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Role of NUCB2/Nesfatin-1 in the Hypothalamic Control of Energy Homeostasis

A. Stengel[1] and **Y. Taché**[2]

[1]Charité Center for Internal Medicine and Dermatology, Division for General Internal and Psychosomatic Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany

[2]Department of Medicine, CURE Digestive Diseases Research Center and Center for Neurobiology of Stress, Digestive Diseases Division UCLA, and VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA

Abstract

Hunger and satiety are regulated in a complex fashion by a few food intake stimulatory (orexigenic) and a multitude of inhibitory (anorexigenic) factors produced in the periphery (mainly in the gastrointestinal tract) or directly in the brain. Within the brain, the hypothalamus plays a pivotal role as a production site of food intake regulatory factors. Importantly, this site integrates peripheral and central signaling factors to orchestrate food intake and in the long term body weight. Our knowledge on these regulatory pathways is not static but rather rapidly changing as new factors as well as upand downstream signaling pathways of already known transmitters are uncovered. Hypothalamic nucleobindin2 (NUCB2), the precursor of nesfatin-1, was first described in 2006 and nesfatin-1 found to be a novel anorexigenic modulator of food intake and body weight. The initial report stimulated several groups to investigate the biological actions of nesfatin-1 and subsequent studies delineated the underlying brain mechanisms involved in its food reducing effect. Of interest was the demonstration that NUCB2 also exerts its anorexigenic action in the paraventricular nucleus of the hypothalamus and is regulated at this site by changes in metabolic status with a diurnal rhythm inversely related to that of feeding in rats. The present review describes the current state-of-knowledge on central nesfatin-1's effects on food intake and body weight and highlights important missing links regarding cellular signaling mechanisms involved in nesfatin-1's action.

Keywords

body weight; food intake; hypothalamus; NUCB2; nesfatin-1; obesity; satiation

Correspondence. ***Y. Taché, PhD***, Center for Neurobiology of Stress, CURE Building 115, Room 117, VA GLA Healthcare System, 11301 Wilshire Blvd, CA 90073 Los Angeles, USA, Tel.: +1/310/312 9275, Fax: +1/310/268 4963, ytache@mednet.ucla.edu.

Conflicts of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

Introduction

Identification of nesfatin-1 and processing of nucleobindin2

Using an elegant screening approach of differential gene regulation by the peroxisome proliferator-activated receptor gamma agonist, troglitazone, Mori and his team identified a responsive gene expressed in medulloblastoma (HTB185) and adipose (3T3-L1) cells as well as in the rat brain [1]. This gene, identified as nucleobindin2 (NUCB2), encodes a 24-amino acid (aa) signal peptide followed by a 396-aa protein [1] and is highly conserved across mammalian and non-mammalian vertebrates indicative of its physiological importance [2].

The structure of NUCB2 appears to predict the post-translational cleavage by the enzyme prohormone convertase (PC)-1/3 into the N-terminal nesfatin-1 (aa 1–82), mid nesfatin-2 (aa 85–163), and the C-terminal nesfatin-3 (aa 166–396) [1]. Several biological actions and pharmacological effects have been described in response to the administration of nesfatin-1. Those are not reproduced by nesfatin-2 and nesfatin-3, in particular the anorexigenic effect, supporting the notion that the biological activity of NUCB2 is contained in the N-terminal peptide [1]. However, it is to note that nesfatin-2 and nesfatin-3 have received less attention so far and more studies are needed in order to establish or rule out whether these cleavage products have biological activity.

In the initial report, the authors identified – besides the full length 50-kDa NUCB2 – the mature nesfatin-1 as a 9.7 kDa peptide in a pooled sample of rat cerebrospinal fluid as well as in rat hypothalamic extracts using a specific nesfatin-1 antibody (ab24) in a competitive ELISA [1]. Later on, nesfatin-1 was also found in human plasma using a nesfatin-1 specific sandwich-type ELISA [3]. However, all other studies using a commercial antibody (Phoenix Pharmaceuticals, Inc.) did not detect endogenous mature nesfatin-1, whereas the full length NUCB2 and exogenous synthetic nesfatin-1 were clearly identified by Western blot [4–6]. Based on these data, it is not clear whether the postulated cleavage of NUCB2 to nesfatin-1 occurs in vivo or whether the divergent results are merely due to different antibodies used (purified nesfatin-1 ab24 antibody vs. Phoenix NUCB2/nesfatin-1 antibody). Based on evidence that the unprocessed full length NUCB2 is biologically active as it exerts the same anorexigenic effect as nesfatin-1 [1], NUCB2 seems likely to serve as the endogenous biologically active form and it may be more proper to refer to the product as NUCB2/nesfatin-1.

The immunohistochemical phenotype of NUCB2/nesfatin-1 expressing cells in the brain showed the immunoreactivity in the cytoplasm of the cell bodies as well as primary dendrites, while no labeling was observed in axons and nerve terminals [1, 4, 7–10]. This specific pattern of intracellular distribution gives rise to the speculation of a primary intracellular mode of action of NUCB2/nesfatin-1. However, an extracellular signaling role of secreted NUCB2/nesfatin-1 cannot be ruled out. This is based on evidence that primary dendrites and cell bodies are also able to secrete transmitters and peptides [11, 12]. In addition, in the periphery, NUCB2/nesfatin-1 is located in secretory vesicles of endocrine cells in the rat stomach and pancreas [5] and human stomach [13] providing morphologic evidence for a humoral mode of action of NUCB2/nesfatin-1.

Although our knowledge on the pleiotropic actions of NUCB2/nesfatin-1 in the brain is increasing, the associated receptor remains yet to be established. Existing evidence using Ca^{2+} flux as an index suggested that NUCB2/nesfatin-1's action in neurons is mediated by a Gi/o-protein-coupled receptor as the response is abolished by pertussis toxin [7, 14]. In particular, in vitro studies showed that nesfatin-1 stimulates Ca^{2+} influx into neurons of the rat hypothalamus [7, 15], rat dorsal motor nucleus of the vagus nerve (DMV) [16], and mouse vagal afferent nodose ganglion [17]. Results obtained using inhibitors specific for subtypes of Ca^{2+} channels indicate that the nesfatin-1-induced elevation of $[Ca^{2+}]_i$ exhibits pharmacological characteristics of either N-, L-, and/or P/Q channels. In mouse nodose ganglia, the $[Ca^{2+}]_i$ response was blocked by the N-type Ca^{2+} channel blocker, ω-conotoxin GVIA [17], while the Ca^{2+} influx into rat hypothalamic neurons was blocked by L-type, ω-conotoxin GVIA or verapamil and P/Q Ca^{2+} channel blockers, ω-conotoxin MVIIC [7, 15]. Other reports indicate that full length nesfatin-1 and the mid fragment of nesfatin-1 stimulated the activity of the cAMP response element (CRE) reporter in mouse NB41A3 neuroblastoma cells, an effect inhibited by an L-type Ca^{2+} channel blocker [14]. Lastly, radiolabeled ^{125}I-nesfatin-1 was shown to bind to these neuroblastoma cells and to the mouse hypothalamus [14] giving rise to a cell surface expression of the assumed Gi/o-protein-coupled receptor.

Localization of NUCB2/Nesfatin-1 in the Brain

In the first report, NUCB2 mRNA and protein was detected in rat hypothalamic nuclei involved in the regulation of food intake, namely the supraoptic nucleus (SON), paraventricular nucleus (PVN), lateral hypothalamic area (LHA), and arcuate nucleus (Arc) [1]. Other reports expanded the brain expression of NUCB2/nesfatin-1 to the insular cortex, central amygdaloid nucleus, periventricular nucleus, tuberal hypothalamic area, dorsomedial hypothalamic nucleus, the medullary raphe nuclei, ventrolateral medulla (VLM), Edinger-Westphal (EW) nucleus, locus coeruleus (LC), cerebellum, DMV, nucleus of the solitary tract (NTS), and preganglionic sympathetic and parasympathetic neurons of the spinal cord in rats [4, 7–9, 18–20], mice [10], and pigs [21]. Besides the detection in well-established nuclei, in mice NUCB2/nesfatin-1 immunoreactivity was also detected in a so far undescribed area, named the intermediate dorsomedial area of the hypothalamus [10]. Future studies will be necessary to unravel the function of this brain nucleus. The pattern of NUCB2/nesfatin-1 distribution in brain centers not only regulating food intake but also responsive to stress and regulating autonomic functions support broader actions of NUCB2/nesfatin-1.

Double labeling immunohistochemistry showed that the majority of neurons expressing NUCB2/nesfatin-1 were also positive for urocortin (~90 %), melanin-concentrating hormone (MCH, ~80 %), cocaine- and amphetamine-regulated transcript (CART, ~70 %), α-melanocyte-stimulating hormone (α-MSH, ~60 %), pro-opiomelanocortin (POMC, ~60–80 %), vasopressin (~50 %), neuropeptide Y (NPY, ~40 %), oxytocin (~40 %), growth hormone-releasing hormone (GHRH, ~30 %), corticotropin-releasing factor (CRF, ~20 %), thyrotropin-releasing hormone (TRH, ~20 %), somatostatin or neurotensin (~10 %) and serotonin [4,7,8,15,18,19,22]. In keeping with the focus of the review, the interaction

between brain NUCB2/nesfatin-1 and mediators mentioned above will be limited to those relevant to the regulation of hunger and satiety.

Inhibition of Food Intake by Central NUCB2/Nesfatin-1

Oh-I et al. reported in their first seminal study that full length NUCB2 or nesfatin-1 injected into the third brain ventricle in chronically cannulated rats dose-dependently reduced the dark phase food intake under ad libitum feeding conditions in rats [1]. Other reports likewise showed that nesfatin-1 injected at low pmol doses (5–20 pmol) into the brain at different levels, namely into the lateral, third or fourth ventricle of chronically cannulated rats or acutely into the cisterna magna of briefly anesthetized rats, induced a robust reduction of dark phase food intake in rats [15,23–28]. Likewise, an injection of nesfatin-1 into the lateral brain ventricle (intracerebroventricular, icv) of chronically cannulated mice induces a food intake suppressive effect, which was observed at 2-h post injection and remained visible over an 8-h observation period [27]. Similar to rodents, acute icv injection of nesfatin-1 in goldfish reduces food intake [6,29] indicating that the anorexigenic effect of nesfatin-1 is not restricted to mammalian species but rather a conserved function of a phylogenetically old peptide [2]. The lateral brain ventricle injection of nesfatin-1 at a low dose of 5 pmol had a delayed onset (maximum effect of 87% reduction during the third hour post injection) and a long duration of action (6 h) in rats [25], which was extended to 48 h upon icv injection at a higher dose (25 pmol) [28]. The change in eating behavior upon icv injection of lower doses of nesfatin-1 is not related to the induction of other confounding behaviors such as altered locomotor activity [1, 25, 28] or grooming including scratching, licking, and washing [25]. However, at higher doses (25–80 pmol) – in addition to the anorexigenic effect – icv nesfatin-1 also induces anxiety-like behaviors as assessed by an increased startle response and less time spent in the open arms of an elevated plus maze in rats [30, 31].

Interestingly, when nesfatin-1 was injected into the 3rd or 4th brain ventricle or into the cisterna magna of rats, the kinetic of dark phase feeding suppression was different with a rapid onset of the anorexigenic effect already observed during the 1st hour post injection [1, 25] indicative of different sites of action of nesfatin-1 in mid- vs. hindbrain. Subsequent studies investigated responsive hypothalamic sites using microinjection into the brain parenchyma. Nesfatin-1 delivered directly into the PVN or at a higher dose into the lateral hypothalamic area reduces the dark phase food intake, while microinjection of the peptide into the ventromedial hypothalamus had no effect [32]. These data support the PVN as a primary hypothalamic target site involved in the anorexigenic effect of nesfatin-1.

The inhibitory effect of nesfatin-1 on food consumption was recently further characterized using an automated monitoring system that every second records food intake of solid rodent chow in undisturbed animals. In mice, the acute icv injection of nesfatin-1 reduced food intake via an increase of satiation (as reflected in a reduction of meal size) and satiety (as reflected in a decrease in meal frequency and prolonged inter-meal intervals) [24]. Similarly, the mid fragment nesfatin-1$_{30–59}$ injected icv under identical conditions induces satiation, whereas satiety was not altered [33]. The different alterations of feeding microstructure exerted by full length nesfatin-1 and mid fragment nesfatin-1$_{30–59}$ may point towards additional receptor binding sites only activated by full length nesfatin-1 and necessary to

exert an effect on both, satiation and satiety. In contrast, the Nand C-terminal fragments, nesfatin-$1_{1–29}$ and nesfatin-$1_{60–82}$ had no effect indicative that the mid fragment contains the active core of the peptide [33]. While these studies provide insight into the structure-activity relationship, it is still to be established whether NUCB2/nesfatin-1 is processed into a mature bioactive nesfatin-$1_{30–59}$ in vivo since so far the N- and C-terminal as well as mid fragment peptides are derived from computer-predicted cleavage sites.

Growing evidence supports that the anorexigenic action of nesfatin-1 is not merely a pharmacological effect but rather reflects a physiological property of NUCB2/nesfatin-1. Nesfatin-1 acts at very low (pmol) doses to reduce the dark phase food intake, the photoperiod associated with eating in rodents [1]. One study showed that endogenous blockade of brain NUCB2/nesfatin-1 signaling by injecting the anti-nesfatin-1 antibody ab24 acutely or an anti-NUCB2 antisense oligonucleotide into the 3rd ventricle for a period of 10 days increases food intake and body weight gain in male Wistar rats [1]. Likewise, the selective and transient knockdown of NUCB2 levels in the PVN using NUCB2 shRNA microinjected bilaterally into the rat PVN increases cumulative daily food intake for 2 days in parallel with changes in PVN NUCB2 levels [34]. In addition, the 3rd brain ventricular injection of anti-nesfatin-1 IgG during the light phase increased food intake while having no effect in the dark phase suggesting a role of NUCB2/nesfatin-1 in the circadian suppression of food intake during the light photoperiod [34]. Similar to the anorexigenic effect, anti-nesfatin-1 antisense oligonucleotide injected into the lateral brain ventricle increases the drinking response to angiotensin II providing new insight to the role of hypothalamic NUCB2/nesfatin-1 in the regulation of water intake [35]. However, in two other studies, the anti-NUCB2 morpholino oligonucleotide injected icv over a period of 2 days in male rats or 7 days in young female rats did not alter food intake while hypothalamic NUCB2 content was reduced by 29 % in male and 75 % in female rats [35, 36]. These discrepancies may be due to the different treatment conditions (third ventricle vs. icv, 10 days vs. monitoring for 2 days) or sex differences (male vs. female).

Regulation of Brain NUCB2/Nesfatin-1

Further underlining the role of NUCB2/nesfatin-1 as a physiological modulator of feeding, NUCB2 mRNA and protein are regulated by the metabolic status with a decrease in the PVN and SON after fasting and an increase following re-feeding in rats [1, 8, 36] and goldfish [6]. Importantly, studies indicated that NUCB2 mRNA expression in the PVN is subject to a diurnal rhythm associated with eating pattern in lean rats which is impaired in Zucker-fatty obese rats [34]. NUCB2 mRNA rises during the early light phase associated with the suppression of eating and declines in the afternoon with low levels maintained in the dark-phase during the eating period. Moreover, in Zucker-fatty rats there was a blunted rise in NUCB2 mRNA expression in the PVN during the light phase associated with hyperphagia which can be corrected by the icv injection of nesfatin-1 [34]. Similar to the acute influence of diurnal or feeding status, chronic changes of body weight are associated with changes in hypothalamic NUCB2/nesfatin-1 expression. In a mouse model prone to develop obesity, the Tsumura Suzuki diabetic mice, hypothalamic NUCB2 mRNA and protein were decreased when compared to nondiabetic controls that could contribute to the hyperphagia observed in these mice.

Transposition of these experimental studies to the clinical arena showed that chronic changes in body weight impact on the circulating levels of NUCB2/nesfatin-1 with decreased plasma levels in female patients with anorexia nervosa [38] and increased levels in both male and female obese patients [39, 40] leading to a positive correlation with body mass index (BMI) [38, 39]. However, the ratio of cerebrospinal fluid/plasma NUCB2/nesfatin-1 was negatively correlated with BMI and body fat mass due to the strong rise of plasma NUCB2/nesfatin-1 with increasing BMI, while cerebrospinal fluid NUCB2/nesfatin-1 levels were only slightly increased [40]. In contrast to these findings, one study reported a negative association of plasma nesfatin-1 levels with BMI in nonobese males [3]. In obese children serum NUCB2/nesfatin-1 levels were reported to be significantly lower than those of the healthy controls resulting in a negative correlation of nesfatin-1 with standard deviation scores of BMI but not with insulin resistance index in the obese [41]. Whether different assessment methods (ELISA recognizing NUCB2 and nesfatin-1 vs. sandwich-type ELISA recognizing only nesfatin-1), the body weight range (normal weight vs. underweight and obesity) and/or sex differences (females vs. males) contribute to these discrepancies warrants further investigations.

Interaction of Central NUCB2/Nesfatin-1 with Other Transmitters

It was established early on that the anorexigenic effect of NUCB2/nesfatin-1 is independent of leptin signaling as shown by the retained food intake suppressive activity in leptin receptor deficient Zucker rats [1, 15]. In addition, injection of leptin did not modulate the expression of NUCB2 mRNA in the hypothalamus [1]. However, the extensive co-localization of NUCB2/nesfatin-1 with a multitude of brain transmitters suggested the interplay with several other mediators possibly modulating or mediating nesfatin-1 anorexigenic effect.

Convergent findings established that oxytocin and melatonin are involved in the downstream anorexigenic signaling of nesfatin-1. Injection of nesfatin-1 into the 3rd brain ventricle activates oxytocin positive neurons in the PVN as assessed by Fos expression and Ca^{2+} influx into these neurons [15]. In vitro studies showed that nesfatin-1 induced oxytocin release from PVN neurons [15]. Conversely, blockade of oxytocin signaling using the oxytocin receptor antagonist, H4928, injected into the 4th brain ventricle blocked the food intake suppressive effect of nesfatin-1 [15, 26]. The nesfatin-1-oxytocin signaling is likely to involve POMC as downstream mediator based on the following observations: oxytocinergic nerve terminals originating from the PVN project to the brainstem, especially the NTS [42] in close proximity to neurons containing POMC [15]. In addition, injection of oxytocin stimulates the release of POMC in the NTS [15]. POMC protein is cleaved into several neurotransmitters including α-MSH known to activate the melanocortin 3/4 receptor [43]. The icv or 3rd brain ventricle injection of the α-MSH-melanocortin 3/4 receptor antagonist, SHU9119, completely blocked the anorexigenic action of nesfatin-1 [1, 31]. In addition, a bidirectional interaction is suggested by the demonstration that a 3rd brain ventricle injection of α-MSH upregulates the expression of NUCB2 mRNA in the PVN [1]. Collectively these data delineate a hypothalamic-brainstem NUCB2/nesfatin-1-oxytocin-POMC-α-MSH signaling system to reduce food intake.

Another brain transmitter well known to exert anorexigenic properties, the CRF signaling system [44, 45], contributes to the downstream mediation of nesfatin-1 anorexigenic action. Nesfatin-1 stimulates the excitability of CRF positive PVN neurons in vitro [46]. This finding was corroborated in vivo. The 3rd ventricular injection of nesfatin-1 increases the CRF content in the PVN [47] and plasma ACTH and corticosterone levels [48] indicating a stimulation of the hypothalamus-pituitary-adrenal gland axis. In addition, blockade of CRF_2 receptors using the CRF_2 antagonist, astressin$_2$-B, or the CRF_1/CRF_2 antagonist, α-helical $CRF_{9–41}$, completely blocked or blunted, respectively the anorexigenic action of icv injected nesfatin-1 [25, 47]. However, when nesfatin-1 was injected at the level of the hindbrain into the cisterna magna, astressin$_2$-B did not modulate the anorexigenic effect [25]. Taken together, these data support the involvement of CRF receptor signaling pathways in nesfatin-1 anorexic action mainly in the PVN consistent with nesfatin-1 prominent site of action and physiological role at this site [32, 34, 49].

Another study suggested an involvement of TRH in the food intake suppressive effect of nesfatin-1 based on the observation of a blunting of nesfatin-1 anorexigenic action after 3rd brain ventricular injection of a TRH antibody [47] and that nesfatin-1 injected into the 3rd ventricle increases the expression of hypothalamic TRH mRNA [47].

In addition to the peptidergic pathways mentioned above, histamine and serotonin (5-HT) were implicated in the downstream anorexigenic signaling of nesfatin-1 [47, 50]. First, the 3rd ventricular injection of nesfatin-1 increases the turnover of hypothalamic histamine and histamine stimulates the expression of NUCB2/nesfatin-1 in the PVN [47] giving rise to a feed forward loop between these two transmitters. Second, H_1 receptor knockout (KO) mice or rats with blockade of histidine decarboxylase using α-fluoromethyl histidine display a blunted anorexigenic action of nesfatin-1 [47]. Since blockade of central CRF signaling using α-helical $CRF_{9–41}$ injected into the 3rd brain ventricle blunts the nesfatin-1-induced anorexigenic effect and the stimulation of histamine turnover in the PVN [47], the histamine signaling pathway is likely to act downstream of CRF. With regard to brain serotonin signaling, it was found that 5-HT_{2C} KO mice display a reduced hypothalamic NUCB2 and POMC expression compared to their wild type littermates which was associated with an increased food intake [50]. Conversely, injection of the 5-$HT_{1B/2C}$ agonist, *m*-chlorophenylpiperazine, upregulates the expression of hypothalamic NUCB2 mRNA associated with a reduction of food intake [50]. These findings should be corroborated in future studies.

In addition to the interaction with several other anorexigenic hormones, nesfatin-1 may also interfere with orexigenic pathways namely, NPY. Administration of nesfatin-1 onto arcuate neurons results in a hyperpolarization of NPY positive neurons in vitro [51], an effect likely contributing to nesfatin-1's anorexigenic effect.

Summary and Perspective

The past years witnessed mounting evidence establishing that nesfatin-1 is a novel centrally acting anorexigenic modulator of the dark phase food intake in rodents. Recent evidence also supports the physiological role of PVN NUCB2/nesfatin-1 in the circadian feeding

pattern of rodents. NUCB2/nesfatin-1 is likely not only involved in the acute reduction of food intake but also in the long term control of body weight. The finding that the anorexigenic function of NUCB2/nesfatin-1 – in opposition to many other food intake regulatory hormones – is not modulated by leptin signaling, highlights its possible use as a target in the drug treatment of obesity, a metabolic condition where leptin resistance is frequently observed. Although our knowledge of NUCB2/nesfatin-1 interactions with several peptidergic anorexigenic pathways greatly increased during the past years, the identification of the nesfatin-1 receptor(s) will be an important step to advance the understanding of the mechanism of action at the cellular level, and the development of new pharmacologic tools to target this pathway.

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