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Mathematical modeling of insulin secretion and the role of glucose-dependent mobilization, docking, priming and fusion of insulin granules

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Abstract

In this paper we develop a new mathematical model of glucose-induced insulin secretion from pancreatic islet β -cells, and we use this model to investigate the rate limiting factors. We assume that insulin granules reside in different pools inside each β -cell, and that all β -cells respond homogeneously to glucose with the same recruitment thresholds. Consistent with recent experimental observations, our model also accounts for the fusion of newcomer granules that are not pre-docked at the plasma membrane. In response to a single step increase in glucose concentration, our model reproduces the characteristic biphasic insulin release observed in multiple experimental systems, including perfused pancreata and isolated islets of rodent or human origin. From our model analysis we note that first-phase insulin secretion depends on rapid depletion of the primed, release-ready granule pools, while the second phase relies on granule mobilization from the reserve. Moreover, newcomers have the potential to contribute significantly to the second phase. When the glucose protocol consists of multiple changes in sequence (a so-called glucose staircase), our model predicts insulin spikes of increasing height, as has been seen experimentally. This increase stems from the glucose-dependent increase in the fusion rate of insulin granules at the plasma membrane of single β -cells. In contrast, previous mathematical models reproduced the staircase experiment by assuming heterogeneous β -cell activation. In light of experimental data indicating limited heterogeneous activation for β -cells within intact islets, our findings suggest that a graded, dose-dependent cell response to glucose may contribute to insulin secretion patterns observed in multiple experiments, and thus regulate in vivo insulin release. In addition, the strength of insulin granule mobilization, priming and fusion are critical limiting factors in determining the total amount of insulin release.

Keywords

Insulin secretion; Insulin granule dynamics; Multiple pool; β -cells; Diabetes

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1. Introduction

Insulin is stored in granules in the β -cells that reside in the pancreatic islets of Langerhans, and these granules are secreted via exocytosis in response to elevated glucose levels. The concept of insulin granules' belonging to functionally different pools has been proposed as a way to explain the characteristic biphasic insulin secretion pattern that occurs in response to a sudden step increase in glucose concentration (Rorsman and Renstrom, 2003; Wang and Thurmond, 2009). The biphasic nature of the insulin response was first observed by Curry et al. (1968), and later experiments have shown that in Type 2 diabetes the amount of insulin secreted during the first phase, in particular, is severely reduced (Nagamatsu et al., 2006; Ohara-Imaizumi et al., 2004; Rorsman and Renstrom, 2003). Within models based on the multiple-pool concept, the first-phase, rapid spike of released insulin is attributed to secretion of granules from a pool of readily releasable granules, while the subsequent, slowly increasing phase of insulin release involves granules which, via a series of glucose-induced reactions, become release-ready over time (Rorsman and Renstrom, 2003; Wang and Thurmond, 2009). Replenishment of the readily releasable pool (RRP) could involve physical translocation, or *mobilization*, of granules from the cell interior, with movement occurring along microtubules within the β -cell. Replenishment could also depend on remodeling of filamentous actin (F-actin) (Wang and Thurmond, 2009), and/or chemical reactions, so-called *priming*, possibly reliant on ATP-dependent granular acidification (Barg et al., 2001; Wang and Thurmond, 2009). While originally the RRP was thought to comprise a subset of granules that are pre-docked at the plasma membrane (Rorsman and Renstrom, 2003; Wang and Thurmond, 2009), recent experiments using total internal reflection fluorescence microscopy (TIRFM) have revealed that the first phase, though mostly originating from pre-docked granules, may also involve granules that approach the plasma membrane from the cell interior and almost instantaneously undergo fusion, the so-called "newcomers" (Nagamatsu et al., 2006; Ohara-Imaizumi et al., 2004, 2007; Shibasaki et al., 2007). Moreover, insulin secretion during the second phase may be contributed mainly by newcomers (Ohara-Imaizumi et al., 2004). Hence stable docking does not appear to be a prerequisite for granule fusion (Allersma et al., 2006; Burchfield et al., 2010; Degtyar et al., 2007; Holz and Axelrod, 2008; Kasai et al., 2008; Nagamatsu et al., 2006; Ohara-Imaizumi et al., 2004, 2007; Shibasaki et al., 2007; Verhage and Sorensen, 2008). However, the ability to retain an adequate pool of docked granules seems important since Type 2 diabetes is associated with both a smaller number of docked granules and a significantly reduced first-phase insulin secretion (Nagamatsu et al., 2006; Ohara-Imaizumi et al., 2004).

Although a normal β -cell contains a large pool of insulin granules (about 10,000), under normal physiological conditions only a small fraction will ever be secreted (Rorsman and Renstrom, 2003). For instance, the total insulin secretion in a 120-minute period of exposure to normal glucose stimulus accumulates to only about 650 granules (less than 10 percent of the total amount) (Bertuzzi et al., 2007). Since one important pathophysiological feature of Type 2 diabetes is insufficient insulin secretion under normal physiological stimulation, it is of clinical interest to investigate the insulin granule trafficking dynamics, in order to understand the limiting factors that prevent the β -cells from tapping into the vast granule reserve. Recently, insulin secretion dynamics have been explored using mathematical

models based on the idea that the granules belong to different pools (Bertuzzi et al., 2007; Chen et al., 2008; Pedersen and Sherman, 2009; Pedersen et al., 2008). For example, building on the rather detailed model in Chen et al. (2008), in which fusion and insulin secretion are Ca^{2+} -dependent, Pedersen and Sherman (2009) showed how the inclusion of a pool of highly Ca^{2+} -sensitive insulin granules (Wan et al., 2004; Yang and Gillis, 2004) allows for second-phase insulin secretion that stems from fusion of newcomers.

The earliest mathematical model of insulin secretion based on the multiple-pool concept was developed in Grodsky (1972). This model includes one small, labile insulin compartment from which insulin is rapidly released upon stimulation, and one stable compartment from which refilling of the labile compartment gradually occurs. To explain the staircase experiment, in which insulin is secreted in spikes of increasing heights as the glucose concentration is increased in a step-wise fashion, Grodsky further proposed that the labile insulin is not stored in a single homogeneous compartment (which presumably would be depleted and not allow secretion of more insulin at higher glucose steps). Instead, the labile insulin is stored in “packets” whose individual sensitivity to glucose follows a bell-shaped distribution. Secretion of insulin from these packets rapidly occurs when the glucose concentration exceeds their sensitivity threshold.

A later model similar to the Grodsky model in that it assumes a heterogeneous activation of insulin secretion has been presented in Pedersen et al. (2008). However, instead of assigning individual activation thresholds to “packets of insulin,” Pedersen et al. based their model on experimental observations suggesting that individual cells within a population of β -cells may exhibit different activation/recruitment thresholds (Pedersen et al., 2008). The authors assumed that at a given level of glucose, only a fraction of the β -cells are active and secrete insulin with the same rate constant. Moreover, the percentage of activated cells is given by a glucose-dependent sigmoidal curve. The model is able to reproduce the above mentioned staircase experiment, the reproduction of which hinges on the model’s assumption of heterogeneous recruitment thresholds within the β -cell population.

In general, the mechanisms underlying the glucose-dependent insulin release response are not fully understood and two non-exclusive models have been proposed: (1) individual β -cells respond to similar recruitment thresholds, and increase the magnitude of their response as glucose is increased; and (2), individual β -cells respond to different recruitment thresholds, without changing the magnitude of their response as glucose is increased (Nesher and Cerasi, 2002; Salgado et al., 2000). Experimental observations supporting the notion that β -cells may activate at different glucose levels include the investigation presented in Jonkers and Henquin (2001), in which the percentage of active β -cells (i.e. cells showing a rise in cytoplasmic Ca^{2+} -concentration) was shown to increase with increasing glucose concentrations. However, while maximal recruitment occurred at relatively low glucose concentrations, especially when cells were connected in clusters (as β -cells normally are, within islets), insulin secretion continued to increase as glucose was further increased (Jonkers and Henquin, 2001). Moreover, insulin secretion was temporally and quantitatively correlated with the average cytoplasmic Ca^{2+} -concentration, which gradually increased in response to augmented glucose levels. Thus the β -cells did not respond in an all-or-nothing

fashion, and insulin secretion dynamics could not be completely explained by glucose-induced heterogeneous recruitment (Jonkers and Henquin, 2001).

Because of the varying results and difficulties in experimentally testing the first hypothesis in particular (Salgado et al., 2000), in this paper we present a model to investigate it mathematically. By assuming that the rate of fusion within each β -cell is an increasing function of the glucose concentration, we obtain model solutions that allow for successive insulin peaks of higher magnitude during the staircase experiment, even as the pools of insulin granules themselves are being depleted. Furthermore, supported by recent experimental observations, we include fusion from pre-docked granules as well as newcomers. While basing model assumptions on biological evidence, our modeling approach is aimed at simplicity, and we therefore only introduce the main regulatory factor, glucose, as a driving force behind insulin granules dynamics. Because of the relative simplicity of our model, we hope to gain some understanding of the mechanisms behind the very intricate insulin secretion patterns that may be observed.

We organize the rest of our paper as follows. In the next section we develop our model, which consists of a system of time-dependent ordinary differential equation (ODEs). We then analyze our model in Section 3, and conclude in Section 4 with a discussion of our results.

2. Model development

Although insulin secretion is dependent on multiple biological processes and signaling pathways, the main regulatory factor is glucose (MacDonald et al., 2005; Rorsman and Renstrom, 2003; Wang and Thurmond, 2009). In response to increasing extracellular glucose levels, for example after a meal or an intravenous injection in a clinical setting, glucose is transported into the β -cells via glucose transporters located at the cell surface (MacDonald et al., 2005; Rorsman and Renstrom, 2003; Wang and Thurmond, 2009). Inside the β -cells, glucose is subsequently metabolized, leading to an increase in the ATP/ADP ratio and closure of ATP-sensitive K^+ -channels situated at the plasma membrane. The closure of these channels results in depolarization of the cell membrane, opening of voltage-operated Ca^{2+} -channels and influx of Ca^{2+} (MacDonald et al., 2005; Rorsman and Renstrom, 2003; Wang and Thurmond, 2009). The rise in cytoplasmic Ca^{2+} induces fusion of insulin granules with the plasma membrane. Subsequently, upon sufficient dilation of the fusion pore, insulin is secreted into the extracellular space (MacDonald et al., 2005; Rorsman and Renstrom, 2003; Wang and Thurmond, 2009).

In our model we divide the insulin granules into five subgroups depending on their properties: mobilized unprimed granules, $M_u(t)$; mobilized primed granules, $M_p(t)$; docked unprimed granules, $D_u(t)$; docked primed granules, $D_p(t)$; and fused granules, $F(t)$. The processes that are enhanced by glucose in a dose-dependent way are: mobilization of granules from the core of the β -cell; docking of granules to the cell membrane; priming of unprimed granules, which makes the granules release-competent; and, finally, fusion of primed granules. In line with recent experimental data we assume that the pool of primed, and thus release-ready, granules consists not only of a subpopulation of the docked granules

but also of a subpopulation of the mobilized ones, i.e. $RRP=M_p(t)+D_p(t)$. In Fig. 1 we show a schematic diagram of the model design, variables and parameters.

2.1. Mobilized unprimed granules, $M_u(t)$

We assume that the mobilized granules are situated somewhere between the core of the β -cell (where they are synthesized in the endoplasmic reticulum (Rorsman and Renstrom, 2003)) and the plasma membrane (where they ultimately may fuse). In the absence of glucose, unprimed granules are mobilized from a granule reserve in the core of the β -cell at a constant rate, m_0 . The reserve is assumed to be large compared to the other pools, so that its size can be considered a constant. In addition, it is known that glucose induces changes which aid the transport of granules toward the cell membrane (Wang and Thurmond, 2009). For example, remodeling of the β -cell F-actin web by glucose may allow intracellular granules to reach the plasma membrane more readily (Wang and Thurmond, 2009). We therefore introduce an additional mobilization term $\hat{m}(t)$ which is a saturating function of extracellular glucose, following the Hill equation (which has been utilized widely to model biological processes) (Hill, 1910; Murray, 2002). For glucose-induced mobilization to take place we assume that the level of extracellular glucose, $G(t)$, has to exceed a certain threshold, G_m , and that the number of mobilized granules is below a saturation level, $M_{u,max}$. We also assume that the mobilization is delayed by a time δ_m so that the rate of mobilization enhanced by glucose at time t depends on $G(t - \delta_m)$, and is given by

$$\hat{m}(t) = \tilde{m}(t) \left(1 - \frac{M_u}{M_{u,max}} \right),$$

where

$$\tilde{m}(t) = \begin{cases} \frac{m[G(t-\delta_m)-G_m]^n}{[G_{m50}-G_m]^n + [G(t-\delta_m)-G_m]^n}, & G(t-\delta_m) \geq G_m \\ 0, & G(t-\delta_m) < G_m \end{cases},$$

i.e.

$$\tilde{m}(t) = \frac{m[G(t-\delta_m)-G_m]^n}{[G_{m50}-G_m]^n + [G(t-\delta_m)-G_m]^n} H(G(t-\delta_m)-G_m)$$

where n is the Hill coefficient, m is the maximal rate of stimulated mobilization, G_{m50} is the level of extracellular glucose at which $\tilde{m}$ is half-maximal, and $H(x)$ is the Heaviside step function, $H(x)=0$ if $x<0$, and $H(x)=1$ if $x \geq 0$, which activates mobilization above the background level provided that the concentration of glucose is above the threshold, G_m . The introduction of a time delay, δ_m , is motivated by the fact that the (extracellular) glucose has to enter the cells via facilitated diffusion (which involves cell-membrane-situated glucose transporters, mainly GLUT-1 and GLUT-2) and has to be metabolized before it can exert its influence (Wang and Thurmond, 2009). The actual processes leading to reorganization of the F-actin web and facilitation of granule mobilization may also take some time.

In addition to mobilization it is believed that the RRP is refilled via priming of granules. The exact mechanisms responsible for granule priming are not known, but the process is believed to be enhanced when ATP (Eliasson et al., 1997) or the ATP/ADP ratio is high (Olsen et al., 2003), and it may involve granular acidification (Barg et al., 2001; Renstrom et al., 2002). Since elevated levels of glucose lead to increased levels of ATP via glucose metabolism, we assume that at basal glucose level the unprimed mobilized granules undergo priming at the rate $p_{m0}M_u$, where p_{m0} is a constant, while at levels above G_p additional glucose-stimulated priming occurs with rate coefficient $\hat{p}(t)$ given by

$$\hat{p}_m(t) = \frac{p_m[G(t - \delta_p) - G_p]^n}{[G_{p50} - G_p]^n + [G(t - \delta_p) - G_p]^n} H(G(t - \delta_p) - G_p),$$

where p_m is the maximum rate and G_{p50} is the level of glucose at which the rate of priming of mobilized granules is half-maximal.

We assume that a fraction of the mobilized granules dock at the cell membrane at the rate $(d_0 + \hat{d}(t))M_u$. Similar to the priming rate we assume that d_0 is constant while

$$\hat{d}(t) = \frac{d[G(t - \delta_d) - G_d]^n}{[G_{d50} - G_d]^n + [G(t - \delta_d) - G_d]^n} H(G(t - \delta_d) - G_d).$$

Taken together the above factors affecting the unprimed mobilized pool lead to the following ODE:

$$\dot{M}_u = \underbrace{m_0 + \hat{m}(t)}_{\text{mobilization}} - \underbrace{(p_{m0} + \hat{p}_m(t))M_u}_{\text{priming}} - \underbrace{(d_0 + \hat{d}(t))M_u}_{\text{docking}} \quad (1)$$

2.2. Mobilized primed granules, $M_p(t)$

While relatively recent studies using new imaging techniques such as TIRFM have revealed that granules can travel to the cell membrane and rapidly fuse (within 50 ms), it is not known whether these newcomer granules are already primed when they arrive at the cell membrane or if they dock and become primed in rapid succession. Since, as explained in the previous section, we have assumed that priming of mobilized granules does occur, here we assume that newcomer granules arrive for fusion at the plasma membrane in a primed state. For simplicity we also neglect the possibility that a fraction of the primed mobilized granules may dock and fuse some time later. As with mobilization, priming and docking, we assume that the rate of fusion is $(f_{m0} + \hat{f}_m(t))M_p$, where f_{m0} is the constant glucose-independent basal rate, and

$$\hat{f}_m(t) = \frac{f_m[G(t - \delta_f) - G_f]^n}{[G_{f50} - G_f]^n + [G(t - \delta_f) - G_f]^n} H(G(t - \delta_f) - G_f),$$

where G_{f50} is the level of glucose at which the above-basal rate of fusion of primed mobilized granules is half-maximal, $f_m/2$. By considering the rate of priming (from Eq. (1)), and the rate of fusion, we arrive at the following equation for the evolution of M_p :

$$\dot{M}_p = \underbrace{(p_{m0} + \hat{p}_m(t)) M_u}_{\text{priming}} - \underbrace{(f_{m0} + \hat{f}_m(t)) M_p}_{\text{fusion}}. \quad (2)$$

2.3. Docked unprimed granules, $D_u(t)$

We assume that the change in the number of docked unprimed granules is governed by

$$\dot{D}_u = \underbrace{(d_0 + \hat{d}(t)) M_u}_{\text{docking}} - \underbrace{(p_{d0} + \hat{p}_d(t)) D_u}_{\text{priming}}. \quad (3)$$

The unprimed docked granules undergo priming at the rate $p_{d0}D_u$ at basal glucose level, while at glucose levels above G_p additional priming is induced with a rate coefficient $\hat{p}_d(t)$ given by

$$\hat{p}_d(t) = \frac{p_d [G(t - \delta_p) - G_p]^n}{[G_{p50} - G_p]^n + [G(t - \delta_p) - G_p]^n} H(G(t - \delta_p) - G_p),$$

where the half-maximal above-basal priming rate, $p_d/2$, occurs at glucose concentration G_{p50} .

2.4. Docked primed granules, $D_p(t)$

As above for primed mobilized granules, we introduce fusion from primed docked granules by assuming that it occurs at a rate that is proportional to D_p with a coefficient consisting of a glucose-independent basal rate, f_{d0} , and a glucose-dependent portion, $\hat{f}_d(t)$, where

$$\hat{f}_d(t) = \frac{f_d [G(t - \delta_f) - G_f]^n}{[G_{f50} - G_f]^n + [G(t - \delta_f) - G_f]^n} H(G(t - \delta_f) - G_f),$$

with half-maximal rate $f_d/2$ at glucose concentration G_{f50} . Combining the effects of priming and fusion gives the following equation for the docked primed granules:

$$\dot{D}_p = \underbrace{(p_{d0} + \hat{p}_d(t)) D_u}_{\text{priming}} - \underbrace{(f_{d0} + \hat{f}_d(t)) D_p}_{\text{fusion}}. \quad (4)$$

2.5. Fused granules, $F(t)$

With fusion from both primed mobilized and primed docked granules, the pool of fused granules increases according to the fusion terms in Eqs. (2) and (4). By assuming that the

number of fused granules decreases due to secretion at rate sF , where s is a positive constant, the evolution of the fused granule pool is determined by the following ODE:

$$\dot{F} = \underbrace{f_{m0} + \hat{f}_m(t)}_{\text{fusion}} M_p + \underbrace{(f_{d0} + \hat{f}_d(t))}_{\text{fusion}} D_p - \underbrace{sF}_{\text{secretion}}. \quad (5)$$

We remark that there is some delay between the event of granule fusion at the cell membrane and insulin release into the extracellular space. However, because this delay is on the order of seconds (Rorsman and Renstrom, 2003), which is faster than the time-scale relevant for insulin dynamics, we ignore it and assume that secretion is proportional to the current level of F .

2.6. Insulin secretion rate, ISR

In our model the number of granules secreted by a single β -cell per unit time is given by the term sF in Eq. (5). We assume that the total insulin secretion rate is given by

$$ISR(t) = N_\beta I_g sF(t), \quad (6)$$

where I_g is the insulin content of one granule and N_β is the number of β -cells. In general, if the heterogeneity of the cell population were taken into account, then $F(t)$, and therefore the insulin secretion, would vary between individual cells, say $F_i(t)$ for cell i , with the total insulin secretion given by a summation over all cells. However, since a main focus of this paper is to investigate what types of insulin secretion dynamics are possible from a homogeneous β -cell population, throughout this paper we assume that $F(t)$ is the same in all cells.

3. Results

From Eqs. (1)–(5) we notice that this is a system of linear non-homogeneous differential equations with time-dependent (non-linear) coefficients; i.e., it can be written as

$$\dot{\mathbf{x}} = \mathbf{A}(t) \mathbf{x} + \mathbf{g}(t) \quad (7)$$

where

$$\mathbf{A}(t) = \begin{bmatrix} -p_{m0} - \hat{p}_m(t) - d_0 - \hat{d}(t) - \tilde{m}(t)/M_{u,max} & 0 & 0 & 0 & 0 \\ p_{m0} + \hat{p}_m(t) & -f_{m0} - \hat{f}_m(t) & 0 & 0 & 0 \\ d_0 + \hat{d}(t) & 0 & -p_{d0} - \hat{p}_d(t) & 0 & 0 \\ 0 & 0 & p_{d0} + \hat{p}_d(t) & -f_{d0} - \hat{f}_d(t) & 0 \\ 0 & f_{m0} + \hat{f}_m(t) & 0 & f_{d0} + \hat{f}_d(t) & -s \end{bmatrix} \quad (8)$$

If the extracellular glucose concentration is piecewise constant, then the time-dependent rate coefficients in Eq. (8) are also piecewise constant, and it is possible to obtain an analytical solution of the system. Assuming that the first change in the external glucose concentration occurs at $t=0$ and that δ_m , δ_p , δ_d and δ_f are nonzero, the solution will remain at basal values

(given by Eq. (9) below) for $t \in [0, \delta_{\min}]$, where $\delta_{\min}$ is the smallest of the time delays: $\delta_{\min} = \min\{\delta_m, \delta_p, \delta_d, \delta_f\}$. In general, since the rate coefficients in Eq. (8) are piecewise constant, the solution of Eq. (7) varies over a set of time intervals which are delineated by the external glucose protocol and the parameter values. In each interval, the solution of Eq. (7), with all entries of Eq. (8) being constant but interval-specific, is given by the sum of the particular and the homogeneous solution $\mathbf{x} = \mathbf{x}_p + \mathbf{x}_h$, where the particular solution, $\mathbf{x}_p$, is the steady state solution of Eq. (7) and the homogeneous solution $\mathbf{x}_h \rightarrow 0$ as $t \rightarrow \infty$ (since the eigenvalues of Eq. (8) are negative). In Appendix B we give the solution for a single glucose step with $\delta_m = \delta_p = \delta_d = \delta_f$, which is the simplest case.

In addition to the analytical solution, Eq. (7) may be solved numerically; in this paper we use a Runge–Kutta method (MATLAB's ODE solver ode45). The codes that generate all figures are available upon request. For our model simulations we estimated parameter values based on biological observations when possible; we discuss key parameters throughout the text, and we list the values in each figure legend. Throughout the text we state the glucose concentration in the unit mmol/l, while the glucose concentrations in some experiments, e.g. in O'Connor et al. (1980) are given in mg/dl (100 mg/dl = 5.56 mmol/l). We also refer the reader to the Appendix where we list model variables and parameters, along with their units, physiological value ranges and values used in our simulations, in Tables A1 and A2, respectively.

3.1. Basal secretion

Assuming that at the beginning of each glucose protocol, the system is at the steady state corresponding to a basal glucose level (i.e., for $t < 0$, $G(t) < \min(G_m, G_p, G_d, G_f)$), we use the following initial conditions:

$$\begin{aligned} M_u(0) &= \frac{m_0}{p_{m0} + d_0} \equiv M_{uss}^0 \\ M_p(0) &= \frac{p_{m0} m_0}{f_{m0}(p_{m0} + d_0)} \equiv M_{pss}^0 \\ D_u(0) &= \frac{d_0 m_0}{p_{d0}(p_{m0} + d_0)} \equiv D_{uss}^0 \\ D_p(0) &= \frac{d_0 m_0}{f_{d0}(p_{m0} + d_0)} \equiv D_{pss}^0 \\ F(0) &= \frac{m_0}{S} \equiv F_{ss}^0 \end{aligned} \quad (9)$$

where *ss* stands for steady state. From islet experiments (Fujimoto et al., 2000; Katayama et al., 1995), we estimate basal secretion to be 0.008 ng per minute per islet. By also assuming that a single granule contains 8 fg of insulin (Eliasson et al., 2008), and that an islet contains 1000 cells, the estimated basal secretion corresponds to $sF = 1$ granule per minute per cell, which also implies that the background mobilization is at that level, i.e. $m_0 = 1$ (see $F(0)$ in Eq. (9)). For our simulations below we use $N\beta I_g = 0.01$ in Eq. (6) as a scaling factor to estimate the islet insulin secretion. However, because of intrinsic variation in insulin content and in the number of active β -cells in different animals and species (Reaven et al., 1979), we use the islet insulin secretion value for illustrative purposes only. With one granule being released per minute in the basal state, we obtain the estimate of the secretion rate $s = 30 \text{ min}^{-1}$ by noting that the time between fusion and cargo release is 2 s (Rorsman and Renstrom, 2003); i.e., the secretion rate is $1/(2/60) = 30 \text{ min}^{-1}$.

3.2. Single step

Model solution to a single step glucose increase is given in Appendix B. In Fig. 2, we present a series of curves showing the secretion dynamics from differently sized step increases in glucose concentration. Since there is a roughly 1–2 min delay observed before insulin secretion is initiated by a step increase in extracellular glucose concentration (Rorsman and Renstrom, 2003; Straub and Sharp, 2002), we have fixed $\delta_f = 1$ min. By assuming that the additional delays associated with priming, docking and mobilization are negligible, we additionally chose $\delta_m = \delta_p = \delta_d = \delta_f$ (and we note that granule priming indeed occurs rapidly once the ATP/ADP ratio has been increased, acting with a latency of 400 ms (Eliasson et al., 1997)). Since the data from perfused rat pancreata (Grotsky, 1972; O'Connor et al., 1980) reveal that a step increase from basal to 2.78 mmol/l (or 50 mg/dl) does not result in any secretion over basal levels, whereas a step increase twice as big induces a pulse of insulin, we fixed the threshold for glucose-induced fusion at $G_f = 4.8$ mmol/l. Consistent with the data from Grotsky (1972), O'Connor et al. (1980), we note that a small step increase in the glucose concentration (from basal to 5.55 mmol/l) gives a single insulin pulse that declines to the background secretion level over time. In order to obtain a solution that ultimately declines to the basal level, we assumed that at this glucose level no glucose-induced mobilization takes place (by fixing $G_m = 6.8$ mmol/l), since then the steady-state solution which F (given by Eq. (A2) in Appendix B), and thus the insulin level, which is proportional to F , attain over time is equal to the basal level.

In Fig. 2, we observe that for higher glucose concentrations, the secretion is biphasic with an initial spike followed by a slowly increasing secretion, also consistent with experiments (Grotsky, 1972; O'Connor et al., 1980). From Eq. (A2) we note that without glucose-induced mobilization, the biphasic pattern would be lost, since an increase in secretion during the second phase is only possible if the steady-state level of the fused granule pool is higher than the basal level. In Fig. 2, inset, we note that it takes approximately 1000 min (17 h) for the system to approach its new steady state. Therefore, in normal laboratory studies, where insulin secretion is only observed for a few hours, the system would not have enough time to settle toward its equilibrium. In Fig. 3, we show the time evolutions of the different granule pools, noting that the first phase corresponds to a rapid reduction of the primed pools. From Eq. (5) we note that as glucose is suddenly increased, the extra glucose-induced fusion makes $\dot{F}$ positive since after the time delay of 1 min, $\hat{f}_m$ and $\hat{f}_d$ attain new, higher, constant values which cause the initial rise in F and insulin secretion. However, as F increases, the term $-sF$ becomes important, making $\dot{F}$ negative and causing F and the insulin secretion to decline. Over time D_p and M_p are slowly being refilled, causing $\dot{F}$ to become positive and the insulin secretion to rise.

In Figs. 2 and 3, we note that for these parameter values the system has not yet settled to its glucose-induced steady state at the end of the experiment. In Fig. 4, we have increased the maximum rate of glucose-induced priming of the mobilized unprimed granules and obtained an insulin secretion profile which more rapidly approaches steady state. We note that similar secretion profiles have been observed from rat islets (Straub and Sharp, 2002), whose insulin secretion reached a plateau after approximately 40 min.

In Fig. 5 panel A, we show how the number of fusion events (given by the positive terms in Eq. (5)) varies over time for a step increase of glucose for the parameter values used in Fig. 2. In experiments using TIRFM imaging, the number of fusion events per $200 \mu\text{m}^2$ in normal rat β -cells increased upon stimulation with 22 mmol/l glucose from approximately 2 at basal up to 14 during first phase and about 5 during the second phase (Ohara-Imaizumi et al., 2004). Assuming that a β -cell has a diameter of $13.2 \mu\text{m}$ (Straub et al., 2004) and a total spherical surface area of $547 \mu\text{m}^2$, the number of fusion events in Fig. 5 is comparable to the numbers given in Ohara-Imaizumi et al. (2004). Similar to the results of the experiments in Ohara-Imaizumi et al. (2004) (which were carried out for 15 min), we observe in Fig. 5 panel B that the fraction of fusion events stemming from docked granules is initially high before it declines. Over a longer time period the fraction slowly increases again; however, the contribution from docked granules is still less than in the initial phase. We note that in Fig. 5 we have chosen parameter values so that the docked and mobilized RRP contribute equally to the initial fusion events (in the experiments performed in Ohara-Imaizumi et al. (2007) 75 percent of the fusion events during the first phase originated from docked granules, while the rest originated from newcomers). By varying the parameters it is possible to obtain lower or higher contributions from the different pools. In this simulation the pool of primed mobilized granules gets refilled faster than the pool of primed docked granules, thus making newcomers contribute more significantly to granule fusion during the second phase of insulin secretion. By increasing the rate of glucose-induced docking of the unprimed mobilized granules, $\hat{d}(t)$, compared to priming, $\hat{p}_m(t)$, it is possible to obtain solutions for which the contribution from docked granules is more significant during the second phase and over longer times, as $t \rightarrow \infty$ (simulation not shown). The relative strength of priming versus docking determines whether priming wins the competition (granules mainly being directed to the right from the M_u pool in Fig. 1) or whether docking leads the race (granules mainly diverted downward from M_u in Fig. 1).

We note that in addition to the perfused rat pancreas (Grodsky, 1972; O'Connor et al., 1980) and islets (Fujimoto et al., 2000; Straub and Sharp, 2002; Straub et al., 2004), biphasic secretion has been observed in other experimental systems, for example human islets (Henquin et al., 2006), mouse pancreas (Vikman et al., 2009) and mouse islets (Dishinger et al., 2009); the secretion pattern is species-dependent with, for example, a smaller second phase in mouse islets than in rat islets (Zawalich et al., 2008).

3.3. Potentiation

Another secretion pattern which hinges on the refilling and priming is obtained in so-called potentiation experiments (O'Connor et al., 1980; Straub and Sharp, 2002). In Fig. 6, glucose is first increased and kept at a high level for one hour before being lowered to background levels for 5 min, and then raised to a high level a second time. Since the pools of unprimed granules, M_u and D_u , increase during the first high glucose period (see panel A), refilling of the RRP, M_p and D_p , occurs rapidly once glucose-induced fusion from the primed pools is switched off during the basal glucose phase (see panel B). Because at the end of the basal glucose phase the pool of primed granules is larger than it was initially, the second increase of glucose causes a higher insulin spike, as shown in panel D. In panel C we also note that for these parameter values the fusion events during the second spike of insulin secretion

mainly originate from the primed mobilized pool. As discussed above, by increasing glucose-induced docking it is possible to obtain solutions in which docked granules contribute more for large t and during the second spike. We note that for these particular parameter values there is a large increase in the size of the unprimed mobilized pool, M_u (panel A). We regard the mobilized pools as consisting of granules that are relatively near the plasma membrane without giving any strict definition. However, M_u (and M_p) would certainly include granules that are almost docked, a pool that has been estimated to consist of approximately 2000 granules (Rorsman and Renstrom, 2003). While Straub et al. reported a 50 percent increase in the docked population (from approximately 700 to 1060 granules) 40 min after glucose had been raised from 5.6 mmol/l to 16.7 mmol/l (Straub et al., 2004), we are not aware of any reports on the rise in the almost-docked pool (though many reports discuss the importance of refilling). We remark that by including an additional mechanism in the model in which the secretion machinery becomes sensitized to glucose (perhaps via sensitization to calcium (Ammala et al., 1993; Gembal et al., 1992)) over the exposure time, it is possible to obtain solutions in which the mobilized pool does not become as large while still maintaining potentiation.

3.4. Staircase

The staircase experiment from O'Connor et al. (1980), in which rat pancreata are subject to glucose being increased in a stepwise fashion multiple times, is shown in Fig. 7. An important factor in achieving spikes of increasing height in our model is the dose-dependence of the fusion terms in Eq. (5). If the glucose concentration at which the fusion rates obtain their half-maximal value, G_{f50} , is small, then the observed spikes are more likely to be of decreasing height (simulation not shown). This is because a small value of G_{f50} results in relatively high fusion rates during the first low glucose step. The high fusion rates, which deplete the RRP, make it hard to achieve large increases in the positive terms of $\dot{F}$ which are needed to achieve increasing spikes (see Eq. (5) where the fusion terms are determined by the product of a glucose-dependent rate coefficient and the number of primed granules). We note that compared to the data presented in Fig. 4 from O'Connor et al. (1980), the spikes in the insulin secretion profile obtained from our model solution are less distinct, implying that glucose-induced increases in fusion rates may contribute to, but not fully explain, the observed secretion pattern. The secreted amount of insulin per islet during the spikes of the staircase in Fig. 7 is 0.466 ng versus 0.334 ng secreted during a single step at the final glucose concentration. A similar comparison in O'Connor et al. (1980) revealed the total amount of insulin secreted from the rat pancreas was 0.92 μ g during the staircase versus 1.02 μ g during the single step, amounts which were not statistically different.

With human islets a staircase experiment has been performed by Henquin and colleagues (Dufrane et al., 2007; Henquin et al., 2006). We show our reproduction of the staircase experiment from Henquin et al. (2006) in Fig. 8 for three different values of the maximum rate of glucose-induced mobilization, m . Comparing our simulations to the experimental data, we note that the intermediate value of m (solid line in Fig. 8) most closely resembles the insulin secretion profile from Figure 1 in Henquin et al. (2006). We remark that in Fig. 8 we have changed some of the parameter values compared to previous simulations. For example, we choose a smaller value of the glucose concentration threshold at which the β -

cells become active, so that the glucose response of human islets is shifted to the left compared to rodent islets (Henquin et al., 2006).

3.5. Negative spike

Experiments in which glucose was lowered from a high to a lower, but still stimulatory, level were performed in O'Connor et al. (1980) on the perfused rat pancreas. As the glucose concentration was lowered, a negative spike in the insulin secretion was observed (O'Connor et al., 1980). In Fig. 9, we show a simulation of such an experiment, noting that our model is able to recreate a negative spike. The instantaneous dips that occur in the insulin secretion rate as glucose is lowered are due to instantaneous lowering of the glucose-dependent fusion rates. We remark that the insulin secretion rate is for the most part well approximated by assuming that Eq. (5) is at quasi-steady state; thus, apart from abrupt changes, which are due to instantaneous changes in the fusion rates, the insulin secretion profile mirrors the evolution of the primed pools. The slow refilling of the primed pools that occur during the low glucose phase thus translates into the slow rise in insulin secretion rate seen after the dip.

In the experiments in O'Connor et al. (1980), the second positive spike that occurred (after glucose was raised back to a high level) was higher than the initial spike. Without changing the parameter values, the height of the first spike in Fig. 9 is the same as the spike in Fig. 2 for the corresponding glucose concentration. However, that was not the case in the experiments of O'Connor et al. (1980), where the spike was larger in the case of a single glucose step (see differing spike heights in Figs. 3 and 5 of O'Connor et al. (1980)). The different initial spikes in O'Connor et al. (1980) suggest inherent differences (and thus parameter values) for the pancreata used in the single step experiment versus those used in the experiments exhibiting multiple spikes (our Fig. 9, and Fig. 5 in O'Connor et al. (1980)). Indeed our simulations show that it is possible to obtain solutions in which the second positive spike is higher than the initial one by either decreasing the glucose-induced rate of fusion or increasing the glucose-induced rate of mobilization. In Fig. 10 where we have done the latter we also show how the positive and negative insulin spikes are influenced by the parameter G_{f50} , which determines the range over which the rates of fusion change significantly with glucose. We note that when G_{f50} is small, the initial spike is large (since the fusion rates are more glucose sensitive). In addition, when G_{f50} is small, the subsequent negative and positive spikes are attenuated compared to when G_{f50} is larger, and the second positive spike is lower than the first (contradicting the experimental results in O'Connor et al. (1980)). The attenuation of the negative spikes – i.e., relatively small drops in insulin secretion as glucose is lowered, along with relatively fast rates of recovery – can be explained by relatively high fusion rates at low concentrations (since the half-maximum fusion rates are attained faster when G_{f50} is small). The high fusion rates at low glucose concentrations also deplete the primed pools, instead of allowing them to be refilled (as is the case when G_{f50} is large), thus causing the small second positive insulin spike. In Fig. 11, we show additional glucose protocols from O'Connor et al. (1980) which our model reproduces well with the higher mobilization rate. The dependence of the half-maximal fusion rates is similar to that shown in Fig. 10.

4. Discussion

Previously proposed explanations for the dose-dependent response of β -cells to glucose have generally been based on two non-exclusive possible scenarios: (1) each β -cell (with its intracellular signals leading to insulin release) responds in a graded fashion to an increasing glucose concentration, and single β -cells thus secrete insulin in a dose-dependent way; and (2) each β -cell has its own threshold for glucose sensitivity, with its secretion being either fully on or off on either side of the threshold (Nesher and Cerasi, 2002). In the second view of glucose-induced β -cell activation, an increase in glucose results in an increased percentage of activated cells, thus leading to dose dependency (Nesher and Cerasi, 2002). Although heterogeneity has been observed in the glucose sensitivity of individual β -cells (Heart et al., 2006; Jonkers and Henquin, 2001; Salgado et al., 2000), it is also recognized that the organization of β -cells, and their crosstalk within intact islets, alters their individual responses (Heart et al., 2006; Jonkers and Henquin, 2001; Nittala and Wang, 2008; Nittala et al., 2007). In particular, recruitment or activation of islet β -cells occurs over a narrower glucose range than does recruitment of individual cells (Heart et al., 2006; Jonkers and Henquin, 2001; Speier et al., 2007), possibly restricted by electrical coupling via Cx36-gap junctions (Speier et al., 2007). Furthermore, dose-dependent increases in insulin secretion tend to continue beyond the glucose concentration at which maximal recruitment of islet β -cells occurs (Heart et al., 2006; Jonkers and Henquin, 2001).

We note that the degree of heterogeneity may be species-dependent. For instance, some studies reported that rat islets (Manning Fox et al., 2006) and human islets (Cabrera et al., 2006; Quesada et al., 2006) exhibited less synchrony than mouse islets. On the other hand, cytoarchitectural studies also suggested that human and rat islets may exhibit a more hierarchical organization and contain multiple compartments of cell clusters, where each compartment is anatomically and functionally like a mouse islet. If so, the recruitment heterogeneity could be low in each compartment, making our model applicable to the islet subunits. In studies of glucose-induced Ca^{2+} oscillations in β -cells within intact human islets, it was found that while the signals were not coordinated on a population-wide basis, they were synchronous within clusters of β -cells grouped together (Quesada et al., 2006). These issues, as well as the question of species-dependent relative contributions from heterogeneity versus graded responses to the insulin secretion patterns, and the implications to translational studies, are all interesting topics worth further investigation.

Without the assumption of β -cell heterogeneity, the staircase experiment has previously only been reproduced by signal-limited models which assume that insulin is secreted from a single compartment at a rate determined by the net effect of both potentiating and inhibitory time-dependent signals, the origin of which are unknown (O'Connor et al., 1980). In this paper, we have shown that, in order to reproduce staircase experiments, it is not necessary to assume heterogeneity in the glucose sensitivity threshold of the β -cells, nor is it necessary to include inhibitory signals. While our model solutions are similar to the staircase data from human islets presented in Henquin et al. (2006), we note that the spikes in the insulin secretion profile obtained from our model solution are less distinct than those observed from rat pancreata (O'Connor et al., 1980). Solutions from previous mathematical models that, contrary to ours, include heterogeneous glucose-induced β -cell activation but exclude the

graded dose response of individual cells, have displayed better agreement with the rodent data (Grodsky, 1972; Pedersen et al., 2008). In reality, both heterogeneity and a graded cell response to glucose most likely contribute to observed insulin secretion patterns, possibly dependent on species and experimental set-up. We remark that in the model presented here, the graded dose response is able to reproduce or partially contribute to the insulin spikes during staircase experiments because of our assumption that the rate of granule fusion within each β -cell increases with increasing glucose concentration. The underlying reason for increased fusion rate due to high glucose could depend on multiple factors. As glucose is increased, the membrane potential of the β -cell becomes depolarized due to closure of ATP-sensitive K^+ -channels in the plasma membrane. Glucose has been shown to induce depolarization in a concentration-dependent fashion (see Fig. 4 in Manning Fox et al. (2006)). When the membrane potential increases above a certain threshold, voltage-operated Ca^{2+} -channels start to open and influx of Ca^{2+} occurs, triggering exocytosis of insulin granules (Atwater et al., 1996; Rorsman and Renstrom, 2003). The average cytoplasmic Ca^{2+} -concentration has been shown to gradually increase as glucose is increased in multiple steps (see Fig. 7 in Jonkers and Henquin (2001)) and is temporally and quantitatively correlated with insulin secretion (Jonkers and Henquin, 2001). In addition to increasing fusion rates via calcium, glucose is also known to act via an amplifying pathway which, at already elevated Ca^{2+} -levels, increases the insulin secretion (without further elevating the calcium concentration) (Henquin, 2009). The exact mechanisms behind the amplification are still unknown despite extensive research (Henquin, 2009), but cyclic adenosine monophosphate (cAMP) or protein kinase A (PKA) could be contributing factors (Ammala et al., 1993; Hatakeyama et al., 2006; Idevall-Hagren et al., 2010; Shibasaki et al., 2007). Another possible way in which glucose could affect granule fusion is via an ATP-dependent K^+ -channel-independent pathway linked to single-cell mitochondrial metabolism (Heart et al., 2006). In Heart et al. (2006) the mitochondrial membrane potential (rather than the average cytoplasmic Ca^{2+} -concentration) correlated linearly with glucose-induced insulin secretion when glucose was varied from 4 to 16 mM. However, it is not currently known how this mitochondrial-dependent mechanism might actually translate into glucose-enhanced granule fusion rates.

From our model analysis we noted that the rapid spike in first-phase insulin secretion depends on rapid depletion of the primed pools, whereas the second phase relies on granule mobilization. Furthermore, the strength of granule priming may determine how fast the second phase increases and reaches steady state.

In simulating potentiation, we observed that depending on the strength of glucose-enhanced priming versus glucose-enhanced docking, the contribution of newcomers could potentially be larger than the contribution from docked granules during the second phase. We note that to our knowledge only one study has investigated the contribution of newcomers versus docked granules during two consecutive glucose administrations using TIRFM. In Aoyagi et al. (2009) the authors observed qualitatively similar exocytotic patterns for both peaks in terms of the contribution from newcomers and docked granules. Moreover, the authors did not observe potentiation, but rather a slightly reduced number of fusion events during the second glucose administration, and speculate whether potentiation is possible in dispersed β -cells (which were used in the study). However, we point out that the lack of potentiation in

Aoyagi et al. (2009) could also be due to the short duration of the first stimulus which was only 15 min. As has been discussed in Nesher and Cerasi (1987), and confirmed in our simulations (data not shown), whether the second peak is inhibited or potentiated compared to the first peak depends on the length of time the first stimulus is administered.

We remark that, as in the model shown in Pedersen et al. (2008), our model is not able to reproduce an experiment performed on the perfused rat pancreas (presented in Fig. 12 in O'Connor et al. (1980)), which produces a high spike when a step in glucose concentration is administered after a slow glucose ramp followed by a short time interval at basal glucose (simulation not shown). We are currently investigating multiple possible ways in which our model could be extended to successfully reproduce such experiments. For example, we could include intracellular calcium, and include modeling of β -cell membrane oscillations which drive insulin release. In addition we could include nonlinear effects that impose limits on the number of docked and fused granules. As has been indicated by experiments, the maximum number of docked granules may be limited by a limited number of docking sites at the β -cell plasma membrane (Straub et al., 2004). In our model, so far we only considered the pancreatic sensitivity toward glucose concentration. Also worth consideration are additional terms that model the capacity of the pancreas to respond to the rate of change in glucose, i.e. the “derivative control” mechanism (Licko, 1973; Pedersen et al., 2010; Pedersen et al., 2008). Previous studies have found that derivative control is needed to explain a range of in vivo data (Cobelli et al., 2007), and that it can arise from the heterogeneous recruitment-threshold hypothesis (Grotsky, 1972; Licko, 1973; Pedersen et al., 2010). It is also likely that other feedback loops would affect the granule dynamics. Feedback could for instance be included in terms of paracrine signaling from other pancreatic hormones such as glucagon and somatostatin, which are secreted by α - and δ -cells, respectively.

While modeling of additional signals has also been proposed in Pedersen et al. (2008) as a possible way to reproduce the negative spike seen when glucose is lowered from a high to an intermediate level, we note that our model is capable of recreating the negative spike. As revealed from our model analysis, the dip in the insulin secretion hinges on the rapid decrease in fusion rates as glucose is lowered, combined with a relatively slow refilling of the primed granule pools.

In conclusion, the model presented in this paper has shown how, by affecting the rates of granule fusion, priming, docking and mobilization, a graded cell response to glucose may contribute to the insulin secretion patterns observed in multiple experiments. In particular, we have shown that a model based on a fully homogeneous β -cell population, in which the rate of granule fusion within each β -cell increases with increasing glucose concentration, is able to reproduce the staircase experiment which, largely due to previous mathematical modeling, has been regarded as an indicator of the importance of heterogeneous β -cell recruitment. Considering experimental data of limited heterogeneity of coupled β -cells within islets, our findings suggest that the dose-dependent glucose response of individual cells could be an important factor in determining in vivo insulin release. In addition, our model suggests that insulin granule mobilization is a critical rate limiting factor for insulin release during the second phase, and hence for the total insulin release.

Appendix A: Model variables and parameters

See Tables A1 and A2.

Table A1

State variables in our model.

Variable	Dimension	Description
M_u	1	Number of unprimed mobilized granules
M_p	1	Number of primed mobilized granules
D_u	1	Number of unprimed docked granules
D_p	1	Number of primed docked granules
F	1	Number of fused granules
t	T	Time

Table A2

Model parameters For a subset of the parameters, estimated value ranges have been discussed in the main text of this paper. If no value range is given, we were not able to obtain an estimate despite an extensive literature search.

Parameter	Unit	Value range	Values used for the simulations in this study	Parameter description
m_0	min^{-1}	1–2	1	Basal mobilization rate of unprimed granules
m	min^{-1}		1–360	Maximum glucose-induced mobilization rate of unprimed granules
G_{m50}	mmol/l		10–14	Glucose concentration at which mobilization of unprimed granules is half-maximal
G_m	mmol/l	3–5	2.8–6.8	Glucose concentration at which glucose-induced mobilization of unprimed granules is activated
δ_m	min		1	Time delay for mobilization in response to glucose
n			1–1.5	Hill coefficient
$M_{u,max}$	1		4000–7000	Threshold value of M_u when glucose-induced mobilization of granules toward the plasma membrane ceases
p_{m0}	min^{-1}		0.004	Basal rate of priming of unprimed mobilized granules
p_m	min^{-1}		0.001–0.04	Maximum glucose-induced priming rate of unprimed mobilized granules
G_{p50}			10–14	Glucose concentration at which priming of unprimed granules is half-maximal
G_p	mmol/l	3–5	2.8–6.8	Glucose concentration at which glucose-induced priming of unprimed granules is activated
δ_p	min^{-1}		1	Time delay for priming in response to glucose
d_0	min^{-1}		0.004	Docking rate of unprimed mobilized granules
d	min^{-1}		0.001	Maximum glucose-induced docking rate of unprimed granules
G_{d50}	min^{-1}		10–14	Glucose concentration at which glucose-induced docking is half-maximal
G_d	mmol/l	3–5	2.8–4.8	Glucose concentration at which glucose-induced docking of unprimed granules is activated

Parameter	Unit	Value range	Values used for the simulations in this study	Parameter description
δ_d	min		1	Time delay for docking in response to glucose
f_{m0}	min^{-1}		0.016–0.032	Basal rate of fusion of primed mobilized granules
f_m	min^{-1}		0.32–1.6	Maximum glucose-induced fusion rate of primed mobilized granules
G_{f50}	mmol/l		7–24	Glucose concentration at which fusion of primed granules is half-maximal
G_f	mmol/l	3–5	2.8–4.8	Glucose concentration at which glucose-induced fusion of primed granules is activated
δ_f	min	1–2	1	Time delay for fusion in response to glucose
p_{d0}	min^{-1}		0.004	Basal rate of priming of unprimed docked granules
p_d	min^{-1}		0.001–0.04	Maximum glucose-induced priming rate of unprimed docked granules
f_{d0}	min^{-1}		0.016–0.032	Basal rate of fusion of primed docked granules
f_d	min^{-1}		0.32–1.6	Maximum glucose-induced fusion rate of primed docked granules
s	min^{-1}	30	30	Rate of insulin secretion

Appendix B: Model solutions under a single step glucose increase

By assuming that the step change in external glucose concentration occurs at $t=0$, and that $\delta_m=\delta_p=\delta_d=\delta_f$, the solution of Eq. (7) is given by the initial conditions in Eq. (9), for $t\in[0, \delta_f]$, while for $t\in[\delta_f, \infty]$ the solution is given by:

$$\begin{aligned}
 M_u(t) &= M_{uss} + c_1 e^{-\lambda_1 t} \\
 M_p(t) &= M_{pss} + (p_{m0} + \hat{p}_m(\delta_f)) \frac{c_1 e^{-\lambda_1 t}}{(\lambda_2 - \lambda_1)} + c_2 e^{-\lambda_2 t} \\
 D_u(t) &= D_{uss} + \frac{(d_0 + \hat{d}(\delta_f)) c_1 e^{-\lambda_1 t}}{(\lambda_3 - \lambda_1)} + c_3 e^{-\lambda_3 t} \\
 D_p(t) &= D_{pss} + \frac{(d_0 + \hat{d}(\delta_f)) \lambda_3 c_1 e^{-\lambda_1 t}}{(\lambda_3 - \lambda_1)(\lambda_4 - \lambda_1)} + \frac{\lambda_3 c_3 e^{-\lambda_3 t}}{(\lambda_4 - \lambda_3)} + c_4 e^{-\lambda_4 t} \\
 F(t) &= F_{ss} + \frac{(d_0 + \hat{d}(\delta_f)) \lambda_4 \lambda_3 c_1 e^{-\lambda_1 t}}{(s - \lambda_1)(\lambda_3 - \lambda_1)(\lambda_4 - \lambda_1)} + \frac{\lambda_4 \lambda_3 c_3 e^{-\lambda_3 t}}{(s - \lambda_3)(\lambda_4 - \lambda_3)} + \frac{\lambda_4 c_4 e^{-\lambda_4 t}}{(s - \lambda_4)} \\
 &\quad + \frac{\lambda_2 p_{m0} + \hat{p}_m c_1 e^{-\lambda_1 t}}{(\lambda_2 - \lambda_1)(s - \lambda_1)} + \frac{\lambda_2 p_{m0} + \hat{p}_m c_2 e^{-\lambda_2 t}}{(s - \lambda_2)} + c_5 e^{-st}
 \end{aligned} \tag{A1}$$

where

$\lambda_1 = d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f) + \tilde{m}(\delta_f) / M_{u,max}$, $\lambda_2 = f_{m0} + \hat{f}_m(\delta_f)$, $\lambda_3 = p_{d0} + \hat{p}_d + \hat{p}_d(\delta_f)$, $\lambda_4 = f_{d0} + f_d(\delta_f)$, and the new steady state corresponding to the step glucose concentration is given by

$$\begin{aligned}
 M_{uss} &= \frac{m_0 + \tilde{m}(\delta_f)}{d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f) + \tilde{m}(\delta_f) / M_{u,max}} \\
 M_{pss} &= \frac{(p_{m0} + \hat{p}_m(\delta_f))(m_0 + \tilde{m}(\delta_f))}{(f_{m0} + \hat{f}_m(\delta_f))(d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f) + \tilde{m}(\delta_f) / M_{u,max})} \\
 D_{uss} &= \frac{(d_0 + \hat{d}(\delta_f))(m_0 + \tilde{m}(\delta_f))}{(p_{d0} + \hat{p}_d(\delta_f))(d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f) + \tilde{m}(\delta_f) / M_{u,max})} \\
 D_{pss} &= \frac{(d_0 + \hat{d}(\delta_f))(m_0 + \tilde{m}(\delta_f))}{(f_{d0} + \hat{f}_d(\delta_f))(d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f) + \tilde{m}(\delta_f) / M_{u,max})} \\
 F_{ss} &= \frac{(d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f))(m_0 + \tilde{m}(\delta_f))}{s(d_0 + \hat{d}(\delta_f) + p_{m0} + \hat{p}_m(\delta_f) + \tilde{m}(\delta_f) / M_{u,max})}.
 \end{aligned} \tag{A2}$$

Furthermore, by assuming continuity of the solution at $t=\delta_f$, we obtain the constants

$$\begin{aligned}
 c_1 &= (M_{uss}^0 - M_{uss}) e^{\lambda_1 \delta_f} \\
 c_2 &= \left(M_{pss}^0 - M_{pss} - \frac{(p_{m0} + \hat{p}_m(\delta_f)) c_1 e^{-\lambda_1 \delta_f}}{(\lambda_2 - \lambda_1)} \right) e^{\lambda_2 \delta_f} \\
 c_3 &= \left(D_{uss}^0 - D_{uss} - \frac{(d_0 + \hat{d}(\delta_f)) c_1 e^{-\lambda_1 \delta_f}}{(\lambda_3 - \lambda_1)} \right) e^{\lambda_3 \delta_f} \\
 c_4 &= \left(D_{pss}^0 - D_{pss} - \frac{(d_0 + \hat{d}(\delta_f)) \lambda_3 c_1 e^{-\lambda_1 \delta_f}}{(\lambda_3 - \lambda_1)(\lambda_4 - \lambda_1)} - \frac{\lambda_3 c_3 e^{-\lambda_3 \delta_f}}{(\lambda_4 - \lambda_3)} \right) e^{\lambda_4 \delta_f} \quad (A3) \\
 c_5 &= \left(F_{ss}^0 - F_{ss} - \frac{(d_0 + \hat{d}(\delta_f)) \lambda_4 \lambda_3 c_1 e^{-\lambda_1 \delta_f}}{(s - \lambda_1)(\lambda_3 - \lambda_1)(\lambda_4 - \lambda_1)} - \frac{\lambda_4 \lambda_3 c_3 e^{-\lambda_3 \delta_f}}{(s - \lambda_3)(\lambda_4 - \lambda_3)} \right. \\
 &\quad \left. - \frac{\lambda_4 c_4 e^{-\lambda_4 \delta_f}}{(s - \lambda_4)} - \frac{\lambda_2 (p_{m0} + \hat{p}_m(\delta_f)) c_1 e^{-\lambda_1 \delta_f}}{(\lambda_2 - \lambda_1)(s - \lambda_1)} - \frac{\lambda_2 c_2 e^{-\lambda_2 \delta_f}}{(s - \lambda_2)} \right) e^{s \delta_f}.
 \end{aligned}$$

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HIGHLIGHTS

- ▶ We developed a new mathematical model of glucose-stimulated insulin secretion.
- ▶ The model reproduces results from multiple experimental systems.
- ▶ The staircase experiment can be explained without introducing β -cell heterogeneity.
- ▶ Insulin secretion dynamics depend on a graded response to glucose by β -cells.
- ▶ Rate of granule mobilization is a critical limiting factor of second phase insulin release.

Fig. 1. Schematic diagram of our model

Unprimed insulin granules are mobilized toward the cell membrane from a constant reserve in the cell interior at basal rate m_0 (all basal rate coefficients are denoted by $*_0$) and additionally at rate $\hat{m}(t)$ in response to elevated glucose levels (all above-basal rate coefficients are denoted by $\hat{*}(t)$). Unprimed granules dock at the cell membrane at rate $(d_0 + \hat{d}(t)) M_u$ and become primed at rate $(p_{d0} + \hat{p}_d(t)) D_u$. Fusion then occurs at rate $(f_{d0} + \hat{f}_d(t)) D_p$. In addition, the mobilized unprimed granules may skip the docking step and instead enter a primed state from which they may fuse directly. Priming and fusion from the mobilized pool occur at rates $(p_{m0} + \hat{p}_m(t)) M_u$ and $(f_{m0} + \hat{f}_m(t)) M_p$, respectively. The insulin secretion rate is proportional to the fused pool, sF .

Fig. 2.

Series of curves showing the insulin secretion in response to varying step increases of glucose applied at time $t=0$ as in experiments performed on rat pancreata in O'Connor et al. (1980). After an initial delay of 1 min the insulin secretion increases from its basal level. While a low step increase in glucose results in a pulse that declines monotonically over time, higher glucose concentrations result in a biphasic response with a slowly increasing secretion phase occurring after the decline of the initial insulin spike, the height of which increases with increasing glucose concentrations. Parameter values: $n=1$, $m_0=1$, $m=60$, $M_{u,max}=7000$, $G_{m50}=G_{p50}=G_{d50}=G_{f50}=14$, $\delta_m=\delta_p=\delta_d=\delta_f=1$, $d_0=p_{m0}=p_{d0}=0.004$, $d=0.001$, $p_m=p_d=0.001$, $f_{m0}=f_{d0}=0.8/25$, $s=30$, $f_m=f_d=50f_{m0}$, $G_f=G_d=4.8$, $G_m=G_p=6.8$.

Fig. 3.

Series of curves showing the number of mobilized unprimed granules (panel A), the number of primed mobilized granules (panel B), the number of docked unprimed granules (panel C), and the number of docked primed granules (panel D) in response to varying step increases of glucose applied at time $t=0$. After an initial delay of 1 min the increase of glucose from its basal level affects granule dynamics. For high glucose concentrations the compartments of release-ready granules (see panels B and D) increase after an initial decline, giving rise to a biphasic insulin response as shown in Fig. 2. Parameter values are the same as in Fig. 2.

Fig. 4.

Series of curves showing the insulin secretion in response to varying step increases of glucose applied at time $t=0$. After an initial delay of 1 min the insulin secretion increases from its basal level. Compared to Fig. 2, the higher rate of priming of mobilized granules makes the insulin secretion rate approach steady state more rapidly. Parameter values are the same as in Fig. 2, but with $p_m=0.1$.

Fig. 5.

(A) Two curves showing total number of fusion events (solid line), and number of fusion events stemming from docked granules (dotted line), following a step increase in glucose from basal to 16.7 mmol/l applied at $t=0$. (B) Series of curves showing the fraction of fusion events stemming from docked granules for different step increases in glucose concentration applied at $t=0$. For the lowest glucose concentration (dashed line) the fraction of fusion events that are due to docked granules remains constant over time. For higher glucose concentrations the fraction of fusion events from the docked pool shows a rapid decline and a slowly rising phase; for these parameter values the initial insulin secretion stems equally from docked and mobilized granules, while mobilized granules contribute more to the second phase. Parameter values are the same as in Fig. 2.

Fig. 6.

A set of curves (solutions of Eqs. (1)–(5)) which by reproducing an experiment performed on rat pancreata (Fig. 6 in O'Connor et al. (1980)) shows how two intermittent periods of high glucose concentrations separated by a period of basal glucose concentration lead to a second spike of insulin secretion which is larger than the first (so-called potentiation). (A)–(B): Potentiation is due to refilling of the unprimed pool of mobilized granules (dashed line) and the unprimed pool of docked granules (dotted line), shown in panel A, which during the intermittent basal glucose phase rapidly refills the primed pools, shown in panel B. Compared to its initial size, the total primed pool is larger just before the glucose concentration is raised a second time. (C)–(D): The second insulin spike (panel D) is larger than the first because of an increased number of fusion events (panel C) due to the extensive refilling of the RRP that has taken place. While fusion during the first spike is contributed to equally by docked granules (dotted line) and mobilized granules (dashed line), the second spike is mainly derived from the latter pool. Parameter values are the same as in Fig. 2. The glucose protocol is indicated by the background color and annotated at the top of each figure, in unit mmol/l.

Fig. 7. Simulation of the staircase experiment performed on rat pancreata (Fig. 4 in O'Connor et al. (1980)), in which glucose is increased sequentially in steps every five minutes. (A)–(B): While the unprimed pools of granules (panel A) increase during the experiment, the primed pools (panel B) decrease with each period of increasing glucose concentration. (C)–(D): For each step increase in glucose concentration, the fusion rates of the primed granules are enhanced, causing insulin spikes to be secreted at each step (panel D). The fusion events (panel C) are from both docked and mobilized granules. Parameter values are the same as in Fig. 2. The glucose protocol is indicated by the background color and annotated at the top of each figure, in unit mmol/l.

Fig. 8.

Simulation of the staircase experiment in which glucose is increased sequentially in steps.

Experimental protocol taken from human islet studies (Fig. 1 in (Henquin et al., 2006)).

Parameter values: $n=1.5$, $m_0=1$, $m_{u,\max} = 4000$, $G_{m50} = G_{p50} = G_{d50} = G_{f50} = 10$,

$\delta_m = \delta_p = \delta_d = \delta_f = 1$, $d_0 = p_{m0} = p_{d0} = 0.004$, $d = 0.001$, $p_m = p_d = 0.04$, $f_{m0} = f_{d0} = 0.8/50$, $s = 30$,

$f_m = f_d = 20f_{m0}$, $G_m = G_f = G_d = G_p = 2.8$. The glucose protocol is indicated by the background color and annotated at the top of each figure, in unit mmol/l.

Fig. 9.

Model solution (of Eqs. (1)–(5)) representing the experiment performed on rat pancreata (Fig. 5 in O'Connor et al. (1980)). (A) The unprimed pool of mobilized granules (dashed line) and the unprimed pool of docked granules (dotted line), monotonically increase during the experiment. (B) In contrast to the unprimed pools, the primed pools undergo depletion during the phases of high glucose, and refill when the glucose levels are low. (C) While docked and mobilized primed granules contribute equally to fusion at the beginning, over time most of the fusion events are due to mobilized granules. (D) When glucose is lowered, a dip occurs in the insulin secretion; this is due to decreased rates of fusion. As the primed pools recover during the low-glucose phase, the insulin rate increases gradually and levels off until glucose is increased a second time, causing a positive spike due to instantaneously increased fusion. Parameter values are the same as in Fig. 2. The glucose protocol is indicated by the background color and annotated at the top of each figure, in unit mmol/l.

Fig. 10.

Model solutions depicting how the insulin pattern in Fig. 9 is altered by a higher rate of glucose-induced granule mobilization, m , assuming three different values for the glucose concentration at which the fusion rates are half-maximal, G_{f50} . Compared to Fig. 9 the high mobilization causes a higher second spike since the primed pools are being replenished at a faster rate during the low glucose concentration (solid line). A low value of G_{f50} (dashed line) gives a large first spike, but attenuates subsequent spikes. In contrast, a high value of G_{f50} (dotted line) yields a small initial spike, but enhanced subsequent spikes. $m=360$; all other parameter values are the same as in Fig. 2. The glucose protocol is indicated by the background color and annotated at the top of each figure, in unit mmol/l.

Fig. 11.

Model solutions of insulin secretion in an experiment performed on rat pancreata (presented in Figs. 7 and 8 in O'Connor et al. (1980)) in which, twice in succession, glucose is changed to an intermediate, stimulatory level and then raised to an even higher level. The glucose levels are the same in (A) and (B), but in (B) the first glucose interval is halved. The faster application of the second glucose concentration in (B) implies that less refilling of the primed pools has taken place and that subsequent insulin spikes are slightly smaller than in (A). The conclusions regarding the impact of G_{f50} on the solutions are the same as in Fig. 10. $m=300$; all other parameter values are the same as in Fig. 2. The glucose protocol is indicated by the background color and annotated at the top of each figure, in unit mmol/l.