OPTICAL STIMULATION OF QUANTAL EXOCYTOSIS ON TRANSPARENT MICROCHIPS

Xiaohui Chen

Dr. Kevin D. Gillis, Dissertation Supervisor

ABSTRACT

Photorelease of caged Ca^{2+} is a uniquely powerful tool to study the dynamics of Ca^{2+}-triggered exocytosis from individual cells. Using photolithography and other microfabrication techniques, we have developed transparent microchip devices to enable photorelease of caged Ca^{2+} together with electrochemical detection of quantal catecholamine secretion from individual cells or cell arrays as a step towards developing high-throughput experimental devices. A 110 nm – thick transparent Indium-Tin-Oxide (ITO) film was sputter-deposited onto glass coverslips, which were then patterned into 24 cell-sized working electrodes (~20. μm by 20. μm). We loaded bovine chromaffin cells with acetoxymethyl (AM) ester derivatives of the Ca^{2+} cage NP-EGTA and Ca^{2+} indicator dye Fura-4F, then transferred these cells onto the working ITO electrodes for amperometric recordings. Upon flash photorelease of caged Ca^{2+}, a uniform rise of $[Ca^{2+}]_i$ within the target cell leads to quantal release of oxidizable catecholamines measured amperometrically by the underlying ITO electrode. We observed a burst of amperometric spikes upon rapid elevation of $[Ca^{2+}]_i$ and a "priming" effect of sub-stimulatory $[Ca^{2+}]_i$ on the response of cells to subsequent $[Ca^{2+}]_i$ elevation, similar to previous reports using different techniques. We conclude that UV photolysis of caged Ca^{2+} is a suitable stimulation technique for higher-throughput studies of Ca^{2+}-dependent exocytosis on transparent electrochemical microelectrode arrays.