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Review

Is secretoneurin a new hormone?

Vance L. Trudeau [a,*], Christopher J. Martyniuk [b], E Zhao [a], Hongxia Hu [c], Hélène Volkoff [d], Wayne A. Decatur [e], Ajoy Basak [f]

[a] Department of Biology, Centre for Advanced Research in Environmental Genomics, University of Ottawa, 30 Marie Curie, Ottawa, ON, Canada
[b] Department of Biology and Canadian Rivers Institute, University of New Brunswick, Saint John, NB, Canada
[c] Beijing Fisheries Research Institute, No.18, Jiaomen Rd., Fengtai, Beijing, PR China
[d] Departments of Biology and Biochemistry Memorial University of Newfoundland, St. John's, NL, Canada
[e] Center for Molecular and Comparative Endocrinology, University of New Hampshire, Durham, NH, USA
[f] Ottawa Hospital Research Institute, Chronic Diseases Program, University of Ottawa, Ottawa, ON, Canada

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ABSTRACT

Numerous small potentially bioactive peptides are derived from the selective processing of the ~600 amino acid secretogranin II (SgII) precursor, but only the 31–42 amino acid segment termed secretoneurin (SN) is well-conserved from sharks to mammals. Both SNa and SNb paralogs have been identified in some teleosts, likely arising as a result of the specific genome duplication event in this lineage. Only one copy of the putative lamprey SgII (188 amino acids) could be identified which gives rise to a divergent agnathan SN that contains the signature YTPQ-X-LA-X_7-EL sequence typical of the central core of all known SN peptides. In rodent models, SN has regulatory effects on neuroinflammation and neurotransmitter release, and possesses therapeutic potential for the induction of angiogenesis. The wide distribution of SN in neuroendocrine neurons and pituitary cells suggests important endocrine roles. The clearest example of the endocrine action of SN is the stimulatory effects on pituitary luteinizing hormone release from goldfish pituitary and mouse LβT2 gonadotroph cells, indicative of an important role in reproduction. Several lines of evidence suggest that the SN receptor is most likely a G-protein coupled protein. Microarray analysis of SN effects on dispersed goldfish pituitary cells in vitro reveals novel SN actions that include effects on genes involved in notch signaling and the guanylate cyclase pathway. Intracerebroventricular injection of SN increases feeding and locomotory behaviors in goldfish. Given that SgII appeared early in vertebrate evolution, SN is an old peptide with emerging implications as a new multifunctional hormone.

1. Introduction

When does a candidate molecule become a bona fide hormone? Here, we present the case for secretoneurin (SN). The neuropeptide SN is a short conserved sequence in the central core of the rather larger secretogranin-II (also called chromogranin C) precursor protein. Secretoneurin has multiple physiological actions [23,28,41,78], some of which tend to place it among classically defined hormones [27]. Importantly, the discovery of the evolutionarily conserved stimulatory effects of SN on pituitary luteinizing hormone (LH) release [6,74,77] forces the question of whether SN itself meets the criteria to be called a hormone.

There are many common working definitions, but what are the select criteria and framework to use for the investigation, categorization and therapeutic applications of potential new hormones? As we start the second 50-year period of the science of comparative endocrinology, we are at a point when indeed many new bioactive chemical messengers are being discovered. Can some of these messengers be considered hormones? One of the more obvious recent examples is the explosion of the adipokine/cytokine family of hormone-like polypeptide molecules produced by adipocytes. Typified by leptin [17] and adiponectin [59], their endocrine roles in feeding, glucose regulation, and reproduction are becoming well established. One has only to remember the cytokine versus hormone debate (e.g., see definitions in [62]) or the decades of discussion on definitions of peptide hormones versus neuropeptides [18] to realize that there are disagreements in the published literature. The intention here is to present the main evidence for the classification of SN as a hormone. New data on the actions of SN in the goldfish model are also provided in support of this hypothesis.

* Corresponding author. Fax: +1 613 563 5486.
E-mail address: trudeauv@uottawa.ca (V.L. Trudeau).
URL: http://www.teamendo.ca (V.L. Trudeau).

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2. Criteria for characterization of secretoneurin as a hormone

2.1. Source of candidate hormone

Original investigation of frog brain extracts with α-melanocyte stimulating hormone regulating abilities [9] led Vaudry and Conlon [67] to isolate a peptide that Kirchmair and colleagues [36] later named 'secretoneurin'. It is now known that SN arises from the proteolytic processing of the SgII precursor protein in several tissues. Remarkably, precursor processing to SN reaches 89–97% in the brain, 49% in the adrenal medulla, and only 26% in the anterior pituitary of the rat [36]. Secretoneurin is also abundant in neuroendocrine tumors [23,41]. In neuroendocrine tissues, SN is abundant in the hypothalamus and pituitary [23,39,78]. A high level of SN in the median eminence of the rat and infundibular area of the goldfish strongly suggests a conserved hypophysiotropic role. In the goldfish, SN-like immunoreactive fibers and presumptive nerve terminals were found in the periventricular preoptic nucleus, pituitary and the ventrocaudal aspect of the nucleus of the lateral recess [7]. The most conspicuous SN-immunoreactivity (SN-ir) was found in the goldfish magnocellular and parvocellular cells of the preoptic nucleus that project heavily to the neural lobe of the pituitary [7], highly consistent with observations in the laboratory rat. There is conservation of the co-localization of SN-ir with oxytocin/isotocin in neurons in the rat and goldfish preoptic area [7]. There appears to be species differences in the localization of SN-ir with the classical anterior pituitary hormones [15,23,76]. Nevertheless, SN-ir has been colocalized to varying degrees with LH, follicle stimulating hormone (FSH), growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH) and adrenocorticotropic hormone (ACTH) in mammalian systems [15,23]. In the goldfish anterior pituitary, SN-ir is nearly exclusively found in the highly regionalized lactotrophs of the rostral pars distalis [75]. The strong association between SgII, SN-ir and PRL protein in the rat, bovine, goldfish and several PRL-secreting tumor cell lines permits the proposal that the lactotroph is an evolutionarily conserved but not exclusive source of SN-ir products in the vertebrate anterior pituitary. However, much remains to be uncovered as regards the processing and localization of SN-ir in the endocrine cells of the pituitary in the various vertebrate classes. It must be noted that most if not all SN antibodies used in western blots recognize the SgII precursor and the processed fragments containing the SN segment. Given the differential processing pattern in the various tissues, the natures of the processed fragments that are visualized by immunocytochemistry remain largely unknown. Moreover, no SN-ir peptides were detected by western blot in extracts of goldfish interrenal, ovary, and cerebellum yet SgII mRNA was expressed in all these tissues [72]. The selective processing of the SgII precursor by prohormone convertases is tissue- and species-specific, and draws clear parallels with proopiomelanocortin processing for the generation of numerous hormonal and neuroactive peptides [10,19].

Based on these observations, there is good evidence that SN is a candidate hormone produced by selective processing of the precursor protein SgII that is expressed in numerous endocrine organs.

2.2. Nature and structure of candidate hormone

Secretoneurin is a neuropeptide that is moderately conserved in evolution. For known SN forms the size ranges from 31 amino acids in the medaka (*Oryzias latipes*) to 42 in the shark (*Squalus acanthius*). Most conserved are the tetrapod forms of SN, which are all 33 amino acids long and only vary at a few positions. We recently determined that there are 2 forms of SN in the teleosts [76]. Arising from a presumptive gene duplication associated with tetraploidization in teleosts, we previously named these paralogs, SNa and SNb. Domains in the N-terminus ("TNE") and middle ("QYTP" and "LATLEQSVFE(Q)EL") of teleost SNa are identical to the mammalian SN. In marked contrast, two stretches in the middle of teleost SNb ("EQYTPQSLA" and "FE(Q)ELG") are only moderately conserved [76]. Shown in Fig. 1 are examples of the amino acid sequences of SN from select vertebrates. One study of the solution conformation of mammalian SN suggests that conserved amino acids in the N-terminus and middle region may contribute to an α-helical structure [45]. In a circular dichroism study of goldfish SNa peptide, it was suggested that SN has a more complex secondary structure that would include a turn and a β-sheet component in addition to an α-helix [5].

It has been suggested that the granins are calcium-binding proteins [23]. However, goldfish SNa was found to be only weakly associated with calcium. Rather, SN and related shorter fragments exhibit unique cesium-binding abilities [5]. Although the biological significance of cesium-binding to SN is unclear, alteration of its activity through interactions with endogenous ions such as Cu^{+2}, Ca^{+2} or K^{+1} may have important implications in normal and pathological conditions [20].

Most non-conserved substitutions for SN are in the C-terminus region [76]. Using the zebrafish (*Danio rerio*) as an example, the SgIIa gene is located on chromosome 15 and SgIIb gene on chromosome 2. Comparison of zebrafish SNa and SNb serves to illustrate the potential hormone diversity generated by duplication events. While the placement of dibasic cleavage sites at the N- and C-termini of the zebrafish SN sequence within the SgII precursor are conserved, SNa and SNb are respectively 34 and 31 amino acids long, having only 14 identical amino acids. The biological activity of SNb has yet to be determined but would be predicted to be different from SNa.

Searches of the sea lamprey (*Petromyzon marinus*) genome assembly (v6.0) revealed a single copy of a SgII-like gene with a potential SN-related segment. The deduced sequence of the lamprey SgII precursor protein is highly basic (p*I* = 11.78) and only 188 amino acids in length (Fig. 2), making it quite distinct from the known teleost and mammalian SgII. In the lamprey SgII sequence there appears to be no tyrosine sulfation site in the N-terminal region preceding the SN fragment, although there are potential serine/threonine targets for possible post-translational phosphorylation [23]. The highly conserved dibasic cleavage site $K^{107}R^{108}$ defines the N-terminal of the putative lamprey SN within $SgII^{1-188}$. As with shark, no traditional dibasic site could be identified in the C-terminal region; however, sea lampreys possess a genome-wide protein

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Lamprey          VQENIEDEYTPQNLARLQVILQELGFFDRAGGKTPARPESRGV 43
Shark            TNEIVEEQYTPQSLATLESAFRELGKYAGPYKEQGRLEEEHF- 42
Zebrafish SNa    TNENAEEQYTPQKLATLQSVFEELSGIASSKTNT--------- 34
Zebrafish SNb    ATEDLDEQYTPQSLANMRSIFEELGKLSAAQ------------ 31
Frog             TNEIVEGQYTPQSLATLQSVFQELGKLKGQANN---------- 33
Chicken          TNEIVEEQYTPQSLATLESVFQELGKMAGPSNH---------- 33
Human            TNEIVEEQYTPQSLATLESVFQELGKLTGPNNQ---------- 33
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Fig. 1. ClustalW2 alignment of the amino acid sequences for lamprey (*Petromyzon marinus*), shark (*Squalus acanthius*), zebrafish (*Danio rerio*) SNa, zebrafish SNb, Frog (*Rana ridibunda*), chicken (*Gallus gallus*) and human (*Homo sapiens*) SN. The * indicates the most conserved amino acids and the number to the left indicates the length of the peptide. For reference, the YTPQ-X-LA-X_7-EL signature is underlined in the human SN sequence.