Progress in Biophysics and Molecular Biology 107 (2011) 293–303

Contents lists available at SciVerse ScienceDirect

Progress in Biophysics and Molecular Biology

journal homepage: www.elsevier.com/locate/pbiomolbio

Review

Coupling of metabolic, second messenger pathways and insulin granule dynamics in pancreatic beta-cells: A computational analysis

Leonid E. Fridlyand*, Louis H. Philipson

Department of Medicine and Pediatrics, The Kovler Diabetes Center, The University of Chicago, Chicago, IL 60637, USA

ARTICLE INFO

Article history:
Available online 8 September 2011

Keywords:
Calcium
cAMP
Exocytosis
Diabetes
Mathematical model

ABSTRACT

Insulin secretory responses to nutrient stimuli and hormonal modulators in pancreatic beta-cells are controlled by a variety of secondary messengers. We have analyzed numerous mechanisms responsible for regulated exocytosis in these cells and present an integrated mathematical model of cytosolic Ca^{2+}, cAMP and granule dynamics. The insulin-containing granules in the beta-cell were divided into four classes: a large "reserve" granule pool, a smaller pool of the morphologically docked granules that is chemically 'primed' for release or the "readily releasable pool", and a pool of "restless newcomer granules" that undergoes preferential exocytosis. The model incorporates glucose and other aspects of metabolism, the cAMP amplifying pathway, insulin granule dynamics and the exocyst concept for granule binding. The values of most of the model parameters were inferred from available experimental data. The model can generate both the fast first phase and slow biphasic insulin secretion found experimentally in response to a step increase of membrane potential or of glucose. The numerical simulations have also reproduced a variety of experimental conditions, such as periodic stimulation by high K^+ and the potentiation induced in islets by pre-incubation with cAMP pathway activators. The explicit incorporation of Ca^{2+} channels, Ca^{2+} and cAMP dynamics allows the model to be further connected to current models for calcium and metabolic dynamics and provides an interpretation of the roles of the triggering and amplifying pathways of glucose-stimulated insulin secretion. The model may be important in the identification of pharmacological targets for improving insulin secretion in type 2 diabetes.

Contents

Abbreviations: AC, adenylyl cyclase; $[Ca^{2+}]_c$, cytoplasmic Ca^{2+} concentration; DGP, docked granule pool; EPAC, guanyl exchange proteins; f_{MF}, metabolic activation factor; GLP-1, glucagon-like peptide-1; [Glu], glucose concentration; IS, insulin secretion; K_{ATP}, channels ATP-sensitive K^+ channels; NGP, restless newcomer granule pool; PKA, protein kinase A; PM, plasma membrane; TPR, target protein pool for RRP; RRP, readily releasable granule pool; RGP, reserve granule pool; SNARE, soluble NSF attachment protein receptor; SM, supplemental material; TDP, target protein pool for docking and priming; T2DM, type 2 diabetes; VDCCs, voltage-dependent Ca^{2+} channels; VP, plasma membrane potential; VVP, special vesicular protein pool for binding with NGP granules.

* Corresponding author. Department of Medicine and Pediatrics, Section of Endocrinology, The University of Chicago, The Knapp Center for Biological Discovery, 900 E 57th St, Chicago, IL 60637, USA. Tel.: +1 773 702 1661; fax: +1 773 702 9194.
E-mail address: lfridlia@medicine.bsd.uchicago.edu (L.E. Fridlyand).

0079-6107/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pbiomolbio.2011.09.001

1. Introduction

Insulin plays a central role in fuel homeostasis. It is secreted by the islets of Langerhans in the pancreas from β-cells by regulated exocytosis. In β-cells insulin is stored in large dense core vesicles. In regulated exocytosis, secretory granules fuse with the plasma membrane (PM) releasing their contents in the extracellular space through the fusion pore in response to a stimulus.

Exocytosis is tightly controlled by several key signals in pancreatic β-cells. A rise in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) following an increase in the external glucose concentration is the main physiological trigger for fusion of the insulin-containing granules (Eliasson et al., 2008; Seino et al., 2009). However, other intracellular metabolites and messengers, including cAMP, also regulate or modulate Ca^{2+}-triggered exocytosis (Henquin, 2009; Seino et al., 2009; Straub and Sharp, 2002). Gut hormones (incretins) such as glucagon-like peptide-1 (GLP-1), GIP and others activate cAMP signaling and enhance or accelerate exocytosis (Eliasson et al., 2008; Henquin, 2009; Leech et al., 2010; Seino et al., 2009).

Insulin secretion (herein termed IS) in response to a fast stepwise increase in extracellular glucose concentrations is biphasic. When extracellular glucose increases from basal to stimulating glucose a sharp and transient increase in the IS rate is observed in the first few minutes after a lag period, and this is referred to as the first phase. When high glucose concentrations are maintained, this is followed by a sustained rate lasting for a few hours (the second phase of IS). Different metabolic and messenger pathways can affect on these phases (Henquin, 2009; Seino et al., 2009; Straub and Sharp, 2002).

New techniques have recently emerged, such as the use of fluorescent proteins that can be targeted to secretory granules, to enable real-time imaging of granule trafficking in living cells. This has produced a great leap forward in our understanding of the exocytotic events in IS *in vitro* (Eliasson et al., 2008; Jensen et al., 2008; Seino et al., 2009). However, our understanding of the causal activating and potentially interfering interrelationships between different receptors, Ca^{2+}, cAMP, other signaling pathways and insulin exocytosis is still incomplete.

In order to describe IS dynamics and test various regulating hypotheses, mathematical models based upon them can be constructed thus allowing us to test whether the simulated results are in accordance with experimental values. Several mathematical approaches in the literature have provided quantitative estimates of the exocytotic processes in pancreatic β-cells (Bertuzzi et al., 2007; Chen et al., 2008; Pedersen et al., 2008; Pedersen and Sherman, 2009; Tsaneva-Atanasova et al., 2010). However, the proposed models still fall short of a comprehensive explanation of existing data and do not include any mechanisms of incretin hormone action or other recent experimental data (see Discussion).

We therefore sought to develop a quantitative, kinetic model of core exocytotic processes of the β-cell and their regulatory mechanisms. Our aim was to develop a mathematical model for the coupled processes of granule dynamics, granule fusion processes and their regulation on a cellular level that can be used for an interpretation of existing results and to design new experiments. We have attempted to integrate and analyze the existing hypotheses using experimental data and thereby evaluate the roles of metabolic, Ca^{2+} and cAMP messenger pathways in the regulation of IS. However, the exocytosis system in β-cells is highly complex and existing experimental data remain inadequate for a sufficiently detailed mathematical consideration. For this reason we were forced to introduce several proposals based on indirect evidence from other systems rather than direct experimental data to fill the numerous gaps in our understanding of these processes. Only in this way can we include terms to make a useful and testable model.

Type 2 diabetes (T2DM) is progressive disease with numerous complications and a huge economic cost. T2DM arises in part due to the failure of β-cells to compensate for an increase in insulin resistance but the basic factors are clearly polygenic and environmental (Ferrannini, 2010; Smith et al., 2010). Defective IS from the pancreatic β-cells clearly contributes to T2DM and monogenic diabetes (Bell and Polonsky, 2001; Rorsman and Renstrom, 2003). For this reason there is considerable interest in understanding how β-cells regulate insulin granule exocytosis. Here we describe development and validation of an *in silico* approach to further understanding this process.

2. Methods

The literature regarding the machinery regulating exocytosis has been recently reviewed (Eliasson et al., 2008; Seino et al., 2009). Here we will briefly analyze the evidence for functional insulin granule pools, the processes of granule trafficking and fusion and their role in regulation of IS. A simplified map of the modeled biochemical steps, second messenger pathways and insulin granule trafficking is schematized in Fig. 1. The detailed equations underlying the mathematical model are presented in Supplemental material (SM).

2.1. Mechanisms of PM depolarization, Ca^{2+} increase and cAMP pathway activation

The consensus model of glucose-stimulated IS (Fig. 1) begins with β-cells glucose uptake via a specific glucose transporter following increased extracellular glucose. Intracellular glucose is promptly phosphorylated by glucokinase, thereby initiating glycolysis and metabolites enter the Krebs cycle. Subsequently, mitochondrial metabolism generates ATP leading to an increase of the ATP/ADP concentration ratio, leading to closure of ATP-sensitive K^+ (K_{ATP}) channels. The resulting decrease in K^+ efflux causes PM depolarization, followed by opening of voltage-gated Ca^{2+} channels (VDCCs) and Ca^{2+} influx into the cell, which triggers exocytosis of insulin granules (Eliasson et al., 2008; Fridlyand and Philipson, 2010). Additional processes can release Ca^{2+} from intracellular stores (Fridlyand et al., 2010; Gilon and Henquin, 2001).