Illuminating the Exocytotic Mechanism using Electrochemical Detectors and Total Internal Reflection Fluorescence Microscopy

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Abstract:

Exocytosis is the process wherein membrane bound packets, or vesicles, of neurotransmitters or hormones are released from the cell. Many cell types share this release mechanism, but the details of the exocytotic mechanism remain a mystery. To study this mechanism, we have developed microelectrode arrays to electrochemically detect the release of molecules from vesicles, while simultaneously monitoring fluorescent labels in the cell with total internal reflection fluorescence microscopy. Using these two techniques, we found that addition of charged peptides to the C-terminal end of synaptobrevin II, one of the key exocytotic proteins, strongly inhibits the exocytotic process.

Summary of Research:

Exocytosis is the release of membrane bound packets of molecules (vesicles) from cells, via fusion of the vesicle membrane with the cell membrane. Many cell types use exocytosis to release neurotransmitters and hormones into the body, but it remains unclear how exocytosis works. We do know that three proteins, collectively called the SNARE complex, are key to the exocytotic mechanism: syntaxin and SNAP-25 in the plasma membrane, and synaptobrevin II (SybII) in the vesicle membrane. These proteins form a coiled-coil motif, which some have postulated tightens or “zips” up, pulling the cell and vesicle membranes into close apposition and opening a pore between the membranes [1].

To study exocytosis, we have developed planar electrochemical detectors fabricated on glass coverslips to detect the release of adrenaline molecules from chromaffin cells of the adrenal gland using amperometry [2,3] (Figure 1). In amperometry, an electrode (traditionally a carbon fiber) is held at a positive potential and positioned near a cell of interest. When a release event occurs in the vicinity of the electrode, adrenaline molecules diffuse to the

Figure 1: The newest version of the four-electrode ECD array. Each 25 × 25 mm coverslip has 14 sets of four electrodes. Inset: An enlarged view showing the active electrode areas at the tips of the Pt wires. This device was insulated with SiO₂.
electrode, where the molecules are oxidized, with each molecule giving up two electrons to the electrode. The electrode detects these release events as transient current spikes with high time resolution [4]. Because the electrode array substrates are glass, we are able to combine electrochemical detection with fluorescence techniques. Total internal reflection fluorescence (TIRF) microscopy allows observation of fluorescent molecules very close to the coverslip surface, greatly reducing background light from fluorophores deeper inside the cell of interest.

To study the specific function of the C-terminal end of SybII, located in the vesicle membrane, we added charged peptides to the C-terminal of the SybII protein, and a green fluorescent protein (GFP) to the opposite end to track the protein. Either two positively charged lysine (K) peptides were added (SybII-KK) or two negatively charged glutamic acid (E) peptides (SybII-EE). A GFP was also added to a wild-type SybII to act as a control. Cells containing these proteins exhibited punctate fluorescence, indicating that the proteins were correctly localized to the vesicles (Figure 2). In cells expressing the wild-type GFP-SybII, disappearance of brightly labeled vesicles could be correlated to amperometric current spikes (Figure 3). For GFP-SybII-KK and GFP-SybII-EE events however, loss of punctate fluorescence could not be correlated to current spikes from the electrochemical detectors. This indicates that vesicles carrying the SybII mutant with added charged residues at the C-terminus are unable to perform exocytosis.

References: