

Public Abstract

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Department:Biological Engineering

Degree:PhD

Title:Integration of single-cell electroporation together with electrochemical measurement of quantal exocytosis on microchips

Exocytosis is a fundamental mode of intercellular communication. Specialized neuroendocrine cells like chromaffin cells contain vesicles that are packed with neurotransmitters. A rise in intracellular calcium concentration drives these vesicles to fuse with the cell membrane and release their contents into the extracellular space via the process of exocytosis. It is important to study the exocytosis mechanism in details as it plays a crucial role in various regular neurological functions as well as in disease conditions like Parkinson's and Huntington's. An electrochemical microelectrode located immediately adjacent to a single neuroendocrine cell can record spikes of amperometric current that result from quantal exocytosis of oxidizable transmitter from individual vesicles. We have developed an efficient method where the same electrochemical microelectrode is used to electroporate an adjacent chromaffin cell and then measure the consequent quantal catecholamine release using amperometry. Trains of voltage pulses can reliably trigger release from cells using gold electrodes. Amperometric spikes induced by electroporation have similar areas, peak heights and durations as amperometric spikes elicited by depolarizing solutions. Uptake of trypan blue stain into cells demonstrated that the plasma membrane is permeabilized by the voltage stimulus. Surprisingly, robust quantal release can be elicited upon electroporation in the absence of calcium in the bath solution. Instead, experiments demonstrate a dependence of the rate of electroporation-induced transmitter release on the extracellular chloride concentration. Using the same electrochemical electrode to electroporate and record quantal release of catecholamines from an individual chromaffin cell allows precise timing of the electroporation stimulus, stimulation of a single cell at a time, and can be used to load membrane impermeant substances into a cell.