changes and psychological factors. It was shown in a study that concentrations of serum nesfatin-1 were significantly higher in major depressive disorder patients (4.22 ± 2.16 ng/mL) than healthy subjects (2.13 ± 1.52 ng/mL) [17]. Also, a decrease was reported in the plasma nesfatin-1 values of the patients with generalized anxiety disorder which were 0.35 ng/mL, in comparison to the values in the control group, which were 0.63 ng/L [5]. The amounts of nesfatin-1 in the brains of individuals who committed suicide varied according to sex [81, reviewed 2]. Thus, it was argued that alterations to the concentration of nesfatin-1 were closely associated with psychological disorders.

Serum nesfatin-1 levels relative to BMI were 5.2 ± 0.9 (<18.5 kg/m^2), 5.6 ± 0.9 (18.5–24.9 kg/m^2), 5.8 ± 1.7 (25–29.9 kg/m^2), 4.2 ± 2.1 (30–39.9 kg/m^2), and 4.4 ± 0.9 ng/mL (>40 kg/m^2) [18]. Nesfatin-1 levels increased from low-weight individuals to those who were overweight, but then decreased from obese individuals to those who were morbid obese [18]. Ramanjaneya et al. [82] found that nesfatin-1 was a depot-specific adipokine produced preferably by the adipose tissue in obese individuals and individuals with arranged dietary deprivation.

It was also reported that nesfatin-1 concentrations were higher in the hyperemesis gravidarum group than in the healthy gravida group. Nesfatin-1 synthesis may have increased as a compensatory mechanism that aims to restore the stomach homeostasis which has been impaired due to pregnancy vomiting [19]. It was also recently reported that advanced cystic fibrosis (CF) and low fat mass (FM), nesfatin-1 plasma levels are significantly increased and inversely correlated with the FM. They assumed that nesfatin-1 exerts its effects independently of TNF-α or IL-6 [20].

The polymorphisms of NUCB2 were first time investigated in a Caucasian population of 1,040 obese and 315 control individuals, and study revealed that there might be an association between three single-nucleotide polymorphisms (SNPs) (rs1330, rs214101, and rs757081), polymorphisms, and (male) obesity, as a correlation was established between the reported SNPs polymorphisms and BMI, weight and fat free mass [23]. The entire coding region of the NUCB2 gene was also screened for mutations in a population of 471 obese children and adolescents. Mutation analysis of NUCB2 exhibited total of seven sequence variants of which four were previously reported as polymorphisms. The remaining three variants included ex9+6G>C, L125H, and K178X and were present only in three unrelated obese individuals (0.6 %) [24]. The NUCB2 gene using polymerase chain reaction (PCR) and direct sequencing in 29 obese children and 24 non-obese children was also screened; five variants were found, including c.1012C>G (Q338E) [25]. Genotyping for c.1012C>G, they also reported that the GG genotype was significantly less frequent in the obese group; the odds ratio for obese subjects carrying CC and CG genotypes was 2.29 (95 % CI 1.17–4.49) in the dominant model, the CC genotype 2.86 (95 % CI 1.41–5.81) in the additive model, and the C allele 1.57 (95 %CI 1.17–2.1) [25]. Based on polymorphism data, it was assumed that heritable defects of NUCB2 gene could be involved in the etiopathology of human obesity.

Major biochemical and physiologic effects

Nesfatin-1, a hypothalamic neuropeptide that regulates appetite, plays key roles in energy homeostasis and metabolism. In fact, the original study that identified nesfatin revealed that PPAR-y ligands increased NUCB2 gene expression in the pre-adipocyte cell line 3T3-L1 [1]. Nesfatin-1 was reported to be able to pass through the blood–brain barrier and to produce anorectic responses after its administration to rodents at pharmacological doses [47]. In another study, the stimulation of the VNS increased the serum nesfatin-1 level, which resulted in a decrease in food intake [53]. This observation is supported by the increased neuronal c-fos expression in the NTS region, which is involved in food intake in response to low-frequency electric impulses [53].

The above data indicate that nesfatin-1 is involved in the central regulation of eating and energy consumption [1]. Previous studies demonstrated that four regions in the hypothalamus, VMH, dorsomedial hypothalamus (DMH), ARC, and LHA, are responsible for the regulation of eating and energy balance [69, 70]. MCH and nesfatin-1 are expressed in LHA and the neighboring areas [reviewed 2, 29, 38, 41]. Nesfatin-1 has attracted significant attention as

a hormone, as it inhibits appetite after its ICV injection. IHC studies show that NUCB2/nesfatin-1 neurons build connections between the hypothalamus and other brain areas [reviewed 2, 29, 38, 41]. This finding indicates that nesfatin-1 plays integrative roles underlying the eating behaviors and that the integrated information is sent to various brain areas from the hypothalamus [2].

Effects on temperature

Nesfatin-1 administered either centrally or peripherally causes a dose-dependent increase in temperature, which varies according to the manner of administration [65]. NUCB2/nesfatin-1 activated by cold (false positive) enables the nuclei in various parts of the brain to adapt to cold. Being co-localized with the prepro-thyrotropin-releasing hormone, NUCB2/nesfatin-1 brings about cold-related changes in the neurons that respond to cold. In other words, NUCB2/nesfatin-1 neurons are susceptible to cold and play critical roles in thermoregulation. Although the exact reason underlying this temperature change is not known, it is assumed to be related to the role of NUCB2/nesfatin-1 in energy consumption and preservation [65].

Effects on reproductive axis

The involvement of nesfatin-1 in the onset of puberty is, in particular, demonstrated by the use of continuous intracerebroventricular infusion of antisense morpholino oligonucleotides against NUCB2. The fact that the expression of NUCB2/nesfatin-1 mRNA is increased during puberty is not sufficient to demonstrate the role of nesfatin in puberty onset [35]. It is known that central pathways and peripheral signals that are responsible for the homeostasis of body weight are guided by the metabolic condition of the organism. The beginning of puberty and fertility are sensitive to the size of fuel (energy) reserves of the body, which are regulated by the key factors that control appetite [35]. Consequently, given that nesfatin-1 is an appetite-inhibiting hormone [1], it should inevitably have an impact on the reproductive axis. NUCB2/nesfatin-1 expression increased in the ovaries and testes of mice in which metabolic syndrome was induced, and this increase was reported to exercise a negative effect on reproduction [reviewed 2, 29, 38]. Furthermore, neuroanatomic studies showed that the hypothalamic expression patterns of NUCB2 mRNA and NUCB2/nesfatin-1 proteins in female rats in puberty were overlapping to a great extent with the patterns reported previously in adult rodents [35]. Although expressions in PVN, LHA, and SON were marked in both groups, the signals were weaker in ARC. It was also observed that the hypothalamic NUCB2/nesfatin-1 expression increased during transition to puberty [that is between day 20 (late infantile) and day 35 (peri-pubertal)], and mRNA levels in the LHA, PVN, and SON displayed a significant increase (as identified by in situ hybridization) together with a three-fold increase in total protein (as identified by Western blot in whole hypothalamic preparations) [35]. Interestingly, the extent of gonadotropin responses to NUCB2/nesfatin-1 increased remarkably under conditions of short-term hunger. As for rats, GnRH, LH-beta, and FSH are all stimulated by nesfatin-1 [35]. NUCB2/nesfatin-1 is involved in the regulation of the HPO axis in gold fish. It is co-localized with the gonadotropin-releasing hormone, which indicates that NUCB2/nesfatin-1 has an effect on gonadotropin release [83]. Intraperitoneal single-dose administration of synthetic goldfish nesfatin-1 (50 ng/g body wt) resulted in a 75 % acute decrease in hypothalamic cGnRH-II and GnRH mRNA. Moreover, an 80 % decrease was recorded in the pituitary LH-beta and FSH-beta mRNA 60 min after the injection [83]. NUCB2/nesfatin-1 is expressed in the follicle cells, but not in the ovaries of zebra and gold fish [83]. When all these data are considered, it is understood that NUCB2/nesfatin-1 has control over the reproductive axis.

Effects on neurons

NUCB2/nesfatin-1 released from POMC/CART neurons directly inhibits NPY/AgRP neurons and causes hyperpolarization via ATP-sensitive potassium channels [51]. This observation is confirmed by the physiological effect resulting from the administration of glibenclamide, which is a potassium channel antagonist. The anorexigenic effect of nesfatin may stem from the hyperpolarization of orexigenic ARC neurons [51]. NUCB2/nesfatin-1 interacts with a G protein-coupled receptor (whose structure is unknown), and by stimulating Ca^{2+} inflow through the activation of protein kinase A (PKA) in specific neuron areas (through the stimulation of transcription factors) using N-type, L-type, or P/Q-type calcium channels; this interaction causes an increase in intracellular Ca^{2+} concentrations [84, 85]. Increased calcium concentration activates PKA, resulting in a cellular response being evoked. Furthermore, calcium, the intracellular concentration of which is elevated in relation to the NUCB2/nesfatin-1 concentration, activates vagal afferent neurons and sends a signal to the brain. Under in vivo conditions, NUCB2/nesfatin-1 activates dorsal motor nucleus of the vagus (DMNV) efferent organ neurons and triggers the calcium signal in the cultured DMNV neurons [84, 85]. As it causes hyperpolarization and depolarization, nesfatin-1 is thought to play a regulating role in PVN [1]. However, this mechanism is not

described on other neuronal population. In others words, it is not yet shown that nesfatin-1 could be considered as a hyperpolarizing neurotransmitter. The nesfatin-1 receptor has not been cloned yet, so we cannot provide an exact explanation for the underlying biochemical mechanism with the available data.

Effects on stress

Acute restraint stress variably activates different regions of the NUCB2/nesfatin-1 neurons in PVN, SON, and NTS [86]. A neuron subset that co-expresses NUCB2/nesfatin-1, CART, and urocortin-1 in the non-pre-ganglionic area of the mouse Edinger-Westphal nucleus, which is known to be involved in the response to various stress paradigms, was activated. These observations suggest that nesfatin-1 can be a part of the repertoire of neuropeptides and effectors that are activated for the adaptation responses to be evoked under stress conditions [86, 87]. In another study, it was reported that the central ICV injection of nesfatin-1 increased the circulating levels of both adrenocorticotropin (ACTH) and corticosterone, both of which are the major elements of the hypothalamic–pituitary–adrenal axis. In addition, bilateral adrenalectomy increased the expression of NUCB2 mRNA in PVN. These data demonstrate that central nesfatin-1 is involved in controlling the stress axis, and NUCB2/nesfatin-1 expression is subject to the negative feedback regulation of the main secretory products of the adrenal gland [reviewed 1, 2, 29, 38]. The NUCB2/nesfatin-1 signal in the brain also modulates the cardiovascular responses that are related to stress adaptation, such as the elevation of the mean artery pressure after ICV injection of nesfatin [88]. It was argued that these hypertensive effects of nesfatin-1 occur through a central OT-melanocortin pathway [reviewed 1, 2, 29, 38].

Effects on ischemia/reperfusion Injury

Nesfatin-1 provides protection against ischemia/reperfusion injury by limiting the infarction size and reducing lactose dehydrogenase release and post-ischemic contracture. A host of biochemical events including translocation inhibitor peptide (PKCε-I), extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), signal transducer transcription-3 activator, and mitochondrial K (ATP) are involved in this protection [89]. Being expressed in the heart, NUCB2/nesfatin-1 affects myocardial performance directly through the pGC-coupled NPR-A, guanylate cyclase (pGC)/protein kinase G (PKG) pathway, and ERK1/2. NUCB2/nesfatin-1 acts upon the cardiac performance by functioning as a peripheral cardiac modulator. These effects of NUCB2/nesfatin-1 are independent from NO. However, the natriuretic peptide receptor A (NPR-A), also known as pGC, PKG, and extracellular signal regulating kinase1/2 are speculated to partially mediate in the process [28].

Effects on blood pressure

Although it does not have any effect on noradrenalin or 5-hydroxytryptamine, nesfatin-1 increased blood pressure via the hypothalamus melanocortin 3/4 receptor [reviewed 2, 75, 90]. This hormone does not affect the cyclic guanosine monophosphate (cGMP) analog and 8-bromo cGMP [90]. Furthermore, NUCB2/nesfatin-1 directly influences the excitability of the majority of medial nucleus of the solitary tract (mNTS) neurons by eliciting either depolarizing (42 %, mean: 7.8 ± 0.8 mV) or hyperpolarizing (21 %, mean: -8.2 ± 1.0 mV) responses [90]. Nesfatin-1 microinjected into the mNTS induces significant increases in both blood pressure and heart rate in rats [31, 90]. Also, nesfatin-1 (10nM, 30 min) significantly inhibited the sodium nitroprusside (SNP)-induced relaxations of smooth muscle in mesenteric artery [90].

Effects on glucose and insulin

Insulin resistance and the amount of insulin production have a direct influence on the glucose use of cells. Hyperglycemia activates serine kinase cascades, which in turn lead to the phosphorylation of the insulin receptor substrate (IRS) (which has tyrosine kinase activity) and proteins. IRS-1 and IRS-2 increase serine phosphorylation and decrease tyrosine phosphorylation. Consequently, the reduced activity of signal molecules such as phosphatidylinositol-3 abates the effect of insulin, which in turn causes insulin resistance [77]. The secretory responses of nesfatin-1 to glucose were lower than its responses to insulin [91]. This indicates that the nesfatin-1 of pancreas origin does not play an endocrinal role in the metabolic regulation, but serves a local function [91].

In a study involving rats with diet-induced obesity, Yang et al. [38] reported that the ICV injection of nesfatin regulated hepatic gluconeogenesis to inhibit hepatic glucose production. Nesfatin-1 does this by either decreasing the synthesis of the phosphoenolpyruvate carboxykinase enzyme, which is a rate-limiting enzyme in gluconeogenesis in the liver, or by increasing the activity of the insulin hormone that causes a decrease in the hepatic glucose production [77]. Furthermore, peripheral insulin injection increases the activation of ARC, PVN LHA, dorsal motor nucleus of the vagus (DMNX), and NTS nesfatin

expressions in the vagal regulating nuclei of the brain [91]. In other words, hypoglycemia activates nesfatin expressions in the hypothalamic neurons. It was also reported that elevated glucose in blood increased the release of nesfatin-1 in the endocrine cells of pancreas [92]. Nesfatin-1 administration to hyperglycemic rats reduces the blood glucose level. Single-dose peritoneal administration of M30 inhibited the food intake 3 hours after the injection, but it did not affect the glucose and lipid concentrations [reviewed 2]. An overall evaluation of the data suggests that the anti-hyperglycemic effect of nesfatin-1 results not only from its endocrine function but also from its inhibition of hepatic glucose production by way of the regulation of glycogen synthesis and gluconeogenesis [80].

Other endocrinal effects

The administration of corticotropin-releasing factor [(CRF) (1)] and CRF (2) receptor antagonists offsets the anorexigenic effects of nesfatin-1. In other words, nesfatin-1 inhibits food intake through CRF receptors [reviewed 29]. Additionally, intraperitoneal administration of CCK induces the fos expression at a rate of 24 % in the NTS neurons and 43 % in the PVN neurons where nesfatin-1 is synthesized [reviewed 2]. In other words, an activation of neurons has been indirectly shown by c-fos expression in response to CCK administration. Therefore, it was claimed that the anorexigenic effect of NUCB2/nesfatin-1 arose from the suppression of orexigenic NPY neurons. This claim is based on the fact that NUCB2/nesfatin-1 causes hyperpolarization in ARC, and most of the hyperpolarized neurons are NPY-positive neurons [30, reviewed 38].

Possible future clinical applications

Human studies have shown that the circulating levels of nesfatin-1 change in several diseases, including epilepsy, diabetes, and anxiety. Therefore, it can be used in the diagnosis of diseases, monitoring of the patient response to treatment, or the progression of the disease both during and after the treatment. Especially, its measurements hold promise as a biomarker in the diagnosis and follow-up of epilepsy. However, the currently available scientific data are scarce and limited to a single group. For a biological marker to figure into clinic practice, it should be subjected to experiments designed by different, independent laboratories that will formulate new hypotheses, tested with reliable bioanalytical methods to be developed, and pass quality control experiments.

As nesfatin-1 or its mid-segment, which is composed of 30 aa and is responsible for the inhibition of food intake, was reported to be capable of limiting the food intake of leptin-resistant animals in a way that is independent of leptin, it can contribute to the treatment of metabolic syndrome and obesity, which are major health problems of our age. Understanding the biologically rooted eating behavior at the molecular level may pave the way for pharmacological treatments or the use of nesfatin-1 as a vaccine against obesity.

Another possible clinical use of nesfatin-1 may be heralding birth or inducing birth as it increases oxytocin release. Also, the correlations with ICV levels of NUCB2/nesfatin-1 are not still well understood. Any gaps in the knowledge in these areas should be addressed with future studies.

Conclusion

Nesfatin-1 derives from precursor NEFA/nucleobindin2 (NUCB2), regulates appetite in the physiological range in a time- and feeding status-dependent manner, but it assumes a role or roles in several pathophysiological events when it exceeds the threshold value of appetite inhibition and is synthesized in many tissues and exercises endocrinal and metabolic effects. In addition to being identifiable in rodent and human species, nesfatin-1 levels were different in a variety of pathological conditions ranging from diet-induced obesity to type-2 diabetes. That is, nesfatin-1 was involved in the regulation of insulin secretion. Overall, however, why nesfatin-1 increases in some diseases, decreases in others, or shows a similarity with controls is still exactly unknown but remains an important research topic for the future.

Conflict of interest I certify that there is no conflict of interest in this paper.

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