MICROANALYTICAL INVESTIGATION OF CELL TO CELL SIGNALING PEPTIDES AT THE CELLULAR AND SUBCELLULAR LEVELS

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Cell to cell signaling peptides (SPs) play key roles in the modulation and integration of nervous system activity. Analysis of SPs in cells and tissues is challenging due, in part, to their broad range of concentrations, the large variety in physicochemical properties among SPs, and the dynamic changes in their expression which is dependent on the physiological state of an organism. Moreover, investigation of the function-related behavior of SPs in the nervous system requires methods of microanalysis that allow the monitoring of synthesis, transport, and release of these analytes at the single cell and even subcellular levels.

We have developed a broad range of approaches that allows detection and characterization of SPs in vertebrate and invertebrate tissues, cells, and subcellular structures. These approaches consist of unique sample preparation techniques, analyte separation and detection methods, and specialized data analysis with *in silico* prediction of individual SP structures. The sample preparation techniques allow the isolation and preparation of individual organelles, neuronal processes, and cells. Preselected structural elements of mammalian and invertebrate nervous systems can be targeted using these techniques. Depending on sample and analyte properties, different separation and detection techniques are implemented including CE-LIF, CE-MS, CE-radionuclide detection, SPE-CE, SPE-MS, and microfluidic-MS. As proof of concept, these approaches are successfully applied to investigate SP content in individual secretory vesicles, release of SPs from different areas of a single cell, and spatial profiling of the localization of these compounds along a single neuronal process.

Several strategies are developed and applied to characterize unknown SPs including tandem MS approaches and hybrid approaches using bioinformatics. Multiple SPs are usually encoded by an associated prohormone gene. The prohormone is enzymatically processed into the bioactive SPs. Because of the number of processing steps, it becomes difficult to predict SPs from a novel gene. We have developed an approach for the *in silico* prediction of SP structure which is utilized in the Web-based tool Neuropred (neuroproteomics.scs.uiuc.edu/neuropred.html). Neuropred generates a list of the most likely SPs encoded by a given prohormone gene. These masses are used in conjunction with the mass spectral data to discover and characterize novel SPs.