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Review

Mathematical modeling and statistical analysis of calcium-regulated insulin granule exocytosis in β-cells from mice and humans

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ABSTRACT

Insulin is released from pancreatic β-cells as a result of Ca^{2+}-evoked exocytosis of dense-core granules. Secretion is biphasic, which has been suggested to correspond to the release of different granule pools. Here we review and carefully reanalyze previously published patch-clamp data on depolarization-evoked Ca^{2+}-currents and corresponding capacitance measurements. Using a statistical mixed-effects model, we show that the data indicate that pool depletion is negligible in response to short depolarizations in mouse β-cells. We then review mathematical models of granule dynamics and exocytosis in rodent β-cells and present a mathematical description of Ca^{2+}-evoked exocytosis in human β-cells, which show clear differences to their rodent counterparts. The model suggests that L- and P/Q-type Ca^{2+}-channels are involved to a similar degree in exocytosis during electrical activity in human β-cells.

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1. Introduction

The polypeptide insulin is released from pancreatic β-cells at high glucose concentrations to lower blood-sugar levels by promoting glucose uptake by muscles and adipose tissues in addition to reducing glucose output from the liver. The release of insulin is biphasic; a first rapid phase of secretion is followed by a second phase with slower release (Curry et al., 1968). Prior to release insulin is contained in secretory granules. These granules are transported to the plasma membrane via the microtubules (Varadi et al., 2002; Ivarsson et al., 2004), and actin networks (Ivarsson et al., 2005). At the plasma membrane the granules are docked and primed through Ca^{2+}, temperature- and ATP-dependent processes (Proks et al., 1996; Renström et al., 1996; Eliasson et al., 1997). Ultrastructural analysis (Olofsson et al., 2002) in single mouse β-cells has revealed that ~700 out of the total ~10 000 granules are docked to the plasma membrane. Of these ~200 are primed and readily releasable.

The main pathway of glucose-stimulated insulin secretion is well-established (Ashcroft and Rorsman, 1989; Misler et al., 1992; Henquin, 2000). Glucose enters the β-cell through GLUT glucose

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transporters and is then metabolized. The resulting increase in the ATP-to-ADP ratio leads to the closure of ATP-sensitive K^+-channels, which allows a leak current to depolarize the plasma membrane and trigger electrical activity. The depolarizations open voltage-dependent Ca^{2+}-channels, and once the intra-cellular Ca^{2+} concentration increases by influx of Ca^{2+} through the Ca^{2+}-channels (Ammälä et al., 1993b) the granules fuse with the plasma membrane and insulin is released.

The understanding of processes involved in exocytosis of insulin granules has increased tremendously during the last 20 years through the introduction of techniques such as carbon fiber amperometry (Smith et al., 1995), TIRF microscopy (Ohara-Imaizumi et al., 2004), and live confocal imaging (Ivarsson et al., 2004), but also capacitance measurements in combination with the patch-clamp technique (Gillis and Misler, 1992). Using the latter method, we and others have suggested that granules can be divided into different functional pools prior to release [see e.g. Eliasson et al. (2008)]. Granules docked and primed at the plasma membrane belong to the readily releasable pool (RRP). A subset of this pool termed the immediately releasable pool (IRP) is situated in close contact to the L-type Ca^{2+}-channels [~10 nm distance; Wiser et al. (1999)]. Granules within the IRP are accordingly situated within the Ca^{2+}-channel microdomains. The majority of the granules belong to a reserve pool (RP) and these need to be transported, docked and primed prior to release. On a cellular level it has been suggested that first phase insulin secretion is equivalent to release of granules from the IRP/RRP and second phase insulin secretion correspond to the release of granules from the RP (Daniel et al., 1999; Eliasson et al., 2008). Interestingly, first phase insulin secretion is lacking in patients with type 2 diabetes (Hosker et al., 1989) why studies investigating the control of the IRP/RRP is of great importance.

In addition to the triggering pathway described above, an amplifying pathway is acting to augment insulin release (Henquin, 2000). The amplifying pathway is also referred to as the KATP-independent pathway, and it is not activated until the triggering pathway has depolarized the membrane and increased intracellular Ca^{2+}. The amplifying pathway can be investigated under depolarized conditions bypassing the KATP-channels such as when stimulating insulin secretion using high K^+ in presence of diazoxide (Gembal et al., 1992) or performing patch-clamp capacitance measurements (Gillis and Misler, 1992; Ammälä et al., 1993b) The most known amplifying factors are ATP (Eliasson et al., 1997) and cAMP (Gillis and Misler, 1993; Renström et al., 1997). Other potentiating factors include Malonyl-CoA (Prentki et al., 1992), glutamate (Maechler and Wollheim, 1999) and NADPH (Ivarsson et al., 2005).

The exocytotic response in pancreatic β-cells can also be enhanced by agents such as the incretins glucagon-like peptide-1 (GLP-1) (Gromada et al., 1998) and glucose-dependent insulinotropic polypeptide (GIP) (Ding and Gromada, 1997). These hormones increase the intra-cellular cAMP-concentration by binding to respective G-protein coupled receptors and activation of adenylate cyclases. Adenylate cyclases can also be activated by the pharmacological compound forskolin to generate cAMP. Potentiation of insulin exocytosis by cAMP involves both activation of protein kinase A (PKA) (Renström et al., 1997) and Epac 2 (Exchange protein associated with cAMP 2) (Ozaki et al., 2000). Using capacitance measurements, we have suggested that PKA stimulates mobilization whereas Epac 2 is involved in rapid exocytosis of IRP/RRP granules (Eliasson et al., 2003). Conditional for rapid cAMP-dependent exocytosis is also the presence of the SNARE-protein SNAP-25, which binds to Epac 2 (Vikman et al., 2009). Epac has further been suggested to bind to piccolo (Fujimoto et al., 2002) and to associate with the plasma membrane sulphonylurea receptor SUR1 (Eliasson et al., 2003).

Here we have carefully reanalyzed previously published data (Eliasson et al., 2003) with attention to the positive effect of forskolin on exocytosis by using a statistical mixed-effects model (Pinheiro and Bates, 2000). We find that the data show a linear relation between depolarization-evoked membrane capacitance increase (ΔC_m) and the Ca^{2+}-charge entering during a depolarization (Q), and argue that this indicates that pool depletion is negligible in response to short depolarizations in mouse β-cells. A mathematical model of exocytosis in human β-cells is also presented. Simulations show that human exocytosis data are well reproduced by assuming that granules fuse away from Ca^{2+}-channels. We find that during electrical activity L- and P/Q-type Ca^{2+}-channels contribute similarly to exocytosis in human β-cells.

Parts of this work have been published previously in abstract form (Pedersen, 2010c).

2. Methods

Capacitance measurements and Ca^{2+}-entry data from mouse β-cells were taken from Eliasson et al. (2003), where details on the experiments are provided. Briefly, using the perforated-patch technique, pulses of increasing length (5–850 ms) were applied to isolated single mouse β-cells, and resulting Ca^{2+}-currents and increases in membrane capacitance were recorded. The data in the absence and presence of 2 μM forskolin was used for the statistical analysis. Ca^{2+}-currents were inspected visually, and data with large leak currents or high amounts of noise were discarded for the analysis.

In a first statistical analysis, we fitted linear models for regression of ΔC_m on Q, taking the differences between cells into account. Because a significant between-cell variation was present within both the control and forskolin-stimulated groups, a second more appropriate analysis was performed by fitting a linear mixed-effects model (Pinheiro and Bates, 2000), with treatment group as fixed effect and cell as random effect, to the data. Mixed-effects models are more appropriate for representing clustered data, such as in our case where several observations are done on each cell. The final model was of the form

$$\Delta C_{m,ij} = (\beta + \beta_{FSK} \cdot FSK_i + b_i)Q_{ij} + \varepsilon_{ij}, \quad \varepsilon_{ij} \sim N(0,\sigma), b_i \sim N(0,\sigma_b), \tag{1}$$

for observation j of cell i, where ε_{ij} is a normally distributed error term, FSK_i is a covariate indicating whether cell i was exposed to forskolin, b_i is a factor allowing for cell-to-cell variation, and the parameters β, β_{FSK}, σ and σ_b are to be estimated. The contrast to traditional modeling lies in the introduction of the mixed-effect via b_i. The statistical software R was used, in particular the lme function of the nlme R-package (Pinheiro and Bates, 2000). Parameter estimates are given with standard errors and p-values from two-sided t-tests.

The mathematical model for human β-cells was implemented in XPPAUT (Ermentrout, 2002). Computer code for XPPAUT can be downloaded from http://www.dei.unipd.it/pedersen. The calcium fluxes were modeled by a two-compartment model. Calcium flows into a submembrane space through voltage-dependent channels, and diffuses into a general cytosolic compartment. Extrusion of Ca^{2+} from the cell occurs from the submembrane compartment with flux $J_{pmca} + J_{ncx}$, and exchange with the ER happens from the cytosolic compartment with flux $J_{serca} + J_{leak}$. This leads to

$$\frac{d\mathrm{Ca}_{mem}}{dt} = f\left(-\alpha \mathrm{I}_{Ca}/v_{mem} - B/f_v(\mathrm{Ca}_{mem} - \mathrm{Ca}_i) - \left(J_{pmca} + J_{ncx}\right)\right), \tag{2}$$

$$\frac{d\mathrm{Ca}_i}{dt} = f(B(\mathrm{Ca}_{mem} - \mathrm{Ca}_i) - (J_{serca} + J_{leak})). \tag{3}$$

Here $f = 0.01$ is the ratio of free-to-bound Ca^{2+}, $\alpha = 5.18 \times 10^{-12}$ μmol/s/pA changes current to flux,