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Modulation of Exocytosis by Protein Kinases and Ca^{2+} in Epithelial Cells:

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In neurons and some endocrine cells, Ca^{2+} plays a pivotal role as the final signal for rapid stimulus-evoked release of neurotransmitters and hormones. In contrast, intracellular signals other than Ca^{2+}, such as protein A and C, were more emphasized the exocytosis in non-neuronal cells. We questioned if the regulatory mechanisms of exocytosis are fundamentally different between cell types. 1) Exocytosis in two endocrine cells, pituitary gonadotrope and pancreatic beta cell, is known to be mainly triggered by Ca^{2+}. Here we found that protein kinase A and C also are potent modulator even in the absence of $[Ca^{2+}]_i$ rise. 2) Exocytosis in pancreatic duct epithelial cells is known to be regulated by cAMP. We demonstrate that, in addition to the second messenger, Ca^{2+} is a potent activator of exocytosis in the cells. In conclusion, the machinery of exocytosis can be regulated by both protein phosphorylation and Ca^{2+} in a variety of cell types. Each cell type adapts an appropriate regulatory pathway depending on the function it needs to perform. In this talk I will focus on the recent data for the epithelial exocytosis.

Methods:

Cells: Dog pancreatic duct epithelial cells were derived from the main pancreatic duct. For single-cell experiments, the cells were plated on small coverglass chips (5 x 5 mm) with an appropriate coating substance.

Biophysical detection exocytosis: We used carbon-fiber amperometry to detect

exocytosis from single cells in real time. Amperometry provides high resolution to detect molecules released from single secretory vesicles and stability for long recordings. Amperometry is usually limited to cells that secrete an endogenous oxidizable molecule. We incubated cells for 40 min at room temperature in high concentrations of dopamine to force the exogenous monoamine to distribute passively into cytoplasm and acidic secretory vesicles. Cells were then transferred to a monoamine-free saline solution and measured. Vesicular release of the loaded dopamine was monitored as pulses of electric current generated by oxidation of the molecules at the tip of a carbon-fiber electrode polarized to +400 mV.

Single-Cell Photometry: The intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with the Ca^{2+}-sensitive fluorescent dye indo-1 AM. The excitation wavelength was 365 nm and fluorescence signals were recorded at 405 and 500 nm, using a pair of photon-counting photomultiplier tubes.