Published in final edited form as:

Prog Biophys Mol Biol. 2011 November ; 107(2): 293–303. doi:10.1016/j.pbiomolbio.2011.09.001.

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

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Abstract

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

Keywords

calcium; cAMP; exocytosis; diabetes; mathematical model

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Introduction

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

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

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

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

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

We therefore sought to develop a quantitative, kinetic model of core exocytotic processes of the β -cell and their regulatory mechanisms. Our aim was to develop a mathematical model for the coupled processes of granule dynamics, granule fusion processes and their regulation on a cellular level that can be used for an interpretation of existing results and to design new

experiments. We have attempted to integrate and analyze the existing hypotheses using experimental data and thereby evaluate the roles of metabolic, Ca^{2+} and cAMP messenger pathways in the regulation of IS. However, the exocytosis system in β -cells is highly complex and existing experimental data remain inadequate for a sufficiently detailed mathematical consideration. For this reason we were forced to introduce several proposals based on indirect evidence from other systems rather than direct experimental data to fill the numerous gaps in our understanding of these processes. Only in this way can we include terms to make a useful and testable model.

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

2. Methods

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

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

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

The mechanisms of coupling membrane potential (V_p) with glucose changes are complex and our understanding is still incomplete. For this reason we developed a simplified mechanical description for steady-state V_p regulation with the changes of glucose level or other factors (Eqs. 1–2, SM), although we have developed more complex models for action potential activity in β -cells (see refs. (Fridlyand et al., 2009; Fridlyand et al., 2003)). Simple model of $[\text{Ca}^{2+}]_c$ regulation (Eqs. 3–5, SM) in pancreatic β -cells was based on our previous mathematical models and describes Ca^{2+} entry through voltage-dependent Ca^{2+} channels

and remove by Ca^{2+} pumps on PM and Ca^{2+} sequestration (see refs. (Fridlyand and Philipson, 2010; Fridlyand et al., 2003)).

We also modeled a change of a specific metabolic activation factor (f_{MF}) that we introduced to describe an influence of glucose changes on IS other than the regulation of $[\text{Ca}^{2+}]_c$ (see below). We proposed that the increase in f_{MF} depends on glucose concentration (Eq. 6, SM). The proposed mechanism of action of this factor will be described below.

Cyclic AMP serves as an almost universal signal that modulates or regulates exocytosis in various secretory systems including the pancreatic β -cell (Leech et al., 2010; Seino et al., 2009). However, the precise mechanisms of all of its action are still unclear. In pancreatic β -cells specific G proteins can activate or inhibit adenylyl cyclases (AC) that catalyze cAMP synthesis. In turn, cAMP signaling is attenuated by phosphodiesterase (PDE)-catalyzed degradation (Fig. 1). In this article we have used our previous β -cell mathematical model describing AC and PDE activity underlying Ca^{2+} -dependent cAMP regulation (Fridlyand et al., 2007) (Eqs. 7–15, SM).

Cyclic AMP activates protein kinase A (PKA), a multifunctional regulatory enzyme while also binding to EPAC guanyl exchange proteins (Epac 1 and 2) (Holz, 2004; Leech et al., 2010; Seino et al., 2009). These processes were modeled based a previous model (Smolen et al., 2006) as shown in the supplementary material (Eqs. 16–21, SM). Different effectors of PKA and EPAC(s) regulate the potentiation of exocytosis in β -cells (see for example (Gilon and Henquin, 2001; Leech et al., 2010; Seino et al., 2009)). However, the molecular details of these processes are not completely understood so that in our model the terminal products of the cAMP activation pathway are the activated forms of PKA and EPAC, which influence the processes of insulin granule dynamics (further discussed below).

2.2. Granule dynamics

The number of insulin-containing granules in the total population of β -cells is large relative to the number required to respond to a single meal and is on the order of about 10,000 per cell. The granules are present in the cell interior or docked at or very near the PM. The insulin-containing granules in the β -cell can be divided into three classes: a large “reserve” granule pool (RGP) (~90%), a smaller “exocytosable” pool of morphologically docked granules (DGP) (~10%), and the pool that is chemically ‘primed’ for release or the “readily releasable pool” (RRP) (0.3–2.2%) (Rorsman and Renstrom, 2003). Results from total internal reflection fluorescence (TIRF) microscopy has added a new pool to this list, in particular the “restless newcomer granules” which are localized inside the cell and have specific properties leading to preferential release (Kasai et al., 2008; Ohara-Imaizumi et al., 2002; Shibasaki et al., 2007). We will refer this fourth pool as the “restless newcomer granule pool” (NGP). We suggested that all granules display similar properties and their belonging to different pools can be determined by different specific proteins bound to the surface of granules. For this reason we have also considered the dynamics of these putative protein binding and adaptor pools.

2.2.1. Fusion—In general, exocytosis requires the fusion of two separate unit membranes, the vesicle and PM, to allow a discharge of the vesicular content. The cytoplasmic domains

of both vesicular and target membrane proteins interact with each other through the formation of highly stable coiled-coil proteins in order to bring the two separate membranes into close apposition (Eliasson et al., 2008). The PM target proteins may include syntaxin 1A, syntaxin-4 and SNAP23/25 (Jewell et al., 2010). The insulin-granule vesicular membrane proteins include the vesicle associated membrane protein 2 (VAMP2/syntaxobrevin) as the predominant isoform required for IS (Jewell et al., 2010) and, for example, the intrinsic granule membrane protein ICA512 (IA-2), whose is cleaved upon granule exocytosis and IA-2beta (phogrin) (Henquin et al., 2008; Trajkovski et al., 2008).

RRP and DGP are localized near the PM, however they can be functionally distinguished. RRP granules appear to be “stand-by granules” waiting for fusion. RRP granules in rodents have been said to be tightly or closely associated with L-type Ca^{2+} channels, as the process of release of primed granules needs a local increase in calcium in microdomains. This is most likely contributed by voltage-dependent Ca^{2+} influx near the release site, triggering exocytosis, and not by cytosolic Ca^{2+} elevation stemming from release of Ca^{2+} from intracellular stores (Eliasson et al., 2008; Trus et al., 2007). It is possible however that the translocation of STIM1 protein-containing membranes to the PM upon release of ER calcium stores can also play a role in increasing sub-PM Ca^{2+} in microdomains (Tamarina et al., 2008). P/Q-type Ca^{2+} channels may play similar role in human beta cells (Braun et al., 2009). We modeled this mechanism to display a dependence of RRP fusion on the opening of VDCCs (Eqs. 22–23, SM).

PKA may specifically activate the first phase of glucose-induced exocytosis without affecting the second phase in intact islets or small clusters of mouse β -cells (Hatakeyama et al., 2006). In our model the first phase of IS is determined mainly by RRP fusion (see later). Since PKA can facilitate L-type channel opening (Szczak et al., 2008) a mechanism involving PKA activation of the first phase of IS was simulated in our model as a specific acceleration of RRP fusion, activated by VDCCs (Eqs. 22–23, SM).

Imaging of insulin granule dynamics using primary cultured pancreatic β -cells indicated that NGP can be recruited and immediately become fused to the PM without docking and priming (Ohara-Imaizumi et al., 2009a; Ohara-Imaizumi et al., 2007; Shibasaki et al., 2007). We will refer to this process of NGP fusion as “immediate fusion” (Fig. 1). The rate of NGP fusion does not depend directly on the existence of the pools termed RRP and DGP (Ohara-Imaizumi et al., 2009a; Ohara-Imaizumi et al., 2007) and rather may rely on the transport of insulin granules along the microtubule network from the interior of the β -cell to the cell periphery via actin-based myosin motors (Wang and Thurmond, 2009). We modeled this “immediate fusion” as a product of several factors (Eq. 24, SM) as follows:

1. Fusion of all granules depends on activation of VDCCs (Eliasson et al., 2008; Trus et al., 2007), and we suggested that the opening of VDCCs accelerates the immediate fusion of NGP following PM depolarization in a similar manner to VDCCs activation leading to RRP fusion (Eq. 24, SM). However, there seems to be separate Ca^{2+} -channels that are selective for primed or restless newcomer granules. In this regard it was found that R type Ca^{2+} channels may play a role in the second-phase of IS (Jing et al., 2005), where NGP fusion mostly occurs (see later) and it is

possible that R type Ca^{2+} channels (or a corresponding Ca^{2+} microdomain) may control the fusion of NGP granules.

2. A slow and sustained $[\text{Ca}^{2+}]_c$ rise in the cytoplasm induced the fusion of NGP (Ohara-Imaizumi et al., 2009a), although the series of events is not well characterized (Jewell et al., 2010). Synaptotagmins, in particular the isoforms 7 and 9 associated with native β -cell insulin granules, have been identified as putative Ca^{2+} sensors and are involved in exocytosis in β -cells (Gauthier and Wollheim, 2008). Ca^{2+} -dependent phosphorylation of myosin light chain has also been proposed as a regulator of granule trafficking (Niki and Hidaka, 1999). We represent this varied dependence on $[\text{Ca}^{2+}]_c$ through the empirical Hill-type equation for the $[\text{Ca}^{2+}]_c$ potentiating factor (Eq. 25, SM), which regulates the rate of NGP immediate fusion.
3. Metabolic signals (apparently other than an increase in the ATP/ADP ratio) participate in the amplifying pathway that augments the action of Ca^{2+} on IS (Henquin et al., 2009; Jensen et al., 2008). Current signaling candidates include an increase in the cytoplasmic NADPH/NADP⁺ ratio, mitochondrial citrate and other metabolites which increase with the glucose concentration (Fridlyand and Philipson, 2010; Henquin et al., 2009; Jensen et al., 2008; MacDonald et al., 2005). These amplifying pathways have been implicated in the second phase of IS (Deeney et al., 2000; Henquin, 2009; Straub and Sharp, 2002). By ignoring the actual specific molecular mechanisms underlying these amplifying pathways we introduced a glucose-induced metabolic activation factor (f_{MF} , Eqs. 6, 24; SM) that accelerates the immediate fusion. The characteristic property of the amplifying pathway is that its effect continues to increase with increased glucose even though $[\text{Ca}^{2+}]_c$ becomes saturated (Henquin et al., 2009; Jensen et al., 2008). For this reason the half-saturated glucose concentration for this factor was accepted as 17 mM, corresponding to the dependence of IS in second phase on [Glu] (see for example: (Henquin et al., 2006)).
4. Analyses of insulin granule dynamics indicate that the potentiating effect of cAMP on glucose-induced fusion events also involves NGP, and EPAC(s) may activate NGP fusion (Shibasaki et al., 2007). For this reason, a process of EPAC activation of NGP fusion was also simulated in our model through an EPAC potentiating factor (f_{iep} , Eq. 26, SM).

2.2.3. Priming and docking—Molecular docking mechanisms for insulin granules are poorly defined but granules in the DGP may arise from NGP granules (Shibasaki et al., 2007). They can bind reversibly to the exocyst protein complexes on the cytoplasmic face of the PM. These complexes become unbound during RRP granule fusion and can then be available to bind with restless newcomer granules forming new docked granules (Eliasson et al., 2008; Jahn and Scheller, 2006; Yasuda et al., 2010). To reflect this process we introduced a conserved “target protein pool” for DGP (TPD) that determines the total count of RRP and DGP granules and free TPD protein complexes (TPD_f) that become free during RRP granule fusion. In this case the rate of a docking can be estimated by combining NGP and free TPD (Eqs. 27–28, SM).

Syntaxin 1A and Rim2 α may be the representative TPD proteins. Mice with syntaxin 1A deficiency or Rim2 α knockout show fewer docked granules and have a selectively impaired insulin release during the first phase, that is determined mainly by RRP fusion (see below) (Ohara-Imaizumi et al., 2007; Yasuda et al., 2010). In this example, syntaxin 1A and/or Rim2 α may be bound to both docked and primed granules.

It has been suggested that docked granules cannot be released until they are primed, at that point becoming RRP granules (Yasuda et al., 2010). However, the exact nature of the priming reaction remains unclear and multiple stable configurations may occur (Takahashi et al., 2010).

Similarly to docking we suggested that the priming process can be regulated by binding of docking granules with some exocyst protein, represented here as specific target protein pool for RRP (TPR) (Eqs. 30–33, SM). We suggested that these proteins become free (TPR_f) during RRP fusion and acquire the ability to complex with granules from the DGP forming new RRP granules (Eq. 34, SM). In this event the maximal number of RRP granules is determined by the quantity of this TPR.

EPAC activation (PKA independent pathway) may increase the number of RRP (Renstrom et al., 1997; Seino et al., 2009; Shibasaki et al., 2007). This mechanism includes activation of the small G protein RAP1 that finally lead to a phosphorylation of secretory granule-associated proteins, thereby increasing the likelihood that these granules will undergo exocytosis in response to Ca²⁺ influx through VDCCs (Dzhura et al., 2011; Seino et al., 2011). We simulated these processes analogously to a description of PKA activation and using the different forms of TPR. We suggested that an inactivated TPR (TPR_i) can be activated by EPAC leading to active free TPR (TPR_f) that can, in turn, bind with DGP forming RRP (Eqs. 30–33, SM).

2.2.4. NGR formation—Insulin is synthesized as preproinsulin in ribosomes attached to the rough endoplasmic reticulum of the β -cells (Halban, 1991; Steiner et al., 2010). Following removal of a 24-residue amino terminal extension, proinsulin is transported from the rough endoplasmic reticulum to the Golgi-complex where is processed to mature insulin by excision of the connecting peptide (c-peptide) within the trans-Golgi-network (Halban, 1991; Steiner *et al.*, 2010). It is still unclear how (pro)insulin is aggregated, sorted and segregated into new granules, along with a complex mixture of small molecules (eg, Ca²⁺, Zn²⁺, ATP) and specific soluble and granule membrane proteins. Newly formed vesicles may be preferentially secreted (Michael et al., 2007). Since NGP are preferentially secreted in our model we suggested that new granules can go initially into the NGP.

Some signaling mechanism must control the biosynthetic rate of insulin granules destined for the NGP because the quantity of these granules is limited. To simulate this process we proposed an existence of a special conserved “vesicular protein pool” (VPP) that can bind with new granules forming NGP. We propose that the proteins from this pool become free (VPP_f) following docking and immediate fusion of NGP and that NGP formation can be determined by combining the free protein from this pool and the count of proinsulin granules (Eqs. 35–37, 41, SM).

Several types of proteins could be included in the VPP. For example, protein anchors and adaptors are required to anchor vesicles to microtubule motors for transport to secretion sites (Park and Loh, 2008). It can be imagined that NGP includes insulin granules that are bound with protein anchors and/or adaptors awaiting signals for transport.

2.2.5. Regulation of RGP—Relatively few intracellular granules undergo secretion under normal conditions, with most remaining in the storage pool for several days before being released or degraded (Halban, 1991). Aged β -cell granules are retired by intracellular degradation mechanisms, either via crinophagy and/or autophagy (Uchizono et al., 2007). To formulate this model we assumed that the RGP forms from NGP releasing elements in the free VPP and that back transformation of RGP into NGP can also occur as RGP are bound with free VPP molecules. Granules in the RGP can slowly degrade intracellularly (Eq. 40, SM).

3. Results

The detailed kinetic mechanism of the exocytosis cascade is still not well understood. However, in order to construct a model we imposed constraints that the model must follow, and then determined if the constraints and basic rate equations were appropriate by following simulated data from several different experimental protocols.

3.1. Resting conditions

At resting state (low glucose levels) the rate of metabolism in β -cells is relatively low. K_{ATP} channels are mostly in the open state and maintain a hyperpolarized membrane potential. VDCCs are closed below their activation threshold. The RRP is at this point is relatively large and determined by the conserved pool of corresponding targeting protein for RRP (TPR_f) available for granule binding. Under these conditions the influx of Ca^{2+} is small, $[Ca^{2+}]_c$ is low and the IS is at a low basal (constitutive) level. The values of the pools and parameters under these resting conditions were obtained by solving the equations at 2 mM glucose (see Fig. 2, left panel). The rate of IS in the resting state is determined mainly by the low RRP fusion rate (coefficient k_{rf} from Eq. 22, SM).

Under these resting conditions the simulated PM potential was -74 mV, $[Ca^{2+}]_c = 0.045$ μ M, RRP = 95 granules and the IS rate was 0.043 granules/sec per cell. Only 1–2 granules/min per cell are released at extracellular glucose concentrations of approximately 3 mmol/l from mouse and rat β -cells, and the pool of immediately releasable granules contains 50–100 granules in the basal state (Rorsman and Renstrom, 2003; Straub et al., 2004). Therefore the chosen coefficients and key assumptions made to construct the model yield a secretion rate that approximates the expected values.

3.2. IS during membrane depolarization at low glucose

High isotonic extracellular K^+ (25 mM) leads to β -cell PM depolarization at low glucose (as does direct voltage-clamp induced depolarization). This depolarization activates VDCCs leading to increased $[Ca^{2+}]_c$ and an activation of fusion processes. The continuous increase in K^+ causes a sustained $[Ca^{2+}]_c$ rise, whereas the IS rate exhibits a large initial peak followed by a slow return to the initial value (Henquin et al., 2006; Mourad et al., 2010;

Ravier and Henquin, 2002). The majority of granules responsible for fusion events induced by K^+ stimulation appeared to be from the RRP or DGP (Shibasaki et al., 2007).

PM depolarization can be simulated in our model by directly adjusting the PM potential (V_p) (Eq. 1, SM) (Fig. 2). The simulated IS pattern after PM depolarization was monophasic and, after the peak, the secretion rate declined toward the basal value. The initial RRP level was limited by the total number of specific free TPR (TPR_f) involved in exocytosis at the exocyst complex. A transient IS increase can be viewed simply as an opening of Ca^{2+} channels that increases the fusion rate of RRP, and the subsequent decrease in the fusion rate can be attributed to the decrease in the RRP (Fig. 2B).

Only small changes in [cAMP] were found by the simulations of direct PM depolarization in comparison with the resting cAMP level (not shown). The immediate fusion rate of NGP was low (Fig. 2) (this corresponds to experimental data obtained at high extracellular K^+ and low glucose (Shibasaki et al., 2007)) because the metabolic activation factor (f_{MF}) was small and constant at low glucose. Increased $[Ca^{2+}]_c$ secondary to PM depolarization alone cannot increase substantially the immediate fusion of NGP in our model without an increase in the metabolic activation factor.

Replenishment of the RRP begins even during a transient increase of IS as indicated by increasing mobilization and priming of the DGP (Fig. 2B). However, the kinetic parameters determined for RRP fusion during a PM depolarization are much larger in our model than for the re-supply process from the DGP. After a transitory increase in IS the following RRP fusion is determined by the priming rate of DGP, which was increased over the rate calculated during resting conditions as a result of increased level of free TPR (TPR_{fr}) that leads to increased rate of IS in comparison with the rate at low glucose and repolarized PM.

3.3. Stepwise increase of extracellular glucose

Several features of the glucose-stimulated response differed from the K^+ -stimulated response. First, there is a significant delay in V_p and $[Ca^{2+}]_c$ (up to several minutes) in rodent and human islets following stepwise increase of extracellular glucose, in contrast to the immediate response to K^+ stimulation (Henquin, 2009; Henquin et al., 2006). This delay is expected because glucose stimulation leading to PM depolarization occurs through a metabolic pathway that includes glycolysis and mitochondrial processes leading to an increased ATP/ADP ratio. This clearly takes more time than immediate PM depolarization activated by high extracellular K^+ . We have introduced this increased time delay using the decreased rate constant (in comparison with K^+ stimulation) in time-dependent differential equations for PM potential (V_p) (Fig. 3) (Eq. 1, SM).

IS from islets in culture is biphasic in response to changes in extracellular glucose concentrations. When glucose is increased from low to stimulating concentrations (10 mM glucose or more) a sharp and transient increase in IS is observed within 2–5 minutes of a glucose challenge (referred to as first phase). When high glucose concentrations are maintained, a second increase or phase is observed (Henquin et al., 2006; Nunemaker et al., 2006; Straub and Sharp, 2002). In response to a glucose challenge (from 5 mM to 20 mM), IS from intact mouse islets increases 15-fold, and approximately 20–30 granules/min per

cell are released at the peak of the first phase of IS after a step increase in glucose. After that a second-phase IS occurs which in mouse islets usually does not exceed the peak of the first phase (Henquin et al., 2006; Nunemaker et al., 2006; Straub and Sharp, 2002).

The first phase of IS can be explained by the closure of K_{ATP} channels, membrane depolarization and an opening of Ca^{2+} channels leading to the exocytosis of a small pool of RRP granules (Rorsman and Renstrom, 2003). The magnitude of the initial IS response is largely determined by the size of the RRP. The end of the first phase of IS might reflect the emptying of the RRP (Ohara-Imaizumi et al., 2009b; Rorsman and Renstrom, 2003). However, first phase secretion may not only involve the RRP but also the “restless newcomer” granules that approach the PM during stimulation only to be immediately released (Shibasaki et al., 2007).

A simulation of granule fusion rates after a step glucose increase at the basal coefficient is shown in Fig. 3. In this case the steady-state V_p was set to -20 mV (the usual bursting plateau) and $[Ca^{2+}]_c$ was set to 0.41 μ M (Fig. 3A) when the extracellular glucose was set at 14 mM. Our model reproduces the characteristic biphasic IS pattern in response to a step increase in glucose, consistent with experimental data. The first phase of IS can be explained by an increase in the open probability of Ca^{2+} channels that increases the fusion of RRP. The subsequent decrease of the fusion rate can be attributed to a decrease in RRP (as discussed above for PM depolarization following high K^+ in low glucose levels). However, our simulation also shows that activation of immediate fusion of granules from the NGP can begin during the first phase of IS, so that some of the secreting granules during this phase can be from the NGP (Fig. 3B).

Activation of immediate fusion of restless newcomer granules can be an important aspect of accelerated IS during the second phase (Ohara-Imaizumi et al., 2009b; Ohara-Imaizumi et al., 2004; Seino et al., 2011). Increased IS in the second phase is determined primarily by increased immediate fusion events of NGP granules during the second phase in our model (Fig. 3B) as a consequence of increased metabolic activation factor and a Ca^{2+} potentiating factor following an increase in glucose level (Fig. 3A). Priming from the DGP provides a sustained basal level of IS under resting conditions and a modest increase in fusion of RRP granules in the second phase at high glucose level (Fig. 3).

The influence of various metabolic factors on second phase secretion have been proposed in several studies (see for e.g. (Henquin, 2009; Straub and Sharp, 2002), where it was found that the number of granules secreted per- β cell during the second phase of IS was increased several fold in control mouse islets (Henquin, 2009; Mourad et al., 2010) following simulation with 15 mM glucose compared with tolbutamide treatment at low glucose (3 mM). These are conditions where the depolarized cell has a high intracellular $[Ca^{2+}]$ but is without the influence of “metabolic activation factor.” Introduction of f_{MF} allow us to describe these data in our model because f_{MF} increased with glucose increase and accelerated NGP fusion.

We have also simulated changes in IS following a stepwise increase of glucose from low to medium and high glucose level (Fig. 4). IS was increased in both phases with increased

glucose levels. This simulated result connects also with increasing f_{MF} with glucose that increased NGP fusion rate (not shown). Increased first phase IS can be explained by the participation of immediate fusion of NGP in first phase of IS (see Fig. 3).

Experiments were performed where single mouse islets have been exposed to diazoxide, an agent that opens K_{ATP} channels and prevents the effect of glucose on V_p . Periodic changes in V_p were imposed by repetitive depolarization with high KCl at permanent low and high glucose levels (Ravier and Henquin, 2002). Under these conditions the difference in permanent glucose levels had little impact on changes in $[Ca^{2+}]_c$, that determines by the changes in membrane potential. However, when the glucose concentration was high the amplitude of insulin pulses in response to KCl periodical increase was also markedly augmented. We were able to simulate these interesting experimental results (Fig. 5). In this case the simulated changes in V_p and $[Ca^{2+}]_c$ were similar at low and high glucose. However, the IS maximal level was increased with increased glucose. These results can be explained by increased f_{MF} with increased glucose that increased the rate of NGP immediate fusion.

Syntaxin1A-knockout β -cells showed fewer previously docked granules with no fusion during the first phase of glucose induced IS; second-phase fusion from restless newcomers was preserved (Ohara-Imaizumi et al., 2007). We can simulate this by a decrease in the target protein pool for docking and priming (TPD) (see above). This leads to decreased RRP and DGP and corresponding block of RRP fusion and a decrease in the first phase of IS (not shown) that corresponds to the experimental data previously mentioned.

3.4. Regulation of IS through cAMP pathway

Although cAMP potentiates GSIS, exactly how cAMP acts in the exocytotic processes of recruitment, docking, and fusion of secretory granules is not clear. On the basis of published experimental data our model was designed so that the cAMP pathway can activate IS at three points (see above): 1. Activated PKA activated directly of RRP fusion (Eq. 22, SM). 2. Activated EPAC accelerated the immediate fusion of NGP (Eq. 24, SM). 3. Activated EPAC increased the quantity of TPR_f (Eq. 30) that in turn increases RRP potential accumulation in resting conditions. We had also taken into account the influence of $[Ca^{2+}]_c$ on cAMP through the activation of calcium dependent AC that increase of cAMP concentration.

At low glucose concentrations (1.0–2.8 mM), that do not induce a PM depolarization, treatment of pancreatic β -cells with agents that raise cAMP alone have little or no effect on fusion of the granules to the PM or on IS (Shibasaki et al., 2007). Simulation of cAMP signaling at low glucose levels in our model also only leads to small increases in IS (not shown). These results were obtained in part because the opening probability of VDCCs were minimal in the hyperpolarized state at low glucose and $[Ca^{2+}]_c$ and the f_{MF} for immediate fusion of NGP were small under these conditions.

Combining an increase in cAMP levels with high extracellular glucose potentiates IS (Fridlyand et al., 2007; Leech et al., 2010; Seino et al., 2009; Shibasaki et al., 2007). In mouse β -cells an elevation of intracellular cAMP potentiates secretion principally by a direct effect on the exocytotic machinery (Seino et al., 2009; Shibasaki et al., 2007) and to a lesser

extent by increasing $[Ca^{2+}]_c$ (Ammala et al., 1993). Inhibition of PKA activity also did not change $[Ca^{2+}]_c$ significantly (Hatakeyama et al., 2006). We attempted to be consistent with these findings and did not directly include regulation of $[Ca^{2+}]_c$ by cAMP pathway activation in our model.

PKA inhibition primarily decreased of the first phase of IS in isolated mouse islets as indicated by the imaging of exocytosis with fluorescent tracers (Hatakeyama et al., 2006; Hatakeyama et al., 2007). Our model reproduces disappears of the first phase at PKA inhibition (Fig. 6) and shows that an inhibition or an amplification of the first phase of IS may be expected if PKA acts predominantly on fusion of primed granules, as we have suggested (Eq. 22, SM).

Both the cAMP-analog 8-Bromo-cAMP and the incretin activators of cAMP pathways (such as GLP-1) potentiate both the first and second phases of glucose-induced IS (Flamez et al., 1999; Seino et al., 2009; Shibasaki et al., 2007). An EPAC selective cAMP-analogue (8-pCPT-2'-O-Me-cAMP) clearly potentiated both the first and the second phase of glucose-induced fusion events in isolated mouse islets (Fujimoto et al., 2009; Kelley et al., 2009). The majority of the granules responsible for this potentiation were newly recruited, and these were seen to immediately fuse to the PM without docking (Shibasaki et al., 2007). We simulated cAMP effects (Fig. 7) by increasing the activity of AC (as a single term, neglecting the numerous AC activities and compartmentation issues (Eq. 13, SM). By doing this the cAMP concentration was 17-fold (about 10 μ M) increased over the basal state. This gave an increase in both phases of IS similar to that observed experimentally (compare Fig. 3 and Fig. 7).

Three different processes take part to increase first phase IS in our model. The first is the increased RRP that occurs under resting conditions as a consequence of accelerated TPR_f formation due to activation of EPAC. The second is the acceleration of RRP fusion following PKA activation. The third is the initial increase in the NGP fusion rate following EPAC activation that is going to occur during the first phase itself (Fig. 7B). Increased IS during the second phase results mainly from acceleration of NGP immediate fusion following EPAC activation. There is also a smaller contribution from acceleration of RRP fusion following PKA activation and by EPAC activation of priming through acceleration of TPR_f formation.

4. Discussion

Here we represent the descriptive mathematical model of pancreatic β -cell exocytosis at the cellular level that allowed to recapitulate the results of IS experiments across a broad time span of minutes to several hours. Our aim was to quantitatively describe granule dynamics and fusion processes for an interpretation of existing data and to design new experiments. However, the model required the mechanical descriptions of several key processes even though their precise mechanisms are still unclear. In this way we introduced several suggestions that are based on indirect evidence. In particular, we suggested several conserved protein systems: 1) A pool of specific target proteins for RRP (TPR) representing the exocyst complex which are able to promote priming of docked granules at the inner

membrane surface; 2) A conserved “target protein pool” for docking and priming components (TPD); 3) A pool of vesicular proteins (VPP) involved in binding with NGP granules. These conserved protein systems determine the sizes of three postulated intracellular granule pools: RRP, DGP and NGP. The large RGP changed little during our simulations of experiments with durations up to several hours and we did not suggest an existence of a special protein pool that can be bound with RGP.

Our simulations confirmed that VDCC activation plays a decisive role in the fusion process for granules in the RRP as well as in the NGP, justifying the inclusion of this term. However, our simulation also showed the importance of introducing a specific $[Ca^{2+}]_c$ potentiating factor that describes the acceleration of immediate fusion of NGP as $[Ca^{2+}]_c$ increases.

Non-electrogenic effects of glucose can amplify IS, also termed the amplifying pathway, originally referred to as the K_{ATP} channel-independent pathway (Henquin, 2009). Based on these data we introduced in our descriptive model the metabolic activation factor (f_{MF}) that activates the immediate fusion of NGP. This allowed us to describe the process of an activation of IS, other than closing of K_{ATP} channels and an increase of $[Ca^{2+}]_c$, through increased glucose metabolism even though the exact nature of this metabolic factor remains unclear.

Among the intracellular signals, cAMP serves as an almost universal signal that modulates or regulates exocytosis in various secretory systems (Leech et al., 2010; Seino et al., 2009). *In vivo*, β -cells also interact with a variety of hormones that may have complimentary or antagonistic actions on IS through their interactions with various parts of the cAMP regulatory system. For example, activation of cAMP cascades via G-protein coupled receptors is the hallmark of incretin secretion-stimulating hormones. In mouse β -cells, cAMP stimulates Ca^{2+} -dependent exocytosis by PKA-dependent and -independent pathways, the latter involving the cAMP-GEF/Epac family of cAMP-binding proteins (Leech et al., 2010; Seino et al., 2009). We simulated both pathways and were capable of reproducing the effect of cAMP pathway activation on insulin secretory dynamics. However, further studies are necessary to clarify the role of GLP-1 in EPAC mediated insulin granule dynamics.

4.1. Comparison with existing models

Cell capacitance measurements allow monitoring of the changes in the cell surface area that result from the fusion of granules with the cell membrane. The exocytosis step where the kinetic mechanism has been quantitatively studied by this method is the fast Ca^{2+} -triggered granule fusion in isolated β -cells (Eliasson et al., 2008; Kanno et al., 2004). However, exocytosis in β -cells measured by the capacitance method in intact islets is much slower than was found in isolated β -cells and better corresponds to measurements of IS in isolated islets (Gopel et al., 2004). For this reason, we used the experimentally measured IS rate in isolated islets rather than capacitance measurements using isolated β -cells to evaluate the model coefficients, so that our model is not directly applicable to single β -cell capacitance measurements.

Kinetic models for granule fusion in pancreatic β -cells were developed from a model for fast exocytosis in chromaffin cells (Chen et al., 2008; Pedersen, 2010; Pedersen and Sherman, 2009). These models may be used for interpretation of data obtained using capacitance measurements where fast fusion processes were measured during the initial several seconds. In a recent model (Pedersen and Sherman, 2009), based on cell capacitance measurements, docked and primed granule pools were divided into two pools with high and low sensitivity to Ca^{2+} . This model also denoted the high Ca^{2+} -sensitive granule pool as a newcomer granule pool. However, this granule pool may be unrelated (or have other properties) to restless newcomer granules (eg. (Kasai et al., 2008; Ohara-Imaizumi et al., 2007; Ohara-Imaizumi et al., 2004) and NGP that we described in our model. This high Ca^{2+} sensitive granule pool (Pedersen and Sherman, 2009) had an ability for fast fusion (within one second) with cell depolarization, while restless newcomer granules (Kasai et al., 2008; Ohara-Imaizumi et al., 2007) and NGP in our model need tens of seconds for beginning a fusion event. This concept of a high Ca^{2+} sensitive granule pool also did not take into account activation of IS by non-electrogenic effects of glucose that we denoted as a metabolic activation factor which also needs tens of seconds for activation.

One view of the exocytosis process suggested that newly arrived vesicles must go through a sequence of steps, docking and priming, before fusing (Rorsman and Renstrom, 2003). Several mathematical models of glucose-stimulated IS were previously proposed reflecting this staged approach (Grotsky, 1972; Nesher and Cerasi, 2002) which were able to reproduce the observed two phases insulin secretion data. Other recent models concentrated on details of the regulation of this sequence of steps and properties of different pools of granules in single cells introducing the readily releasable pool of granules alongside with docked granule pool (Bertuzzi et al., 2007; Chen et al., 2008; Pedersen et al., 2008). Our model also includes this sequence of steps. Our simulations of the of IS at cell depolarization at low glucose levels (where only first phase of IS exists because NGP “immediate fusion” was insignificant (Fig. 2)) resembles the results obtained by others (Bertuzzi et al., 2007; Chen et al., 2008; Pedersen et al., 2008) at the analogous simulation of IS with cell depolarization at low glucose level.

However, recent data suggests that some undocked granules can begin to fuse in several minutes after a glucose increase and this process can determine the IS rate during the second phase and, partially, in the first as well depending on how they are defined (Ohara-Imaizumi et al., 2007; Seino et al., 2011). We modeled these observations by introducing a restless newcomer granule pool (NGP) and corresponding potentiating factors, which influence the rate of NGP fusion. These properties of the NGP fusion were not taken into account in previous mathematical models. Evidently, these models cannot correctly describe the mechanisms of glucose dependent IS if “restless newcomer granules” have a preferential release as it was found in works (Kasai et al., 2008; Ohara-Imaizumi et al., 2002; Shibasaki et al., 2007).

4.2. Model limitations and future development

Our model was developed for a single β -cell. To expand these results to islets we need two key assumptions, first that all the cells in the islet respond identically to a stimulus and

secondly that there is perfect cell to cell coupling that coordinate these responses. While this is clearly not exactly true in every case, pancreatic β -cells in a mouse islet are tightly electrically coupled via gap junctions. The gap junctions serve to synchronize electrical activity and secretion in β -cells so that within an intact mouse islet virtually all β -cells respond simultaneously (Benninger et al., 2008; Kuznetsov et al., 2005; Zhang et al., 2008). For this reason our model may be appropriate to model events in single mouse islets.

However, dose–response curves obtained in isolated β -cells and cell clusters have different glucose thresholds for triggering membrane potential depolarization and the corresponding Ca^{2+} influx that could reflect distinct sensitivities to glucose (Heart et al., 2006; Henquin et al., 2006; Jonkers and Henquin, 2001). Therefore we cannot as yet directly simulate IS for a suspension of isolated β -cells or islets or a whole pancreas preparation with this model. The approach proposed in a recent model (Pedersen et al., 2008) can be used to calculate IS in those situations. In that approach it was assumed that at the β -cells have a range of thresholds for glucose, so while some remain silent, other cells are triggered to depolarize and release insulin. This model modification also allows simulation of experiments using step increases in glucose where the population of responsive cells at every glucose step is important (Pedersen et al., 2008).

Downstream targets of the cAMP pathway in early IS events can include the VDCCs, K_{ATP} and other channels and lipid-associated proteins (Fridlyand et al., 2007; Kang et al., 2006; Leech et al., 2010; Lopez et al., 2010; Seino and Shibasaki, 2005). However, the data are contradicting and in this study we have not modeled the processes of V_p and $[\text{Ca}^{2+}]_c$ regulation in detail. We described them only in straightforward empirical forms, focusing instead on modeling the effect of the cAMP pathway on exocytosis.

Other enhancers of IS include acetylcholine, released from pancreatic parasympathetic nerve endings, and cholecystokinin, which both act via G-protein coupled receptors that activate phospholipase C. These receptors couple to the generation of 1,4,5-inositol-trisphosphate leading to release of Ca^{2+} ions from internal stores, and also generate PM associated diacylglycerol, a potent activator of specific isoforms of protein kinase C (Gilon and Henquin, 2001). The net result of these pathways is an augmentation of glucose-evoked IS, by directly elevating the cytoplasmic concentration of Ca^{2+} ions, as well as through increased participation and efficiency of the proteins in the exocytotic machinery. The effects of phospholipase C activation on IS have been well described in mouse and rat islets (Gilon and Henquin, 2001; Yamazaki et al., 2006; Zawalich et al., 2008). However, the phosphorylation targets that mediate the enhancement of exocytosis by protein kinase C are unknown. Future models incorporating the role of the phospholipase C activation pathway and activation of the GPR40 pathway, for example, await additional research.

The islet data used to fit the model in our study were taken primarily from mouse islets. For this reason the model as developed here does not have the capability to simulate several experimental results obtained with rat islets. For example, a time-dependent potentiation can be defined as the enhancement in IS that results from previous exposure to glucose and other nutrients. This effect is pronounced in rats and slight or non-existent in the mouse (Grotsky et al., 1969; Straub and Sharp, 2002). The underlying mechanisms remain unknown.

However, for example in rat β -cells, glucose stimulates an increase in the number of insulin granules associated with the PM (Straub et al., 2004). Some combination of priming steps is also likely to be involved. Secondly, the data obtained from mouse islets exhibited the typical flat or slightly increasing second phase of IS. Rat islets, in contrast, similar to isolated rat β -cells exhibit a rising second phase that can considerably exceed the peak of the first phase (Eliasson et al., 2008; Straub and Sharp, 2002; Zawalich et al., 2008). The present form of our model also does not simulate these results from isolated rat cells. Further development of the model including PLC pathway activation and other factors may allow a more appropriate simulation of IS behavior in rat islets in the future.

Our model was developed directly on the basis of experimental studies using the data from β -cells and islet studies that we have described by simplifying mathematical equations. The inclusion of a voltage-depending opening of Ca^{2+} channels, special conserved protein pools and potentiating factors gives a simplified physical reality to describe the fusion process without completely defining the exocyst complex. However, potential docking sites as part of an exocyst complex certainly includes multiple other proteins and lipids (Lopez et al., 2010; Yamashita et al., 2010). Estimation of the parameters from measured observations also involves uncertainties inherent in complex systems. The integrated phenomenological model we developed did not include all the possible factors involved in the regulation of IS process, in part because they are simply not sufficiently understood at this time. However, we have attempted to illustrate the utility of the modeling approach for β -cell exocytosis. This model is a scalable system with the potential for additional subroutines as the understanding of additional pathways grows. Dynamic analysis of these processes can help determine the specific roles of messenger's pathways, and suggest experiments to verify competing hypotheses. This initial descriptive mathematical model will be subject to adjustment with new experimental data.

Changes in the efficiency of the docking, fusion and other coupling processes are likely to play a critical role in the normal control of insulin release. They may also be involved in the regulation of secretion by endogenous clocks (Marcheva et al., 2010). These observations help to substantiate the idea that some forms of T2DM involve defective secretory machinery (Eliasson et al., 2008; Henquin, 2009). This is an intriguing possibility that can be further explored using models that combine β -cell metabolism with exocytosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding for this study was provided in part by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DRTC P60DK020595, DK-48494, DK-063493, a Research Grant from the Keck foundation and the Blum-Kovler Foundation and the Kovler Diabetes Center.

Abbreviations

AC adenylyl cyclase

[Ca²⁺]_c	cytoplasmic Ca ²⁺ concentration
DGP	docked granule pool
EPAC	guanyl exchange proteins
f_{MF}	metabolic activation factor
GLP-1	glucagon-like peptide-1
[Glu]	glucose concentration
IS	insulin secretion
K_{ATP}	channels ATP-sensitive K ⁺ channels
NGP	restless newcomer granule pool
PKA	protein kinase A
PM	plasma membrane
TPR	target protein pool for RRP
RRP	readily releasable granule pool
RGP	reserve granule pool
SNARE	soluble NSF attachment protein receptor
SM	supplemental material
TDP	target protein pool for docking and priming
T2DM	type 2 diabetes
VDCCs	voltage-dependent Ca ²⁺ channels
V_P	plasma membrane potential
VVP	special vesicular protein pool for binding with NGP granules

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Fig. 1.

Schematic of the β -cell exocytosis regulatory system. Top: K_{ATP} channels is the ATP-sensitive K⁺ channels, RRP is the readily releasable granule pool, DGP is the docked granule pool, NGP is the restless newcomer granule pool, RGP is the reserve granule pool, TPR_i and TPR_f are the inactive and activated free target protein pool for RRP, TDP_f is the free “target protein” pool for docking and priming. Bottom: cAMP pathway. Ca²⁺/CaM is Ca²⁺-bound calmodulin, Synthesis and degradation of cAMP are catalyzed by adenylyl cyclase (AC) and phosphodiesterase (PDE), respectively. AC was activated by stimulatory G protein and Ca²⁺/CaM and deactivated by inhibited G protein, whereas PDE activity was enhanced by Ca²⁺/CaM, PKA is the protein kinase A; PKAa is the activated PKA, EPAC is the guanyl exchange protein, EPACa is EPAC activated by cAMP; GLP-1 is glucagon-like peptide-1. Solid lines indicate fluxes, and dashed lines indicate inhibitory or stimulatory effects on currents or fluxes.

Fig. 2.

Simulated islet response to voltage-clamp depolarization (or K^+ stimulation) at $t = 0$ sec. Coefficient k_{1V} and k_{2V} (Eq. 1, SM) were increased from 0.015 s^{-1} (Table 1, SM) to 0.045 s^{-1} to accelerate the processes of a transition of plasma membrane potential (V_p), V_{sk} was -8 mV and g_{mCa} (Eq. 3, SM) was $0.4 \mu\text{mol s}^{-1}$. Factor VF that determines V_p (Eq. 2, SM) was increased from 2 to 15 at $t = 0$ sec. (A) V_p , $[Ca^{2+}]_c$ and RRP (in granules per cell) dynamics. (B) Time course of fusion processes: F_{pf} is the fusion rate of RRP (— · — · —), F_{if} is the fusion rate of NGP (·····), F_p is the primed rate of DGP (— · — · —), F_i is the total insulin secretion rate (sum of F_{pf} and F_{if}) (— — —).

Fig. 3. Simulated biphasic insulin secretion (IS) in response to step increase in glucose level at $t = 0$ sec from $[Glu] = 2$ mM to 14 mM. (A) V_p , RRP (in granules per cell) (—), $[Ca^{2+}]_c$ (.....) and the metabolic potential factor (f_{MF}) (— — —). (B) Time course of fusion processes with the definitions as in Fig. 2.

Fig. 4. Biphasic IS in response to step increase in glucose level at $t = 0$ sec (from $[Glu] = 2$ mM to 10 mM (---), and from $[Glu] = 2$ mM to $[Glu] = 20$ mM (—)).

Fig. 5.

Simulated islet response to periodic K^+ stimulation at different glucose levels at blocked K_{ATP} channels. Stimulation begins at $t = 0$ sec. (A). Simulated train of square-wave (V_p) pulses between -65 mV and -10 mV (the potential induced by KCl changes) with a period of 16 min. Coefficient VF (Eq. 2, SM) was periodically changed from 2 to 15. V_{sk} (Eq. 2, SM) was -8 mV and g_{mCa} (Eq.3, SM) was $0.4 \mu\text{mol s}^{-1}$ (B). Simulated $[Ca^{2+}]_c$ changes. (C) The total insulin secretion (F_i) at a glucose level of 5 mM ($f_{MF} = 0.08$). (D). F_i at 14 mM glucose ($f_{MF} = 0.404$).

Fig. 6. Simulated islet response to step increase in glucose level at $t = 0$ sec from $[Glu] = 2$ mM to 14 mM at an activation and inactivation of PKA. Preliminary inactivation of PKA was obtained by the decrease of coefficient k_{ak} (Eq.16, SM) from basal level (1) to 0.01 and PKA activation was obtained by the increase of coefficient k_{ak} from basal level (1) to 10. Relative PKA activation (PKAa, Eq. 16, SM) was 0.036 at inactivated, 0.27 at basal and 0.79 at activated level. IS (as F_i) was simulated in basal conditions (---), at inactivated PKA (.....) and at activated PKA (—).

Fig. 7.

Simulated effect of glucose increase at preliminary activation of cAMP pathway in rest conditions. Coefficient V_{ACG} that determines AC activation (Eq.13, SM) was increased first from the basal level of $0.05 \mu\text{mol s}^{-1}$ to $0.035 \mu\text{mol s}^{-1}$. Concentrations cAMP was $0.62 \mu\text{M}$ at basal level and $10.1 \mu\text{M}$ at preliminary cAMP pathway activation. The glucose level was raised at $t = 0$ sec from $[\text{Glu}] = 2 \text{ mM}$ to 14 mM . (A) V_p , RRP (in granules per cell) (---), $[Ca^{2+}]_c$ (.....) and the metabolic potential factor (f_{MF}) (— · — · —) dynamics. (B) Time course of fusion processes: F_{pf} is the fusion rate of RRP (---), F_{if} is the fusion rate of NGP (.....), F_p is the primed rate of DGP (— · — · —), F_i is the total insulin secretion rate (sum of F_{pf} and F_{if}) (——).