Minireview

The discovery of novel neuropeptides takes flight

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Abstract

Structural data are critical for the elucidation of how peptides are synthesized and how they function. Two recent studies have used nanoscale chromatography together with mass spectrometry to determine the structures of novel neuropeptides in rat and *Drosophila*. The results shed light on neuropeptide synthesis and function(s) in both vertebrates and insects.

Peptides synthesized in the nervous system serve as messengers and as modulators of numerous biological processes. Thus, it is important to understand how neuropeptides are produced and how they act, because inaccurate neuropeptide synthesis or signal transduction may result in organismal dysfunction or death. Knowledge of the structure of naturally occurring neuropeptides is required if we are to decipher how neuropeptide precursors are processed, to delineate their binding to receptors, and to identify their function(s).

The amino-acid sequence of peptide precursors can be deduced from nucleotide sequence, but this approach does not reveal precisely what peptide is ultimately expressed. Although it is possible to predict what peptides may be processed from a precursor from putative proteolytic cleavage sites, not all conventional sites are used and processing can also occur at unconventional sites. In addition, nucleotide sequence does not provide information on the developmental or tissue-specific regulation of neuropeptide-precursor processing. Several neuropeptides can be encoded by a single gene, and processing of individual peptides from a common precursor can differ during development or between tissues.

Another problem with predicting peptides from nucleotide sequence is that this yields only a putative primary sequence and does not identify post-translational modifications, which are often essential for neuropeptide activity. Historically, the primary structure of a peptide was determined by Edman degradation after it was purified to homogeneity, but purification is often a time-consuming procedure. The labile nature of post-translational modifications and the low abundance of neuropeptides also make it particularly challenging to isolate enough of a peptide to determine its structure. Thus, an efficient and effective method is needed if we are to determine the structures of naturally occurring peptides.

Discovery of novel rat neuropeptides

Skold *et al.* [1] recently described a novel approach that combines online nanoscale liquid chromatography (nanoLC) and electrospray-ionization quadrupole time-of-flight (ESI Q-TOF) mass spectrometry to analyze molecules in the mass range of 300-5,000 Da. The combination of nanoLC with ESI Q-TOF mass spectrometry fills a gap in the analysis of gene products: the more usual analytical method used in proteomics, involving two-dimensional electrophoresis combined with mass spectrometry, analyzes larger molecules, typically those over 10 kDa. Now, using the approach described by Skold *et al.*, it is possible to determine both the total amounts of smaller molecules in a complex mixture and their individual structures.

Skold *et al.* [1] used nanoLC ESI Q-TOF mass spectrometry to analyze three regions of the rat brain - the motor cortex, the striatum, and the thalamus. They report that the method was reproducible and sensitive [1]. About 1,500 rat brain peptides and protein fragments were identified, including known

neuropeptides as well as previously unknown protein fragments. The results distinguish between peptides that differ by only one amino acid, making nanoLC ESI Q-TOF mass spectrometry a valuable tool in the analysis of structurally similar, naturally occurring neuropeptides and/or the identification of a mutation. But not all known rat brain peptides were identified - a problem that may result from technical parameters such as sample preparation or preferential ionization and may be resolved with further development of this method.

Drosophila melanogaster neuropeptides

Baggerman *et al.* [2] analyzed the peptide content of the *Drosophila melanogaster* larval central nervous system using nanoLC and ESI Q-TOF mass spectrometry. This work is relevant to mammals because there is similarity between the neuropeptide systems of insects and vertebrates. The insect corpora cardiaca and the vertebrate hypothalamo-hypophysial systems are sites of synthesis, delivery and release of many neuropeptides. Some insect and vertebrate neuropeptides are similar in their structures, signal transduction pathways, synthesis, cleavage, post-translational modifications and activities, whereas others differ between the two groups. Research conducted on insect neuropeptides that are similar to vertebrate neuropeptides may be applicable to higher organisms; and research on insect neuropeptides that are distinct from vertebrate neuropeptides may identify targets for the development of safe and effective pest-management tools. *D. melanogaster* is a powerful system in which to study peptide function and signal transduction because research in behavior and physiology can be combined with biochemistry, genetics, and molecular biology techniques in an animal species whose generation time is short and whose genome sequence is available [3]. The small size of *D. melanogaster* provides a distinct advantage over vertebrates and many other invertebrates when considering space required for housing, and the large number of bioassays established in *D. melanogaster* demonstrates that the small size of the animal is by no means an obstacle to research.

Baggerman *et al.* [2] report evidence for the presence of 28 neuropeptides in an extract of 50 *D. melanogaster* larvae. Some of the neuropeptides had never been purified before, yet could be predicted from nucleotide sequence data, while others were novel and encoded by genes that had not yet been annotated. The results were confirmed by the identification of several known, previously isolated *D. melanogaster* neuropeptides. The analysis of the *D. melanogaster* nervous system using this novel approach [2] did not identify all the known, previously isolated neuropeptides [4], which may again be due to technical parameters that may be resolved with further application and development of the new method.

Previously, Uttenweiler-Joseph *et al.* [5] analyzed hemolymph, the equivalent to blood in mammals, from wild-type and immune-challenged *D. melanogaster* in order to detect molecules involved in the immune response using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. When combined with high-performance liquid chromatography (HPLC) and protein sequencing by Edman degradation, this method yielded the structures of several *D. melanogaster* immune-induced molecules. Now, nanoLC ESI Q-TOF mass spectrometry can be used to display the peptide content of blood, cells, and tissues in normal and induced states in order to delineate how peptides are synthesized and processed, how they signal, and how disruption of these processes affects the organism as a whole.

The development of a method that allows us to determine the structures of naturally occurring peptides in a mixture is a significant advance. The combination of nanoLC and ESI Q-TOF mass spectrometry holds great promise for the identification of novel neuropeptides. Its application in experimentally versatile model systems for which genome sequence data are available and in which mutants can be generated provides a powerful approach for the delineation of peptide synthesis and function(s), and we can expect further new insights in the near future.

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References

1. Skold K, Svensson M, Kaplan A, Bjorkesten L, Astrom J, Andren PE: **A neuroproteomic approach to targeting neuropeptides in the brain.** *Proteomics* 2002, **2:**447-454.
2. Baggerman G, Cerstiaens A, De Loof A, Schoofs L: **Peptidomics of the larval *Drosophila melanogaster* central nervous system.** *J Biol Chem* 2002, DOI 10.1074/jbc.M206251200.
3. Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, *et al.*: **The genome sequence of *Drosophila melanogaster*.** *Science* 2000, **287:**2185-2195.
4. Nichols R: **Isolation and expression of the *Drosophila* drosulfakinin neural peptide gene product, DSK-I.** *Mol Cell Neurosci* 1992, **3:**342-347.
5. Uttenweiler-Joseph S, Moniatte M, Lagueux M, Van Dorsselaer A, Hoffmann JA, Bulet P: **Differential display of peptides induced during the immune response of *Drosophila*: a matrix-assisted laser desorption ionization time-of-flight mass spectrometry study.** *Proc Natl Acad Sci USA* 1998, **95:**11342-11347.