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INSTITUTIONEN FÖR KEMI OCH MOLEKYLÄRBIOLOGI

Electrochemical and Microscopic Analysis of Chemical Signalling in Biological Systems

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Abstract

Cellular communication is a prerequisite in multicellular organisms in order to survive. Many times, this communication occurs through the highly controlled and regulated release of chemical signals through a process known as exocytosis. This process consists of organelles known as vesicles fusing with the plasma membrane to release the content inside to the extracellular space. Although the basic underpinnings of this process are known, exactly how it is regulated is still largely unknown.

This thesis covers studies performed on mammalian cell lines and invertebrate neurons with the aim to further understand regulation of exocytosis. Several complementary methodologies have been utilised to study this regulation from different points of view. The electrochemical technique of amperometry has the benefit of being able to track the exocytotic process with high temporal resolution and makes it possible to quantify both how many molecules are stored in a vesicle as well as how many are released. Several imaging methods, such as fluorescence, electron microscopy and mass spectrometry, provided high quality spatial information to complement the electrochemical techniques.

The papers included in this thesis have involved studies of how exocytosis is regulated in PC12 and chromaffin cells, along with how it is affected by pharmacological treatments as well as more intrinsic factors such as the secretory activity of the cell. In paper I, storage and release of dopamine was determined both using amperometry and imaging mass spectrometry. In paper II, the drug tamoxifen was observed to regulate both transmitter storage and release. ATP also was shown to regulate transmitter storage and release as demonstrated in paper III. Paper IV further provides an additional role for ATP as regulating vesicle content in combination with norepinephrine. Repetitive stimuli regulate exocytosis by causing cells to release larger fractions of their stored content as seen in paper V. In addition, a method previously developed in our group, intracellular electrochemical vesicle cytometry, was adapted and applied to measurements of vesicle content in the more complex and considerably smaller biological model system of the *Drosophila* neuromuscular junction in paper VI.

In the biological model systems studied here, exocytosis appears to most often occur through vesicle fusion and closure that only allows some of the vesicular content to escape. By only partially releasing the molecules stored inside vesicles, the chemical signalling can be regulated and adapted. The presence of this feature opens new possible drug targets in order to medically alter dysfunctional chemical signalling in diseases as well as a possible key to understand how memories are formed.