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A subcellular model of glucose-stimulated pancreatic insulin secretion

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When glucose is raised from a basal to stimulating level, the pancreatic islets respond with a typical biphasic insulin secretion pattern. Moreover, the pancreas is able to recognize the rate of change of the glucose concentration. We present a relatively simple model of insulin secretion from pancreatic β -cells, yet founded on solid physiological grounds and capable of reproducing a series of secretion patterns from perfused pancreases as well as from stimulated islets. The model includes the notion of distinct pools of granules as well as mechanisms such as mobilization, priming, exocytosis and kiss-and-run. Based on experimental data, we suggest that the individual β -cells activate at different glucose concentrations. The model reproduces most of the data it was tested against very well, and can therefore serve as a general model of glucose-stimulated insulin secretion. Simulations predict that the effect of an increased frequency of kiss-and-run exocytotic events is a reduction in insulin secretion without modification of the qualitative pattern. Our model also appears to be the first physiology-based one to reproduce the staircase experiment, which underlies ‘derivative control’, i.e. the pancreatic capacity of measuring the rate of change of the glucose concentration.

Keywords: multiphasic secretion; granule trafficking; recruitment; derivative control; mathematical model

1. Introduction

The pancreatic β -cells are responsible for producing insulin, which is secreted in response to elevated plasma glucose levels. The main signalling pathway in glucose-stimulated insulin secretion is fairly well understood and includes metabolism of glucose, an augmented ATP-to-ADP ratio leading to closure of ATP-sensitive potassium (K_{ATP}) channels, electrical activity, influx of calcium and finally exocytosis of insulin-containing granules (Henquin 2000). Another amplifying pathway is far less understood (Henquin 2000) but could involve increased mobilization and priming of granules, which would increase the readily releasable pool (RRP) of granules (Henquin *et al.* 2002; Rorsman & Renström 2003).

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The granules located in the RRP are immediately available for release once calcium enters the cell, due to a series of preparatory steps and their location in the vicinity of calcium channels (Rorsman & Renström 2003).

Almost four decades ago, Grodsky and co-workers (Curry *et al.* 1968; Grodsky 1972) investigated the kinetics of insulin secretion in response to a variety of glucose patterns in order to get an insight into the regulation of insulin-containing granules. Insulin is released in a characteristic biphasic manner when the glucose concentration is raised swiftly from subthreshold to stimulating levels. This pattern is observed in single islets of Langerhans (Barbosa *et al.* 1998; Del Guerra *et al.* 2005), the isolated pancreas (Curry *et al.* 1968; Grodsky 1972) and *in vivo* (Cobelli *et al.* 2007). The physiological role of first-phase secretion includes controlling postprandial glucose excursions (Del Prato 2003). The early phase of this characteristic pattern is lost in diabetics at an early stage (Del Prato *et al.* 2002; Gerich 2002), a defect that appears to have its origin on the level of single islets (Del Guerra *et al.* 2005).

The studies on isolated rat pancreases led to the first models of insulin secretion (Grodsky 1972; Cerasi *et al.* 1974; O'Connor *et al.* 1980), which were able to reproduce the observed insulin patterns. Owing to the limited knowledge of the biology of the β -cells at that time, these models were speculative rather than based on well-characterized intracellular mechanisms. In particular, Grodsky (1972) introduced the threshold hypothesis for the granules, where each granule has a certain glucose threshold above which it releases its content. This assumption has gained little support from experiments, thus questioning the model. Also, the biological variables playing the roles of the postulated signals underlying the model by Cerasi *et al.* (1974) have not been revealed.

Since the 1970s, the knowledge about intracellular processes in the pancreatic β -cells has been increased tremendously, which allowed the development of models of glycolysis, mitochondria, cAMP and calcium oscillations, and bursting electrical activity (Westermarck & Lansner 2003; Bertram *et al.* 2007; Fridlyand *et al.* 2007). However, only very recently have models of exocytosis and insulin release appeared (Bertuzzi *et al.* 2007; Chen *et al.* *in press*). Insulin granule movement has also recently been modelled as a random walk process (Shibasaki *et al.* 2007). These models share the notion of distinct pools of granules with the early models by Grodsky, but founded on a better characterized cell biological background. This includes a RRP of granules, which is refilled by the recruitment of granules from a reserve pool to the cell membrane, docking to or near the calcium channels, and ATP- and cAMP-dependent priming (Rorsman & Renström 2003). When the calcium level in the microdomain at the mouth of the calcium channels is raised due to depolarization, exocytosis occurs, and the insulin molecules can escape to the extracellular space. However, this does not always occur due to so-called 'kiss-and-run', where the granules reseal before the large insulin molecules have left the intergranular space (Obermüller *et al.* 2005; Rutter & Hill 2006).

These recent quite detailed models can reproduce a large set of phenomena and have, for instance, been used to investigate both the point at which an amplifying signal is likely to work and the step responsible for the slow second phase of insulin secretion (Bertuzzi *et al.* 2007; Chen *et al.* *in press*). However, none of these models are able to reproduce the staircase experiment, which originally led Grodsky to the development of the threshold hypothesis

(Grotsky 1972). The models are based on the events within a single cell, and have no thresholds in the sense of Grotsky, which explains their inability to simulate the staircase experiment.

We present a model that integrates the detailed intercellular description with the recruitment of cells as observed in experiments (Jonkers & Henquin 2001; Heart *et al.* 2006). The key to reproducing the staircase experiment is the heterogeneity of the β -cells (Pipeleers *et al.* 1994), and especially the fact that the cells become active at different glucose levels as revealed by calcium measurements, but once active the single-cell calcium levels do not change (Heart *et al.* 2006) or change little (Jonkers & Henquin 2001). The relatively simple model reproduces a series of recent experiments in addition to the experiments by Grotsky, and can therefore serve as a core model for glucose-stimulated insulin secretion, which can be integrated into larger models of the glucose–insulin system (Cobelli *et al.* 2007; Dalla Man *et al.* 2007).

2. Model development

In agreement with two recent models (Bertuzzi *et al.* 2007; Chen *et al.* in press), we regard the insulin-containing granules as belonging to different pools (Cobelli *et al.* 2007) as suggested from biological experiments (Eliasson *et al.* 1997; Henquin *et al.* 2002; Rorsman & Renström 2003) in accordance with the notion in chromaffin cells (Horrigan & Bookman 1994; Gillis *et al.* 1996). After insulin synthesis and packaging, the secretory granules are added to a large internal ‘reserve pool’ from which the granules move to the membrane. Here, the granules undergo docking and priming before becoming readily releasable. The granules belonging to the RRP can then fuse with the membrane when the plasma membrane calcium channels open at stimulatory glucose levels. After fusion (exocytosis) has occurred, the fusion pore can either reseal quickly (kiss-and-run) or allow the release of insulin. It has been shown that the granules can kiss-and-run exocytose several times, but seem not to undergo full fusion and, consequently, not to release insulin following a kiss-and-run event (MacDonald *et al.* 2006). Moreover, it has been suggested that granules are internalized and that their membrane proteins are recycled following kiss-and-run (Rutter & Hill 2006). We therefore assume that the granules after kiss-and-run return to the reserve pool. This scheme is summarized in figure 1*a*. Besides controlling calcium influx, we assume that glucose regulates mobilization from the reserve pool to the membrane. This is likely to happen via messengers such as Ca^{2+} (Jing *et al.* 2005) and ATP, which drives myosin V (Ivarsson *et al.* 2005). Note that we allow reinternalization of mobilized granules that are still not docked to the membrane.

We simplify the model by merging mobilized and docked granules into a single ‘intermediate pool’. Also, we will consider the reserve pool as being infinite, thus neglecting synthesis of new granules and changes in the size of the reserve pool due to mobilization, reinternalization and crinophagy. The latter hypothesis is supported by the observation that approximately 80 per cent of the granules are located in the centre of the cell (Rorsman & Renström 2003), and the fact that modifying the biosynthesis rate appears to have little effect on secretion over the first 2 hours after an increased glucose stimulus (Bertuzzi *et al.* 2007).

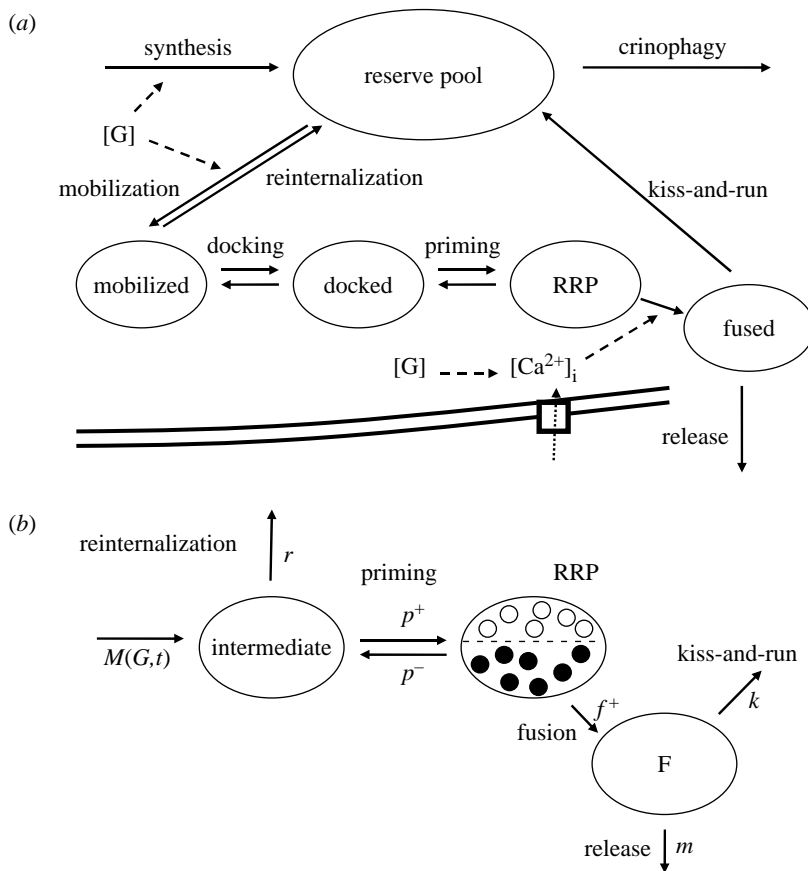


Figure 1. (a) Overview of granule pools and assumed glucose control points. Glucose ($[G]$) is assumed to control synthesis and mobilization. In addition, glucose promotes calcium influx through voltage-gated calcium channels, which raises the intracellular calcium concentration ($[Ca^{2+}]_i$)—the trigger of exocytosis. For further details, see the main text. (b) Schematic of the model. The RRP has been divided into readily releasable granules located in silent cells with no Ca^{2+} influx, exocytosis and release (open circles) and in triggered cells (filled circles). Insulin is released from fused granules with rate constant m independent of the glucose concentration and the threshold for activity of the cell. r is the rate constant of reinternalization.

To compare with the experiments on the intact pancreas, we need to consider all β -cells in the pancreas. The β -cells have different glucose thresholds for triggering Ca^{2+} influx (Hellman *et al.* 1994; Jonkers & Henquin 2001; Heart *et al.* 2006). Above this threshold, $[Ca^{2+}]_i$ changes little with glucose (Hellman *et al.* 1994; Jonkers & Henquin 2001; Heart *et al.* 2006). We therefore assume that at a certain extracellular glucose concentration (G), a fraction of the cells are silent, while the remaining cells are triggered and release insulin with the same rate constant due to similar Ca^{2+} levels, independently of the single-cell threshold. The percentage of triggered β -cells as a function of the glucose concentration ($\Phi(G)$) is assumed to follow a sigmoidal relationship (Jonkers & Henquin 2001; Heart *et al.* 2006). We denote by $\phi(G)$ the mathematical derivative of $\Phi(G)$, which is a density function describing the fraction of cells with threshold between G and $G+dG$. This implies that $\Phi(\infty) = \int_0^\infty \phi(g)dg = 1$.

Thus, at a given G , only the fraction $\Phi(G) = \int_0^G \phi(g)dg$ of the cells are secreting insulin, while the remaining cells are silent. This can be summarized in a model for all the granules in all the β -cells of the pancreas, but where we now make a distinction in the RRP between readily releasable granules located in triggered cells, and those in silent cells (figure 1b). Thus, at a certain G , only a fraction of the granules in the RRP undergoes fusion and release with fixed rate constants.

Since we will validate our model against *in vitro* studies, we consider $G=0$ mg dl⁻¹ to be the basal level, although unphysiological. *In vivo*, a more physiological basal level would be, for example, 80 mg dl⁻¹. However, we note that very few cells have a threshold lower than 80 mg dl⁻¹.

Assuming that all the cells contain the same amount of readily releasable granules at the basal level $G=0$ mg dl⁻¹, we get a sigmoidal function $H_0(G)$ representing the amount of readily releasable insulin in cells with threshold lower than G , so that the total insulin in the RRP in the basal state is $H_0(\infty) = \int_0^\infty h_0(g)dg$ and $\Phi(G) = H_0(G)/H_0(\infty)$. We denote by $h_0(G)$ the mathematical derivative of $H_0(G)$, which is a density function describing the basal amount of the readily releasable insulin in the cells with threshold between G and $G+dG$. The fraction of cells with threshold between G and $G+dG$ can then be calculated as the basal amount of insulin with threshold G divided by the total amount of insulin in the RRP at the basal level, i.e. $\phi(G) = h_0(G)/H_0(\infty)$.

When a glucose stimulus G is applied, the RRP is described by a time-varying density function $h(g,t)$, indicating the amount of insulin in the RRP in the β -cells with a threshold between g and $g+dg$. In other words, the amount of insulin in the RRP in the cells described by $\phi(g)$. We will use the basal condition, i.e. $G=0$ mg dl⁻¹ and steady state, as the initial state, so that $h(g,0) = h_0(g)$. Note, however, that for $t>0$, this relationship does not necessarily hold, and there is no *a priori* relationship between h and ϕ , except in the basal state.

The time dependence of h is modelled as follows. Granules are primed with rate p^+ and are assumed to lose the capacity of rapid exocytosis with rate p^- . Moreover, if the granule is in a triggered β -cell, it will fuse with rate f^+ . This leads to the equation

$$dh(g,t)/dt = p^+I(t)\phi(g) - p^-h(g,t) - f^+h(g,t)\theta(G-g). \quad (2.1)$$

Here $\theta(G-g)$ is the Heaviside step function, which is 1 for $G>g$ and 0 otherwise, indicating that fusion occurs only when the threshold is reached. I is the total intermediate pool, and the priming flux p^+I distributes among cells according to the function $\phi(g)$, i.e. the fraction of cells with threshold g , as described above. Thus, priming is assumed to occur with the same rate in all cells, but we take into account the fraction of cells with the corresponding threshold.

The secretion rate can be expressed as

$$SR(t) = mF(t),$$

where m is the rate constant of release and F is the size of the fused pool, which follows

$$dF(t)/dt = f^+ \int_0^G h(g,t)dg - kF(t) - mF(t), \quad (2.2)$$

where f^+ is the rate constant of fusion; k is the kiss-and-run rate; and $h(g, t)$ is the density function indicating the amount of insulin in the RRP in the β -cells with a threshold between g and $g + dg$. Therefore, the integral represents the amount of insulin in the RRP in the cells with a threshold below G , i.e. the RRP in triggered cells described by $\Phi(G)$.

The intermediate pool develops according to the equation

$$dI(t)/dt = M(G, t) - rI(t) - p^+I(t) + p^- \int_0^\infty h(g, t)dg,$$

where M is the mobilization flux and r is the rate of reinternalization. The last term describes the flux of the granules losing the capacity of rapid exocytosis.

Mobilization is modelled similar to Grodsky (1972). It is assumed to depend on glucose but with a delay τ , and is described by

$$dM(G, t)/dt = (M(G, t) - M_\infty(G))/\tau, \quad (2.3)$$

where the steady-state mobilization $M_\infty(G)$ is a sigmoidal function. The delay introduces a deep trough after the insulin peak, but is not strictly necessary, and is indeed set close to zero when modelling the data by O'Connor *et al.* (1980; see below). Intriguingly, the delay could correspond to remodelling of the cortical actin network, which happens with a delay of 5–20 min and is known to be important for mobilizing the granules to the cell membrane and the second phase of insulin secretion (Wang *et al.* 2007).

At the basal ($G=0$ mg dl⁻¹) steady state, we have $F_0=0$ μ g, $M_0=M_\infty(0)$, $H_0(\infty)=(p^+/p^-)I_0$ and I_0 satisfies

$$0 = M_0 - p^+I_0 - rI_0 + p^- \int_0^\infty h_0(g)dg = M_0 - (p^+ + r - p^-(p^+/p^-))I_0,$$

implying $I_0 = M_0/r$.

We use the following expression for steady-state mobilization:

$$M_\infty(G) = cG^{nM}/((K_{mM})^{nM} + G^{nM}) + M_0, \quad (2.4)$$

and a similar expression for the amount of readily releasable insulin in the cells with threshold lower than G

$$H_0(G) = H_{\max}G^{nH}/((K_{mH})^{nH} + G^{nH}).$$

Parameter values are determined as follows. The rate constant of release m is taken from Grodsky (1972). Fusion is known to be much faster than release (Obermüller *et al.* 2005) meaning that f^+ should be significantly larger than m . We use $f^+ = 10m$, but remark that the simulations are rather insensitive to the exact value (see §4). Kiss-and-run has been estimated to occur in 25 to 60 per cent of fusion events (Obermüller *et al.* 2005; MacDonald *et al.* 2006). We choose the parameter $k=0.75m$ corresponding to kiss-and-run in 43 per cent ($k/(k+m)$) of the total fusion events. Jonkers & Henquin (2001) found that half of the cells were activated at approximately 8 mM, which is approximately equal to 150 mg dl⁻¹ glucose; so we set $K_{mH}=150$ mg dl⁻¹, the same value used by Grodsky (1972) for his ‘releasable insulin distribution’. $H_{\max}=1.65$ μ g is also taken from Grodsky (1972). When implementing the model, one needs to substitute the infinities in the upper limit of some of the integrals with some

Table 1. Parameter values used to reproduce the experiments by Grodsky (1972). (The values are based on arguments presented in the main text.)

parameter (unit)	value
m (min^{-1})	0.62
f^+ (min^{-1})	6.2
k (min^{-1})	0.47
p^+ (min^{-1})	0.021
p^- (min^{-1})	0.11
r (min^{-1})	0.0023
M_0 ($\mu\text{g min}^{-1}$)	0.020
c ($\mu\text{g min}^{-1}$)	1.25
K_{mM} (mg dl^{-1})	185
nM	12
H_{max} (μg)	1.65
nH	3.3
K_{mH} (mg dl^{-1})	150
τ (min)	30
RRP_0 (μg)	1.62
I_0 (μg)	8.91

maximal glucose concentration G_{max} . Since we only model experiments with $G \leq 500 \text{ mg dl}^{-1}$, we set $G_{\text{max}} = 500 \text{ mg dl}^{-1}$, so the total insulin in the RRP in the basal state is

$$RRP_0 = H_0(500) = \int_0^{500} h_0(g) dg = 1.62 \mu\text{g}.$$

The relationship between pool sizes is taken from Olofsson *et al.* (2002) who estimated the RRP to be 50–100 granules per cell, while the docked and ‘almost docked’ are 500–2000 granules per cell. We use $I_0 = 5.5 RRP_0 = 8.91 \mu\text{g}$, and it follows that $p^- = 5.5 p^+$. Since the first phase is due to emptying of the RRP, we need the priming rate to be much slower than the fusion and release. We use $p^+ = m/30$, so $p^- = 5.5 m/30$. Moreover, we have made reinternalization very slow using $r = p^-/50 = 5.5 m/1500$. Finally, from the calculation above $M_0 = r I_0$. τ , nM and K_{mM} were found from the simulations and are given in table 1.

To simulate the experiments from O’Connor *et al.* (1980), we make the following changes. The sigmoidal relationship for mobilization is shifted to the right by increasing $K_{mM} = 250 \text{ mg dl}^{-1}$ (figure 2). Also, $K_{mH} = 250 \text{ mg dl}^{-1}$ is increased, and in addition $H_{\text{max}} = 1.2 \mu\text{g}$ is lowered. Thus, fewer cells are assumed to become active compared with the 1972 experiments (figure 3). As a consequence, $RRP_0 = H_0(500) = 1.09 \mu\text{g}$ is lower. We keep I_0 unchanged, but to maintain steady state, we regulate $p^- = 0.1695$. To have a more rapid refilling, we increase r and M_0 by a factor of 3, and lower $\tau = 0.5 \text{ min}$.

3. Simulation results

As shown in figure 4, our model reproduces the characteristic biphasic secretion pattern in response to a step in the glucose stimulus signal. The peak characterizing the first phase, which lasts approximately 5 min, is due to the emptying

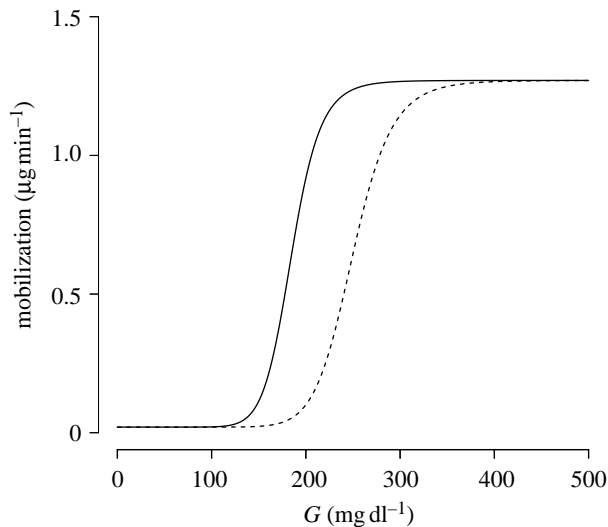


Figure 2. Steady-state mobilization of insulin granules as a function of the extracellular glucose concentration, $M_{\infty}(G)$, with Grodsky (1972) parameters (solid curve; table 1) and O'Connor *et al.* (1980) parameters (dashed curve).

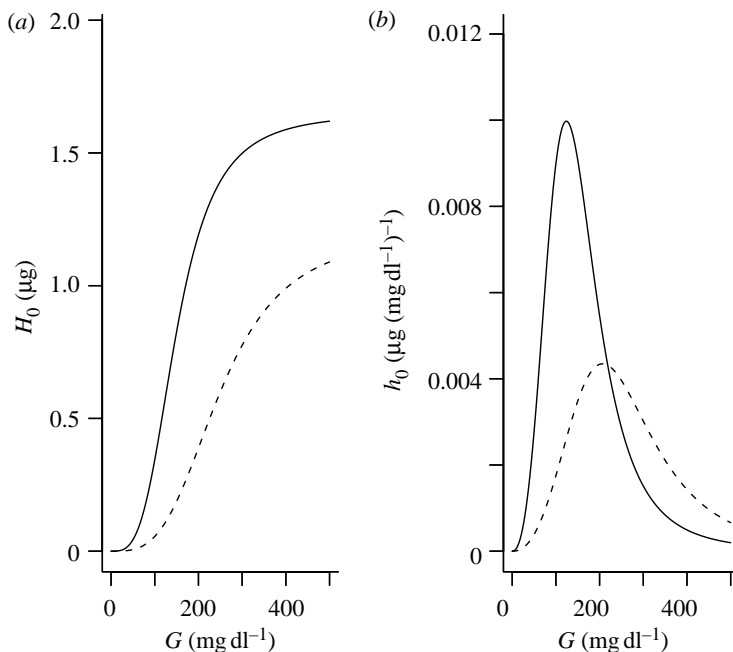


Figure 3. (a) The amount of insulin in the basal state in the RRP of cells with a threshold lower than G , $H_0(G)$, and (b) the corresponding density function, $h_0(G)$, describing the amount of insulin in the RRP of cells with threshold G . The solid curves correspond to the 1972 parameters (table 1), while the dashed curves correspond to the 1980 parameters.

of the RRP in the activated β -cells. The height of the peak increases with the glucose concentration, since more cells are activated at higher glucose levels (figure 3), but the decay has a time constant that is nearly independent of the

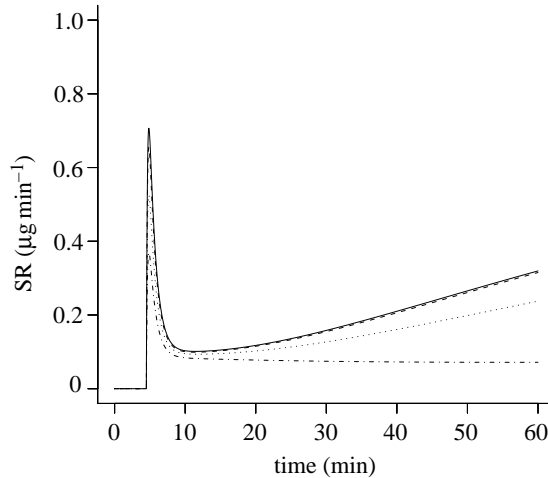


Figure 4. Biphasic insulin release in response to a step in glucose levels at $t=5$ min from $G=0$ to 150 mg dl^{-1} (dot-dashed curve), $G=200 \text{ mg dl}^{-1}$ (dotted curve), $G=300 \text{ mg dl}^{-1}$ (dashed curve) and $G=500 \text{ mg dl}^{-1}$ (solid curve).

glucose concentration due to the fact that the fusion and release rates, f^+ and m , which control the decay, are fixed parameters. The second phase is flat for $G=150 \text{ mg dl}^{-1}$, while it is ascending for higher glucose concentrations. This difference is due to the glucose dependence of the mobilization rate (figure 2).

The so-called slow and fast ramps, i.e. experiments where the glucose concentration is increased in a continuous manner rather than as a step function, were also reproduced by the model (figure 5). When the glucose level was raised slowly (figure 5a), the first-phase peak disappears, while for the fast ramp (figure 5b) the first phase is still clearly present.

Another important capacity of the pancreas is the so-called potentiation, i.e. the fact that after a prolonged glucose stimulus, the pancreas is hypersensitive to further stimulation. Again, this is simulated satisfactorily by our model (not shown). After 60 min of stimulus at $G=300 \text{ mg dl}^{-1}$, the glucose concentration was lowered to zero for 5 min, after which G was raised back to 300 mg dl^{-1} . In response to the second square wave, an insulin spike reaching approximately 1.0 µg min^{-1} was observed, which should be compared with the secretion rate of approximately 0.6 µg min^{-1} in response to the first glucose pulse (cf. figure 4). The second larger peak is due to refilling of the docked and RRP during the 60 min of glucose stimulus, such that the RRP is increased at $t=60$ min just before the second stimulus, compared with the beginning of the experiment.

Finally, the crucial staircase experiment was simulated (figure 6). For increasing steps of glucose concentrations, further cells are recruited into their active state and secrete insulin. This allows the second and third peaks to appear, even though the RRP of the cells activated at the lower glucose levels empties during the first peak, which creates the trough after the peak. As discussed by Grodsky (1972) and Licko (1973), this recruitment of heterogeneous ‘packets’, which in our model are the β -cells, with threshold depending on the glucose concentration, underlies derivative control, i.e. the fact that the pancreas senses not only the present glucose concentration but also its rate of change.

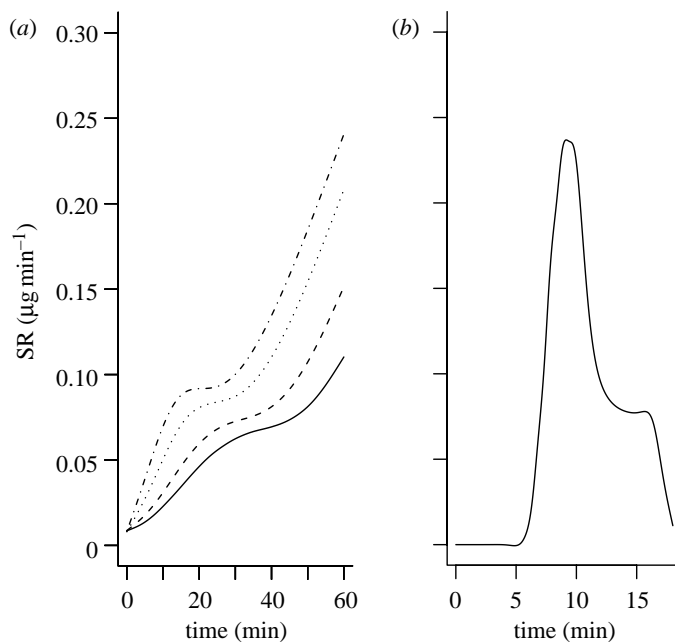


Figure 5. Insulin secretion in response to (a) slow and (b) fast ramps. To simulate insulin secretion in response to (a) slow ramps, the glucose concentration was increased linearly, from $G=45 \text{ mg dl}^{-1}$ at $t=0$ min to $G=250 \text{ mg dl}^{-1}$ (solid curve), $G=300 \text{ mg dl}^{-1}$ (dashed curve), $G=400 \text{ mg dl}^{-1}$ (dotted curve) or $G=500 \text{ mg dl}^{-1}$ (dot-dashed curve) at $t=60$ min. For secretion with a (b) fast ramp, glucose was absent until $t=5$ min, and was then increased linearly to $G=210 \text{ mg dl}^{-1}$ at $t=10$ min. G was then kept constant at 210 mg dl^{-1} until $t=16$ min before being lowered instantaneously to $G=0 \text{ mg dl}^{-1}$.

After having observed that our model simulates the early data satisfactorily, we follow Grodsky and co-workers (O'Connor *et al.* 1980) and test the model on more complicated stimulus patterns. When glucose is quickly reduced to a lower but stimulating level, a negative spike in the insulin secretion trace is observed (O'Connor *et al.* 1980). Our model produces a small drop in insulin secretion after glucose is lowered from 500 to 150 mg dl^{-1} (figure 7), but not nearly as large as the experimentally observed decrease. The model by Grodsky (1972) has the same defect (O'Connor *et al.* 1980). However, the peaks and hypersensitization are simulated satisfactorily. We adjusted parameters to reproduce quantitatively the lower peaks in the experiments from O'Connor *et al.* (1980) compared with the data from Grodsky (1972). Also, mobilization was made more rapid in order to simulate the fast appearance of a second phase observed by O'Connor *et al.* (1980). Notably, in contrast to the model by Grodsky (1972), we were able to reproduce the correct relationship between the two spikes occurring at $G=500 \text{ mg dl}^{-1}$, i.e. a larger second peak after only 10 min at $G=150 \text{ mg dl}^{-1}$ (figure 7b). This is due to the faster refilling of the RRP resulting from the increased mobilization rate. It therefore seems that the incapacity of the Grodsky model to reproduce these data can be explained to a large extent by assuming that the pancreata used in the experiments presented by O'Connor *et al.* (1980) have a larger mobilization rate. However, when

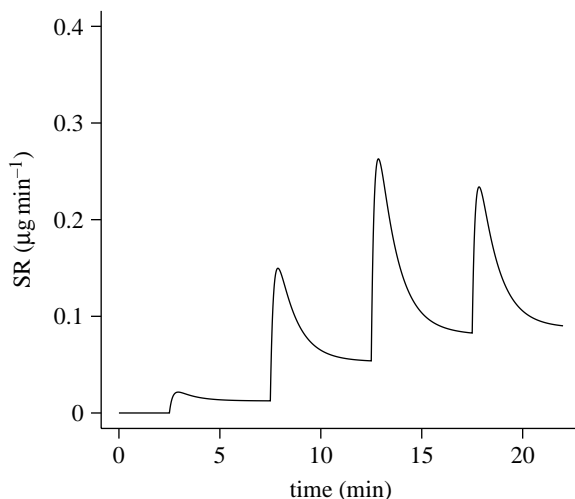


Figure 6. The staircase experiment. The glucose level was raised from the initial basal level, $G=0 \text{ mg dl}^{-1}$, to $G=50 \text{ mg dl}^{-1}$ ($3 \text{ min} \leq t < 8 \text{ min}$), $G=100 \text{ mg dl}^{-1}$ ($8 \text{ min} \leq t < 13 \text{ min}$), $G=150 \text{ mg dl}^{-1}$ ($13 \text{ min} \leq t < 18 \text{ min}$) and $G=200 \text{ mg dl}^{-1}$ ($18 \text{ min} \leq t \leq 22 \text{ min}$).

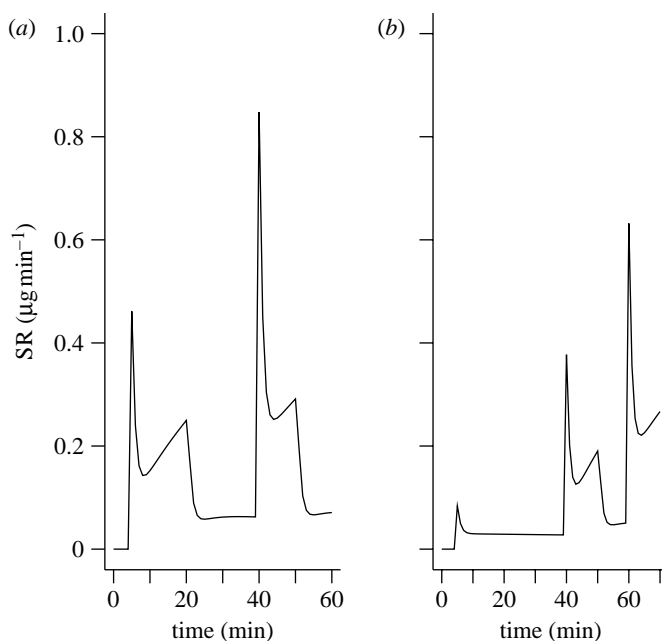


Figure 7. Simulations of insulin secretion during a sequence of high and intermediate glucose stimulations reported by O'Connor *et al.* (1980). (a) G was raised to 500 mg dl^{-1} from $t=5$ to 20 min , where it was lowered to $G=150 \text{ mg dl}^{-1}$. This level was kept constant until a second stimulus of $G=500 \text{ mg dl}^{-1}$ was given between $t=40 \text{ min}$ and $t=50 \text{ min}$. Then glucose was lowered to $G=150 \text{ mg dl}^{-1}$ for the remaining time. (b) Glucose concentration was raised to $G=150 \text{ mg dl}^{-1}$ between $t=5 \text{ min}$ and $t=40 \text{ min}$, where it was increased to $G=500 \text{ mg dl}^{-1}$ for 10 min . At $t=50 \text{ min}$, G was then lowered to 150 mg dl^{-1} until $t=60 \text{ min}$, after which it was kept constant at 500 mg dl^{-1} for the rest of the protocol.

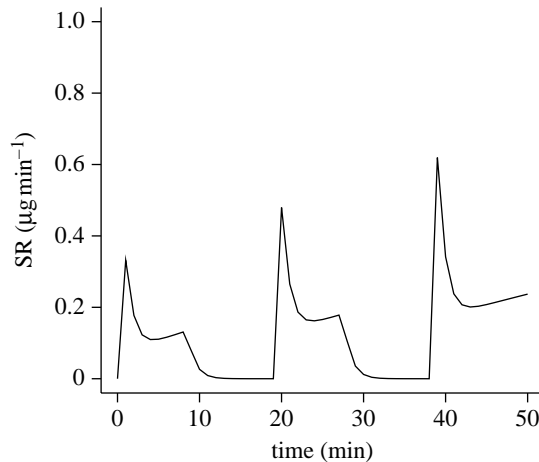


Figure 8. Simulated experiment from O'Connor *et al.* (1980). Glucose pulses of 300 mg dl^{-1} were administrated at $t=0 \text{ min}$ and $t=20 \text{ min}$, each lasting 7 min, and at $t=40 \text{ min}$ for the rest of the study. Between the glucose pulses, G was zero.

stimulating intermittently with 300 mg dl^{-1} , the pancreas responds with peaks of similar height, thus showing no potentiation, while our simulations show increasing magnitude of the spikes (figure 8), indicating that our refilling rate might be too large for this experiment. Finally, our model fails grimly in reproducing the hypersensitization resulting from a ramp at low glucose levels prior to a low concentration glucose pulse (not shown). The ramp consisted in a linear increase from $G=40 \text{ mg dl}^{-1}$ at $t=0 \text{ min}$ to $G=100 \text{ mg dl}^{-1}$ at $t=20 \text{ min}$. Then, G was kept constant at 100 mg dl^{-1} until $t=35 \text{ min}$, where it was lowered to $G=0 \text{ mg dl}^{-1}$. The $G=100 \text{ mg dl}^{-1}$ square wave was given at $t=40 \text{ min}$. The resulting insulin release pattern was virtually flat, in contrast to the spike observed experimentally in response to the square wave (O'Connor *et al.* 1980). The model by Grodsky (1972) had the same flaw (O'Connor *et al.* 1980). The reason is the very low rate of mobilization during the ramp, which is insufficient for refilling the RRP and promoting a large insulin peak in response to the glucose pulse. Hence, there appears to be a need for additional signals participating in the potentiation of the pancreas (O'Connor *et al.* 1980).

Our model is capable of reproducing recent experiments by Henquin *et al.* (2002), where groups of islets were placed in a medium containing $250 \mu\text{M}$ diazoxide, which opens K_{ATP} channels and disrupts the signal from glucose to the membrane potential and calcium influx. Hence, glucose can be assumed to have no effect on fusion, while it does effect mobilization and other putative steps refilling the RRP through the amplifying pathway (Henquin 2000). By adding no or a low concentration ($G=3 \text{ mM}$) of glucose in addition to diazoxide, Henquin *et al.* (2002) were therefore able to control refilling independently of exocytosis. Fusion, and consequently insulin secretion, was triggered by depolarizing by high concentrations of potassium, either continuously or intermittently. A similar experimental protocol, but with a higher glucose concentration ($G=10 \text{ mM}$), yielded similar results as the low glucose-level experiments (Ravier *et al.* 1999).

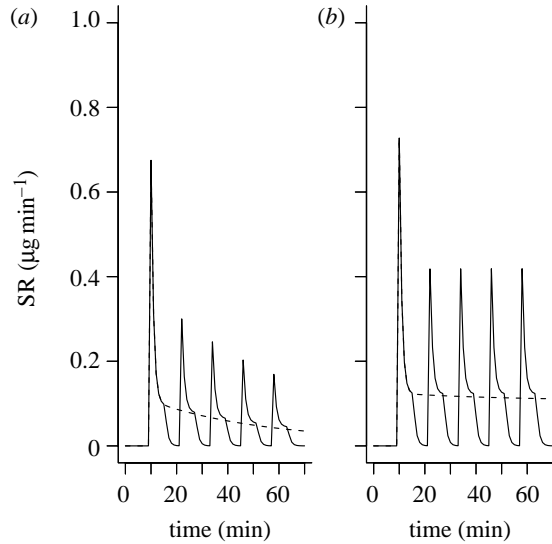


Figure 9. Potassium-stimulated insulin secretion in the presence of diazoxide and (a) no glucose or (b) an intermediate glucose concentration. High potassium was administered continuously (dashed curves) or intermittently in 6 min pulses every 12 min (solid curves). For details on the simulation protocol, see the main text.

We model the effect of potassium by assuming that all the cells are triggered. This is obtained by setting $G=500 \text{ mg dl}^{-1}$ in equations (2.1) and (2.2) for the RRP. The effect of glucose on refilling was reproduced satisfactorily by setting $G=0 \text{ mg dl}^{-1}$ (no glucose) or $G=160 \text{ mg dl}^{-1}$ (presence of glucose) in equation (2.3) for mobilization. These simulations were performed with the parameters shown in table 1.

In agreement with the experimental data, it can be observed (figure 9a) how the prolonged stimulation by high potassium, but with no glucose, leads to a biphasic secretion pattern with a large peak corresponding to the emptying of the RRP, and a slower decaying second phase, corresponding to the emptying of the intermediate pool. When potassium is applied intermittently, the RRP refills partially between the potassium pulses, which allows peaks of decreasing height to appear with each potassium administration. The lower magnitude of the peaks is again due to the slow emptying of the intermediate pool, which implies a smaller priming flux.

By contrast, in the presence of glucose, and therefore with increased mobilization rate, the intermediate pool does not empty during the experiment, and a sustained second phase appears when the islets are stimulated continuously by potassium (figure 9b). Moreover, the insulin response to the application of potassium pulses shows a larger first peak, but the subsequent spikes have similar heights, since the priming flux does not decrease during the study, and the RRP therefore refills to the same extent between each couple of potassium pulses.

To illustrate how the model can be applied, we investigated the fact that the fraction of the granules that undergo kiss-and-run increases when the β -cells are exposed to high glucose concentrations and lipids for sustained periods of time (Tsuboi *et al.* 2006; Olofsson *et al.* 2007). We simulated secretion in response to

a 300 mg dl⁻¹ square-wave glucose stimulus using $k=4m$, meaning that kiss-and-run occurs in 80 per cent of fused granules, i.e. approximately twice the frequency of the previous simulations. We found that secretion followed the same quantitative pattern as shown in figure 4, but that the amount of insulin released was reduced by a factor of 2 (not shown). Thus, increased kiss-and-run leads to lower secretion rates, but does not explain the loss of phase one secretion observed in islets from diabetic patients (Del Guerra *et al.* 2005).

4. Discussion

The model presented in this paper appears to be the first one based on well-characterized biological principles, which is able to reproduce the crucial staircase experiment, which led Grodsky (1972) to set forth the threshold hypothesis. Grodsky (1972) mentioned briefly that the insulin ‘packages’ did not necessarily represent the granules but could also represent the cells (see also Pipeleers *et al.* 1994). This idea is in line with the model presented here, and supported by calcium data (Hellman *et al.* 1994; Jonkers & Henquin 2001; Heart *et al.* 2006) not available at the time of Grodsky’s paper.

Our model reproduced most of the available experimental observations satisfactorily, although the dip in insulin secretion after lowering the extracellular glucose concentration from a very high to an intermediate, but stimulating, level (figure 7) and the potentiation at low glucose (not shown) were not reproduced. These flaws were also present in the model by Grodsky (1972), and indicate that the threshold hypothesis is insufficient for explaining these data, but that further signals are required (O’Connor *et al.* 1980). We remark that the calcium levels do not show a dip when lowering glucose from 15 mM (approx. 300 mg dl⁻¹) to 8 mM (approx. 150 mg dl⁻¹; Nunemaker *et al.* 2006), and signals other than calcium must therefore be involved in the negative spike of insulin secretion mentioned above.

Not surprisingly, an increased frequency of kiss-and-run events led to a lower secretion rate, but it did not modify the qualitative pattern; in particular, a clear first insulin peak was still present (not shown). Hence, increased kiss-and-run cannot be directly responsible for the loss of first-phase secretion in diabetics (Del Guerra *et al.* 2005), unless the frequency of kiss-and-run events changes on a minute-to-minute scale during the stimulation. Other signals, such as calcium and NAD(P)H, are indeed modified by culture in high glucose or palmitate (Tsuboi *et al.* 2006; Olofsson *et al.* 2007), which might be of higher importance for a blunted first-phase response than the increased number of kiss-and-run events.

As discussed by Grodsky (1972) and in greater details by Licko (1973), the threshold distribution underlies derivative control, i.e. the fact that the pancreas is capable of sensing the rate of change of the extracellular glucose concentration (dG/dt) and not just the glucose concentration itself (G). Thus, understanding the mechanism underlying the staircase experiment is of importance for understanding the complex dynamics of insulin secretion, especially since a derivative term is necessary to explain a wealth of *in vivo* data (Cobelli *et al.* 2007).

We note that single-cell experiments have been found to show no sign of increased calcium during a staircase stimulation (fig. 3C, Heart *et al.* 2006), although this is not always the case, especially when measuring calcium in cell

clusters (Jonkers & Henquin 2001) and single islets (Bergsten 1998; Nunemaker *et al.* 2006). Increasing calcium levels in clusters and islets could represent the recruitment of single cells, which would also be able to explain the increased amplitude of calcium oscillations at higher glucose concentration. However, the relative time spent at high calcium levels during oscillations in islets, known as the plateau fraction, increases also with glucose (Nunemaker *et al.* 2006). In our model, an increased plateau fraction and/or larger intracellular calcium levels would correspond to an increased fusion rate, f^+ . Our simulations reveal that the insulin secretion patterns at high glucose concentrations change insignificantly when the rate f^+ is increased by even a factor of 5 (not shown), since the release rate m is limiting for secretion from the RRP. Thus, our simplifying hypothesis of a fusion rate independent of G seems justified. Nonetheless, increased plateau fraction and cytosolic calcium might participate in the increased second-phase secretion at high glucose concentration, due to the effect of calcium on mobilization (Jing *et al.* 2005). In our model, this effect is included indirectly through the delayed action of G on mobilization.

A fundamental assumption of our model is the heterogeneity of the β -cell population (Pipeleers *et al.* 1994), more specifically the fact that the cells activate at different glucose levels (Hellman *et al.* 1994; Jonkers & Henquin 2001; Heart *et al.* 2006). *In situ*, the β -cells are coupled by gap junctions in the islets of Langerhans, and this coupling has been reported to reduce cell heterogeneity and promote inter-islet synchronization (Aslanidi *et al.* 2001; Ravier *et al.* 2005; Speier *et al.* 2007), facts that appear to question our fundamental hypothesis. However, asynchronous Ca^{2+} oscillations have been seen in islets from *ob/ob* mice (Liu *et al.* 1998), rats (Manning Fox *et al.* 2006) and humans (Cabrera *et al.* 2006). Moreover, by imposing a glucose gradient across single islets, Rocheleau *et al.* (2004) showed that active cells were not able to activate otherwise silent cells; in other words, a cell was active if and only if its expected single-cell threshold was reached. Similarly, Bennett *et al.* (1996) observed that metabolism, as measured by NAD(P)H, is heterogeneous across an islet until glucose has equilibrated, such that cells that presumably are located at a low glucose concentration show a low NAD(P)H signal. Once glucose has equilibrated, and activated all cells, the NAD(P)H signal is synchronized across the islet. Hence, it appears that gap junction communication is important mainly for synchronizing the active cells within an islet, rather than promoting an all-or-none response of the entire islet. Such synchrony is important for creating a pulsatile secretion pattern (Pedersen *et al.* 2005), a phenomenon not included here, but discussed by Chen *et al.* (in press). In addition, even though coupled cells should show similar calcium levels, it is not given that the cells secrete insulin with the same rate (Jonkers & Henquin 2001), for example, due to different levels of cAMP and ATP, which are important for sensitizing the exocytotic machinery to the calcium signal (Rorsman & Renström 2003). Such heterogeneity could provide the cell-to-cell variations used in the model. We therefore argue that there is some evidence of the possibility that cell heterogeneity persists *in situ*, especially in rats, which was used for the staircase experiment by Grodsky (1972) and modelled here. The same appears to be true for humans, which is of clinical interest.

We would like to mention that our model would remain unchanged if one assumed that islets are completely synchronized and acting as ‘super-cells’, but that the islets have different activation thresholds. The few existing studies on

rat islet populations with the aim of characterizing inter-islet heterogeneity have found that larger islets have a low glucose threshold for insulin secretion compared with smaller islets, and that heterogeneity is also present between islets of similar size (Chan *et al.* 1998; Aizawa *et al.* 2001). Moreover, human islets have unequal distributions of α - and β -cells (Cabrera *et al.* 2006), which might lead to inter-islet differences in glucose sensitivity. We would like to encourage experimentalists to investigate carefully, for example, the calcium response of single islets at various glucose concentrations in order to quantify the activating threshold distribution, as has been done for single cells (Jonkers & Henquin 2001; Heart *et al.* 2006).

We acknowledge that there is a large body of evidence of calcium showing a multiphasic response to step glucose stimulation, and the dynamic calcium signal in general shows a good correlation with insulin secretion (Barbosa *et al.* 1998; Henquin *et al.* 2002). It would therefore be interesting to add a more detailed description of calcium dynamics to the model presented here, although this would complicate the model and make its application to *in vivo* data more difficult. Such an extended model could be based on the approach by Chen *et al.* (in press), and would be useful for studying pulsatile insulin secretion, which is believed to be the result of calcium and, possibly, metabolic oscillations (Barbosa *et al.* 1998; Nunemaker *et al.* 2006; Bertram *et al.* 2007). In addition, a detailed description of intracellular calcium handling would allow the notion of pools with different calcium sensitivities (Wan *et al.* 2004; Yang & Gillis 2004) to be added to the model.

In conclusion, we have presented a physiology-based model that is able to reproduce the staircase experiment, which underlies pancreatic derivative control. This capacity of the pancreas is important for interpreting *in vivo* data, and we therefore suggest that the staircase experiment should be used to test future models of insulin secretion.

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