UNIVERSITY OF GOTHENBURG

The Mechanisms of Exocytosis Studied in Cells and Models with Amperometry

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AKADEMISK AVHANDLING

För filosofie doktorsexamen i Naturvetenskap, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras onsdagen den 5 juni 2013 kl. 10:00 i föreläsningssal KB, Institutionen för kemi och molekylärbiologi, Kemigården 4, Göteborg.

The thesis will be defended in English on Wednesday, the 5th of June 2013, 10 AM in lecture hall KB at Kemigården 4, Gothenburg

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ABSTRACT

Exocytosis is the universal basis for neuronal communication, allowing the controlled release of neurotransmitter molecules from the presynaptic cell. Exocytosis has been widely studied in many systems. However, the details of this vital process are not fully understood. Exocytosis is quantal in its nature in that the neurotransmitter is released in packages. These packages were initially assumed to be comprised of the entire vesicular content. More recently, this simplistic view has been replaced with a more complex one with several different suggested modes of release. Vesicles can, in addition to the irreversible complete collapse of the vesicle into the cell membrane, fuse transiently with the cell membrane in a process that has been termed kiss-and-run. Transient fusion has the advantage of allowing the vesicle not to release its entire content but instead potentially control the fraction of neurotransmitter molecules it lets out. To elucidate the details of the exocytotic process, it is necessary to resolve individual release events. Amperometric detection at micrometer sized electrodes is an excellent tool for this purpose, providing quantitative and detailed kinetic information about single release events. In this thesis I have employed amperometry to study the mechanisms of exocytosis at PC12 cells and in an artificial cell model.

In paper I, detection of exocytosis using amperometry and fast scan cyclic voltammetry at a microelectrode array is compared and evaluated. In paper II, amperometric detection of exocytosis is compared at a disk and a ring-shaped microelectrode, and the results are used to evaluate the diffusion coefficient of dopamine in the extracellular matrix surrounding the cell. In paper III, I define and explore a new feature of amperometric peaks recorded at PC12 cells, the post spike foot. This feature is used to show that changing the lipid composition of the cell membrane can alter the fraction of neurotransmitter released per event. In paper IV the influence of membrane lipid composition on lipid nanotube dimensions is studied using amperometry at a lipid nanotube-liposome network. It has been demonstrated that sorting of membrane lipids based on structure occurs and a dynamic method for controlling lipid nanotube diameter has been established. The same experimental setup is used in paper V, but here it is applied to PC12 cell plasma membrane vesicles and used to study the membrane dynamics of exocytosis. In this system it has been demonstrated that release occurs though two distinct mechanisms; full and partial distension of the initial fusion pore.

KEYWORDS: Exocytosis, Amperometry, Phospholipids, Cells, Cell models

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