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ScienceDirect
Advanced Drug Delivery Reviews 58 (2006) 850–877
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Two-photon excitation imaging of exocytosis and endocytosis and determination of their spatial organization ☆

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Received 7 February 2006; accepted 13 July 2006
Available online 14 August 2006

Abstract

Two-photon excitation imaging is the least invasive optical approach to study living tissues. We have established two-photon extracellular polar-tracer (TEP) imaging with which it is possible to visualize and quantify all exocytic events in the plane of focus within secretory tissues. This technology also enables estimate of the precise diameters of vesicles independently of the spatial resolution of the optical microscope, and determination of the fusion pore dynamics at nanometer resolution using TEP-imaging based quantification (TEPIQ). TEP imaging has been applied to representative secretory glands, e.g., exocrine pancreas, endocrine pancreas, adrenal medulla and a pheochromocytoma cell line (PC12), and has revealed unexpected diversity in the spatial organization of exocytosis and endocytosis crucial for the physiology and pathology of secretory tissues and neurons. TEP imaging and TEPIQ analysis are powerful tools for elucidating the molecular and cellular mechanisms of exocytosis and certain related diseases, such as diabetes mellitus, and the development of new therapeutic agents and diagnostic tools.

Keywords: Insulin secretion; Pancreatic islet; Exocrine gland; Adrenal medulla; Chromaffin cell; Synapse; Pheochromocytoma cell; Compound exocytosis

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Multi-Photon Imaging: Diseases and Therapies", Vol., 58/7, 2006.

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doi:10.1016/j.addr.2006.07.008

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1. Introduction

Exocytic secretion is one of the fundamental cellular mechanisms for the delivery of biosynthetic materials contained in cytosolic vesicles to the extracellular space [1,2]. Exocytosis involves the transport of secretory vesicles to target regions in the plasma membrane, the docking of vesicles to the plasma membrane and fusion of the two biological membranes, which may be followed by compound exocytosis on the fused vesicles and/or endocytosis. Exocytosis is regulated by cytosolic Ca^{2+}, cAMP and