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Review

Direct cellular peptidomics of hypothalamic neurons

Jennifer W. Mitchell [a], Norman Atkins Jr. [b,1], Jonathan V. Sweedler [b,c,d], Martha U. Gillette [a,b,d,e,*]

[a] Dept. of Cell & Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[b] Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[c] Dept. of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[d] Dept. of Molecular & Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[e] College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

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ABSTRACT

The chemical complexity of cell-to-cell communication has emerged as a fundamental challenge to understanding brain systems. This is certainly true for the hypothalamus, where neuropeptide signals are heterogeneous, localized and dynamic. Thus far, most hypothalamic peptidomic studies have centered on the entire structure; however, recent advances in collection strategies and analytical technologies have enabled direct, high-resolution peptidomic profiles focused on two regions of interest, the suprachiasmatic and supraoptic nuclei, including their sub-regions and individual cells. Suites of peptides now can be identified and probed for function. High spatial and analytical sensitivities reveal that discrete hypothalamic nuclei have distinct peptidomic signatures. Peptidomic discovery not only reveals unanticipated complexity, but also peptides previously unknown that act as key circuit components. Analysis of tissue releasates identifies peptides secreted into the extracellular environment and available for transmitting intercellular signals. Direct sampling techniques define peptide-releasate profiles in spatial, temporal and event-dependent patterns. These approaches are providing remarkable new insights into the complexity of neuropeptidergic cell-to-cell signaling central to neuroendocrine physiology.

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1. Introduction

The hypothalamus comprises discrete brain nuclei that regulate and coordinate homeostatic processes, which range from osmotic balance, hunger/satiety and reproductive state to circadian rhythms and sleep. Hypothalamic nuclei, such as the suprachiasmatic nucleus (SCN) and the supraoptic nucleus (SON), are richly peptidergic [1,14]. The physiological functions of these nuclei—circadian regulation by the SCN and water balance, reproduction and affiliative behavior by the SON—depend upon intrinsic cell-to-cell neuropeptide signaling as well as at afferent and efferent innervations.

Several features of neuropeptides make them difficult to study. Peptide gene transcripts encode large prepropeptides, which undergo significant cell type-dependent processing and cleavage. Thus, prepropeptide mRNA expression does not allow one to predict which functional peptides will be produced from the gene. Neuropeptides are difficult to characterize biochemically, are physiologically active at a range of concentrations, some very low, and exhibit broad bioactivity across heterogeneous brain regions, especially throughout the neuroendocrine systems. Unlike classical neurotransmitters, where only one type is expressed per neuron and expression can characterize a brain region, multiple neuropeptides can be expressed in an individual neuron [44], processing products may be targeted differentially to distal cell regions [63] and a brain nucleus often expresses diverse neuropeptides in adjacent neurons [8]. Neuropeptides are released differentially with co-localized classical neurotransmitters, which significantly elevates the complexity and subtlety of neural signaling [39]. For these reasons, increasing our understanding of the full complement of neuropeptide signals is of significant interest to the field of neuroscience systems biology.

Processing the prepropeptide into functional neuropeptides occurs in multiple steps (Fig. 1). The prepropeptide transcriptional product contains both a signal sequence of amino acids and the sequences that will become functional neuropeptides. The signal sequence targets the molecule to the endoplasmic reticulum, where the NH_2-terminal signal peptide is cleaved. The resulting proneuropeptide transits to the Golgi system where it enters the secretory pathway via dense core vesicles (Fig. 1A). Because dense core vesicles lack synaptic docking proteins, which restrict release of clear vesicles containing classical neurotransmitters to synaptic release sites, neuropeptide release can occur at multiple sites in the cell.

* Corresponding author. Address: Dept. of Cell & Developmental Biology, University of Illinois at Urbana-Champaign, 610 S. Goodwin Ave., Urbana, IL 61801, USA. Fax: +1 217 244 1648.
E-mail address: mgillett@illinois.edu (M.U. Gillette).
[1] Present address: Center for Sleep & Circadian Biology, Northwestern University, Evanston, IL, USA.

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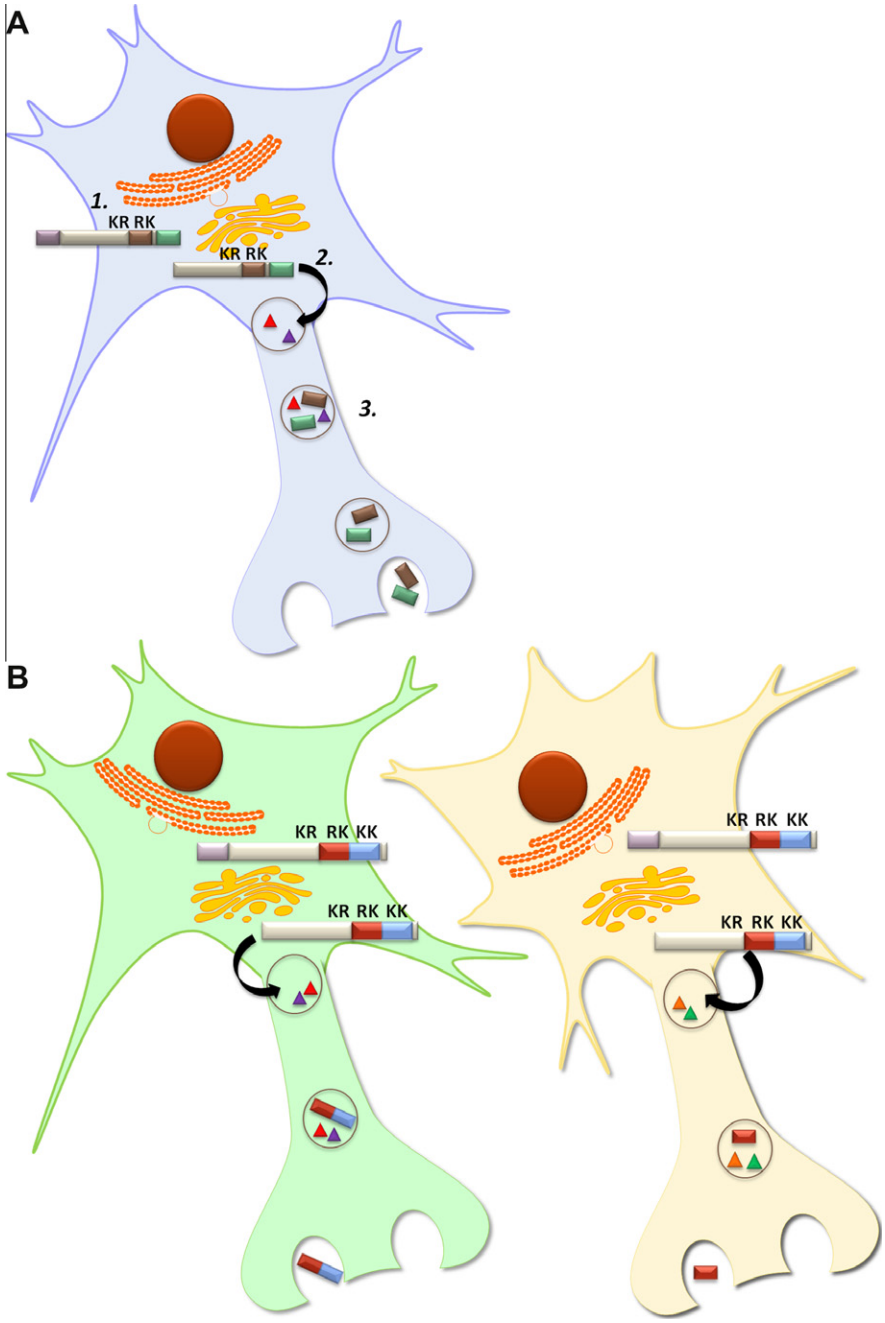

Fig. 1. Prepropeptide precursors undergo sequential steps of processing during which the peptide is generated and targeted. (A) Peptides are produced from larger precursors in multiple steps. 1. Proteolytic processing begins in the endoplasmic reticulum (ER) when the NH_2-terminal signal peptide is cleaved by signal peptidase. 2. The proneuropeptide is routed to the Golgi apparatus and packaged into dense-core secretory vesicles together with processing proteases, termed convertases (colored triangles). 3. As the secretory vesicle matures, proteolytic processing occurs, usually at dibasic cleavage sites (KK, KR, RK). The mature secretory vesicle contains fully processed, biologically active peptide ready for stimulus-initiated secretion by exocytosis. (B) Proneuropeptides may undergo tissue-specific processing by different convertases to generate distinct peptides. The processes illustrated here have been extensively studied [41,51,52,67].

Sequential processing steps convert the proneuropeptide into a collection of neuropeptides within a single vesicle. These proneuropeptide cleavages occur under the control of an array of endogenous intracellular propeptide convertases, which may differ between vesicles so that neuropeptide contents also differ (Fig. 1B).

A single proneuropeptide often produces multiple neuropeptides. Moreover, proneuropeptides may not be processed the same way in different cell types or even within the same cell type under different conditions. Cleavages can occur in a tissue-specific and even region-specific manner. Although proneuropeptides are predominantly cleaved at dibasic residues (lysine–arginine), only a small percentage of dibasic sites actually are cleaved [5]. This is noteworthy because post-translational cleavage and post-translational modifications (PTMs) can affect binding affinities, as well as regulate the bioactivity and stability of the neuropeptide. Antibody-based methods of detecting neuropeptides often cannot discriminate these subtle differences between peptides; therefore, immunohistochemical approaches are inadequate for comprehensively evaluating PTMs and alternate forms of known neuropeptides. They also require *a priori* information regarding potential peptide expression and, thus, are incapable of exhaustively examining the full peptide complement of peptidergic inputs, intrinsic

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