Regulatory Peptides 163 (2010) 18–23

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Review

Nesfatin-1 — Role as possible new potent regulator of food intake

Andreas Stengel, Yvette Taché *

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

ARTICLE INFO

Article history:
Received 8 April 2010
Accepted 11 May 2010
Available online 16 May 2010

Keywords:
Body weight
Brain–gut
Food intake
Gastric emptying
NUCB2/nesfatin-1
Obesity
Stress

ABSTRACT

Nesfatin-1 is an 82 amino acid peptide recently discovered in the brain which is derived from nucleobindin2 (NUCB2), a protein that is highly conserved across mammalian species. Nesfatin-1 has received much attention over the past two years due to its reproducible food intake-reducing effect that is linked with recruitment of other hypothalamic peptides regulating feeding behavior. A growing amount of evidence also supports that various stressors activate fore- and hindbrain NUCB2/nesfatin-1 circuitries. In this review, we outline the central nervous system distribution of NUCB2/nesfatin-1, and recent developments on the peripheral expression of NUCB2/nesfatin-1, in particular its co-localization with ghrelin in gastric X/A-like cells and insulin in ß-cells of the endocrine pancreas. Functional studies related to the characteristics of nesfatin-1's inhibitory effects on dark phase food intake are detailed as well as the central activation of NUCB2/nesfatin-1 immunopositive neurons in the response to psychological, immune and visceral stressors. Lastly, potential clinical implications of targeting NUCB2/nesfatin-1 signaling and existing gaps in knowledge to ascertain the role and mechanisms of action of nesfatin-1 are presented.

Published by Elsevier B.V.

Contents

Abbreviations: 3v, third ventricle; 4v, fourth ventricle; 5-HT, serotonin; α-MSH, α-melanocyte-stimulating hormone; aa, amino acid; CCK, cholecystokinin; CART, cocaine- and amphetamine-regulated transcript; CRF, corticotropin-releasing factor; EW, Edinger–Westphal nucleus; GHRH, growth hormone-releasing hormone; ic, intracisternal; icv, intracerebroventricular; ip, intraperitoneal; LPS, lipopolysaccharide; MCH, melanin-concentrating hormone; NPY, neuropeptide Y, NTS, nucleus of the solitary tract; PC, pro-hormone convertase; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus of the hypothalamus; TRH, thyrotropin-releasing hormone.

* Corresponding author. Center for Neurobiology of Stress, CURE Building 115, Room 117, VA GLA Healthcare System, 11301 Wilshire Blvd, Los Angeles, CA, 90073, USA. Tel.: +1 310 312 9275; fax: +1 310 268 4963.
E-mail address: ytache@mednet.ucla.edu (Y. Taché).

0167-0115/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.regpep.2010.05.002

Nesfatin-1 was discovered in 2006 by Oh-I et al. as an 82 amino acid (aa) polypeptide derived from the calcium and DNA-binding protein, nucleobindin2 (NUCB2) [1]. Besides nesfatin-1, putative post-translational processing of NUCB2 by the enzyme pro-hormone convertase (PC)-1/3 yields nesfatin-2 (aa residues 85–163) and nesfatin-3 (aa residues 166–396) [1], although no biological action has been reported for those peptides so far. Due to its inhibitory effect on food intake, the first cleavage product of NUCB2 was named nesfatin-1, an acronym for *N*UCB2-*e*ncoded *s*atiety and *fat in*fluencing protein [1].

1. Central and peripheral expression of NUCB2/nesfatin-1

1.1. Expression in the brain

NUCB2 mRNA expression was initially detected in rat hypothalamic and brainstem nuclei implicated in the regulation of food intake, namely the arcuate nucleus, paraventricular nucleus (PVN), supraoptic nucleus, lateral hypothalamic area, zona incerta and the nucleus of the solitary tract (NTS) [1]. This expression pattern was similar to that of the protein as assessed by immunohistochemistry for NUCB2 and nesfatin-1 [1]. Subsequent studies extended these observations by detecting NUCB2/nesfatin-1 immunoreactivity in additional hypothalamic and hindbrain nuclei such as the dorsomedial hypothalamic nucleus, tuberal hypothalamic area, periventricular nucleus, arcuate nucleus, Edinger–Westphal nucleus, locus coeruleus, the medullary raphe nuclei and the dorsal motor nucleus of the vagus nerve [2–7]. Lastly, we recently completed the central nervous system mapping of NUCB2/nesfatin-1 with additional forebrain and hindbrain nuclei including the insular cortex, central amygdaloid nucleus, ventrolateral medulla and cerebellum as well as preganglionic sympathetic and parasympathetic neurons of the thoracic, lumbar and sacral spinal cord in rats [7]. As also noticed by others [2,3], we found that NUCB2/nesfatin-1 immunoreactivity was present only in the cytoplasm of cell bodies and proximal processes but not in varicosities and axon terminals [7], suggesting an action of NUCB2/nesfatin-1 as an intracellular modulator. However, cell bodies can also release cellular contents [8,9] raising the possibility that nesfatin-1 may still act as an extracellular regulatory peptide.

The neurochemical content of NUCB2/nesfatin-1 positive cells has been extensively characterized. A large sub-population of hypothalamic NUCB2/nesfatin-1 immunoreactive neurons also expresses immunoreactivity for melanin-concentrating hormone (MCH, ~80% of MCH neurons co-label with NUCB2/nesfatin-1 immunoreactivity), cocaine-and amphetamine-regulated transcript (CART, ~70%), α-melanocyte-stimulating hormone (α-MSH, ~60%), pro-opiomelanocortin (POMC, ~60–80%), vasopressin (~50%), oxytocin (~40%), growth hormone-releasing hormone (GHRH, ~30%), corticotropin-releasing factor (CRF, ~20%), thyrotropin-releasing hormone (TRH, ~20%), somatostatin or neurotensin (~10%) [2–5,10]. Of interest, a recent study showed that hypothalamic arcuate neuropeptide Y (NPY)-containing neurons co-labeled with NUCB2/nesfatin-1 immunoreactivity (~40%) [6]. Moreover, NPY positive fibers were identified in the vicinity of NUCB2/nesfatin-1 immunoreactive neurons [2]. In the midbrain, NUCB2/nesfatin-1 co-localizes with cholinergic and urocortin positive neurons of the Edinger–Westphal nucleus (~90%) [2,11] and in the medulla in serotonin (5-HT)-containing neurons of the raphe [2].

Collectively, existing evidence established a broad distribution of NUCB2/nesfatin-1 immunoreactivity in discrete limbic, hypothalamic, pontine and medullary nuclei co-localized with a number of hypothalamic peptidergic integratory signals regulating food intake and other functions. These neuroanatomical studies greatly widened the knowledge of NUCB2/nesfatin-1's distribution and point towards a broader role for NUCB2/nesfatin-1 that may encompass, in addition to feeding behavior, neuroendocrine regulation, autonomic control of visceral functions, sleep, emotion and pain (Fig. 1).

1.2. Expression in peripheral tissues

The brain and the gut overlap in their peptidergic content [12] and recent studies indicate that nesfatin-1 can cross the blood–brain barrier in both directions in a non-saturable manner [13,14]. Thus, we investigated whether there is a peripheral source of nesfatin-1 and identified NUCB2 mRNA in the rat stomach [15]. Expression levels were 20-fold higher in the gastric oxyntic mucosa than those in the brain and 12-fold compared to other viscera such as the heart as assessed by microarray analysis and confirmed by RT-qPCR [15]. Immunostaining in peripheral tissues substantiated the expression of NUCB2/nesfatin-1 in the rat stomach and additionally in the endocrine pancreas, pituitary gland and testis [15]. In the rat stomach, NUCB2/nesfatin-1 immunosignals are mainly localized in mucosal endocrine X/A-like cells within a distinct sub-population of vesicles different from those containing the orexigenic hormone ghrelin (Fig. 2) [15], suggesting differential regulation and release of ghrelin and nesfatin-1. In the rodent and human endocrine pancreas, NUCB2/nesfatin-1 immunostaining is restricted to insulin positive ß-cells with a sub-cellular cytoplasmic localization distinct from that of insulin [16,17]. Collectively, these data provide support at the cellular level for an exclusive endocrine cell distribution of NUCB2/nesfatin-1 in the pituitary, stomach and pancreas which may impact on the regulation of hormone secretion, food intake and glycemic control in concert with ghrelin and insulin respectively.

1.3. Gaps to fill

An important debate remains regarding the question whether NUCB2/nesfatin-1 are secreted neuroendocrine transmitters. Although post-translational cleavage of NUCB2 to nesfatin-1 is suggested by the presence of PC1/3, an enzyme involved in the processing of NUCB2 in central as well as peripheral tissues containing NUCB2 [1,18], to date mature nesfatin-1 (1–82 aa) has only been reported in the cerebrospinal fluid of rats by Oh-I et al. in their initial study [1], whereas it was not present in hypothalamic protein extracts [1,3]. Subsequent reports did not distinguish between nesfatin-1 and NUCB2 since the antibody, although raised against the full length nesfatin-1, also recognizes NUCB2 [2–7,15,16]. Moreover, we and others were unable to demonstrate endogenous mature nesfatin-1 (9.7 kDa) in the stomach, pancreas or plasma [15,16]. One possible explanation is that the conditions used were not sensitive enough to detect nesfatin-1. However, when synthetic nesfatin-1 peptide (50 ng) was exogenously added, the peptide was identified on the Western blot [15]. As NUCB2 displays biological activity upon third ventricular injection [1], this raises the possibility that the full length NUCB2 may be the biologically active compound that is released. Alternatively, since the structure of NUCB2 contains several motifs involved in intracellular signaling, namely a Ca^{2+}-binding EF hand motif and a nuclear-binding basic helix–loop–helix sequence [19], NUCB2 may have a primarily intracellular role as recently suggested [17].

2. Inhibition of food intake, body weight and digestive functions by nesfatin-1

2.1. Nesfatin-1 injected into the brain inhibits food intake: mechanisms of action

In pioneered studies performed in rats, Oh-I et al. observed a reduction of dark phase food intake following third ventricular (3v) injection of nesfatin-1 at picomolar doses, whereas nesfatin-2 and nesfatin-3 had no effect [1]. Moreover, continuous infusion of nesfatin-1 into the 3v reduced food intake and body weight gain [1].