OPTICAL STIMULATION OF QUANTAL EXOCYTOSIS ON TRANSPARENT MICROCHIPS

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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 acetoxyethyl (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.