Transparent Electrochemical Detectors to Study Exocytosis

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Abstract

Neurons release neurotransmitters in a process called exocytosis, wherein packets of transmitter molecules are released from the cells upon stimulation. Adrenal chromaffin cells, which also undergo exocytosis, constitute a model system for neuronal release. Quantal release of adrenaline from chromaffin cells can be measured using the electrochemical technique of amperometry, historically employing a carbon fiber electrode [1]. To gain information about the exocytotic mechanism, we have developed a method of electrochemical imaging employing transparent microfabricated devices based on amperometry. We have successfully measured electrochemical events using transparent electrodes simultaneously with fluorescence imaging, yielding more information about the exocytotic process.

Summary of Research

Several important cell types, including neurons and chromaffin cells of the adrenal gland, release transmitter molecules via exocytosis—the release of membrane bound packets or “vesicles” of transmitter molecules. Exocytosis can be observed by amperometry, an electrochemical detection method. In amperometry, transmitter molecules released during an exocytotic event are oxidized by a nearby electrode, and the oxidation current is recorded. Historically, carbon fiber electrodes have been employed for this purpose [1].

To gain information about the mechanism of transmitter release, it is desirable to perform simultaneous fluorescence imaging and electrochemical detection of individual release events. We previously demonstrated that amperometric signals from chromaffin cells can be measured using opaque platinum electrodes patterned on a glass coverslip while simultaneously observing fluorescence from the cell between the electrodes. And, that the platinum electrodes behave similarly to carbon fibers [2]. However, to minimize diffusional broadening, the electrode needs to be in close contact with the plasma membrane of the cell of interest. This is particularly important if one wants to study exocytosis from cells such as neurons using amperometry, and particularly difficult if one wants to observe fluorescent labeling in the cells simultaneously.

To overcome this problem, we have developed transparent electrode arrays for simultaneous amperometry and fluorescence imaging through the electrode. Transparent electrochemical detector arrays were fabricated on glass using standard photolithography techniques, and insulated using photoresist. Arrays were fabricated from either indium tin oxide (ITO) [3], or very thin (~ 6 nm) gold (Figure 1). Amperometric signals from bovine chromaffin...
Biological Applications

Cells could be detected with both materials at low noise, and fluorescence changes monitored through the electrodes. Figures 2 and 3 show examples of an electrochemical signal and a fluorescence image from ITO electrode arrays. Amperometric foot signals [4], indicating the initial stages in exocytosis, were detected with both types of electrodes. For arrays with similar geometry, mean charge and half width of the amperometric spikes were $0.89 \pm 0.44 \, \text{pC}$, $34 \pm 9 \, \text{ms}$ (SEM, n = 4 cells) for transparent gold electrodes and $0.38 \pm 0.05 \, \text{pC}$, $32 \pm 2 \, \text{ms}$ (SEM, n = 6 cells) for ITO. The difference in quantal size has a p value of 0.18. The results suggest that amperometric detection of transmitter release with transparent gold electrodes is comparable to that using carbon fibers while ITO may be less efficient.

In the future, we hope to record exocytotic events and fluorescence information directly from neurons. To that end, we are developing electrode arrays suitable for growing neurons across, as shown in Figure 4.

**References**


