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18 Glucose-stimulated insulin secretion – Insights from modelling

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Abstract

Insulin is secreted by the pancreatic β-cells in response to a raised blood glucose concentration. Glucose-stimulated insulin secretion (GSIS) is pulsatile and correlated with bursting electrical activity. We review the current understanding of pancreatic β-cell GSIS from a mathematical modelling perspective. We discuss the glycolysis and the mitochondrial metabolism and metabolic modelling thereof. In particular, we describe the theoretical basis for the hypothesis of an oscillatory glycolysis, which may be the cause of the pulsatile secretion. We also discuss the electrophysiology of

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the β-cell and present insights gained from mathematical modelling in this context. There are important causal connections between β-cell metabolism and electrophysiology. This brings about an unusual challenge for theoretical and computational biologists.

Introduction

The pancreatic β-cells are localized in small clusters of about 2000–3000 cells, called the islets of Langerhans. The physiological task of this cell type is to secrete insulin into the blood in response to a raised blood glucose level, and as such they are crucial for maintaining the glucose homeostasis in the healthy human, since insulin stimulates the glucose metabolism in peripheral tissue and glycogenesis in the liver. Clinically, β-cell disorders are well recognized culprits behind the widespread disease non-insulin dependent diabetes mellitus (NIDDM or type 2 diabetes). The events leading from a raised glucose level to insulin secretion are inter-connected in an intricate biochemical network. This complexity makes it necessary to adopt a quantitative mathematical view in order to gain a good theoretical understanding of glucose-stimulated insulin secretion (GSIS).

This review will illustrate how mathematical modelling and analysis has contributed to the understanding of the biochemistry and physiology of the pancreatic β-cell. Thus, we will outline some mechanisms proposed to take part in GSIS, particularly those which have been modeled quantitatively. We will not describe other stimulants of insulin secretion (such as acetylcholine and glucagon-like peptide-1) and their associated pathways, and mostly limit ourselves to the description of the knowledge of the non-diabetic β-cell. The modelling approaches we will consider are those that explicitly address the biochemistry of the β-cell. Hence, we do not consider purely phenomenological "black box" models of GSIS (a subject which was recently reviewed elsewhere [1]).

From a biochemical perspective, it was early proposed that glucose directly acts as a signal for insulin secretion, via increased cellular metabolism [2]. The experimental testing of this hypothesis gained substantial momentum as pancreatic islets were first isolated in 1967 [3]. During the following years it was demonstrated that the pancreatic islet cells are electrically active [4, 5] and that the ubiquitous cellular messenger Ca^{2+} acts as a signal to promote insulin secretion via the exocytosis of secretory granules [6, 7, 8]. A breakthrough in the elucidation of the coupling between increased metabolism and insulin secretion came in 1984, when Cook and Hales [9] identified the ATP-sensitive potassium (K_{ATP}) channel in the β-cell and Ashcroft et al. [10] showed that these channels close in response to a glucose bolus resulting in depolarization of the cell membrane. In turn, the depolarization leads to the opening of L-type voltage

sensitive Ca^{2+} channels resulting in an increase of the cytosolic Ca^{2+} concentration, $[Ca^{2+}]_i$ [11]. This causal relationship is well-established; the increased ATP concentration precedes the rise in $[Ca^{2+}]_i$ [12, 13, 14, 15]. Thus, Grodsky's metabolism hypothesis was vindicated; here we have a direct link between an increased metabolism (resulting in increased cytosolic ATP/ADP ratio) and electrical activity and insulin secretion. This pathway of GSIS is now known as the K_{ATP}-dependent pathway – see figure 1 which summarizes the discussion so far.

The electrical activity of the β-cell exhibits complex dynamics. The depolarization of the membrane potential triggered by glucose comes in the form of repetitive bursting. The bursting consists of depolarized plateaus of about −30 to −40 mV, with superimposed rapid spikes (action potentials) of about 10–20 mV, and hyperpolarized phases of about −60 to −50 mV [16]. Generally, one speaks of three characteristic burst frequencies [17]. "Fast bursting", with a period of 2–5 s, has been seen to occur in single β-cells. "Medium bursting", with a period of 10–60 s and "slow bursting" with a period of 2–4 min are seen both in single cells and whole islets. Synchronized with these burstings are oscillations in $[Ca^{2+}]_i$. Crucially, insulin secretion is oscillatory as well, with a period of several minutes [18], and synchronized with slow bursting and corresponding $[Ca^{2+}]_i$ oscillations [8].

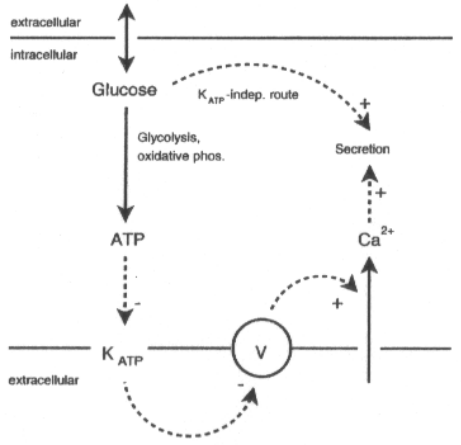

Figure 1. The K_{ATP}-dependent pathway comprises glucose increasing the ATP/ADP ratio which closes ATP-sensitive K^+ channels. This results in a depolarization of the cell membrane (V indicates membrane potential). In response to this, voltage-sensitive Ca^{2+} channels open, which leads to an increased $[Ca^{2+}]_i$, which in turn stimulates insulin secretion. A K_{ATP}-independent pathway acts synergistically.

The oscillations discussed so far are initiated [19] and, in case of the slow oscillations, perhaps driven ([20], see also the discussion below) by ATP-mediated modulation of the K_{ATP} channel. However, this is not the whole story of β-cell GSIS. In 1992 Gembal et al. [21] demonstrated that insulin secretion is augmented by some other, K_{ATP}-independent, pathway, acting synergistically with the K_{ATP}-dependent pathway (see figure 1) but only in the presence of a permissively high $[Ca^{2+}]_i$. Furthermore, insulin secretion is biphasic, with a first phase occurring 1–2 minutes after glucose stimulation, and a second phase after 25–30 minutes [22, 23]. There is evidence that the initial phase is due to the K_{ATP}-dependent pathway, while the second phase is due to the K_{ATP}-independent pathway [24].

We will begin our treatment with a description of how glucose stimulates the β-cell metabolism and describe important modelling efforts of central metabolic pathways, in particular the glycolysis. Then, we discuss how the metabolic signals triggered by glucose propagate to the K_{ATP}-independent and K_{ATP}-dependent pathways, respectively.

Glycolysis

Glucose enters the pancreatic β-cell via GLUT type transporter proteins [25]. Glucose transport is considered to normally occur on a much faster time-scale than downstream events, and glucose concentration may thus be considered to be equilibrated between the extra- and intracellular compartments [26, 27, 14]. Next, glucose enters the glycolysis, the ubiquitous biochemical pathway which consists of 10 enzymes transforming the six-carbon glucose molecule to two three-carbon pyruvate molecules. These are subsequently metabolized mainly aerobically in the mitochondria – the anaerobic alternative, the metabolization by lactate dehydrogenase (LDH), is marginal in the β-cell [28, 29]. Also, the diversion of glycolytic flux to the pentose phosphate pathway is limited [30]. Two glycolytic enzymes have received special attention in the β-cell: glucokinase (GK) and phosphofructokinase (PFK).

Glucokinase

GK is the first enzyme of the β-cell glycolysis and it is an isozyme of hexokinase (isoform D or IV) with, compared to other isoforms, an unusually high half-activation point of 5–10 mM [31, 32, 33] – a half-activation point which happens to be in the range of the physiological glucose concentration. Also, the coupling of the GK reaction with the hydrolysis of an ATP molecule makes it practically irreversible under physiological conditions. Therefore, GK was early hypothesized to have a high control over the glycolytic flux in the β-cell [26, 34]. In a computer modelling effort by Garfinkel et al. [35], a kinetic model of GK was found to be able to fit data on the glycolytic rate measured in

islets, lending support to this view. Some years later, a full model of the β-cell glycolysis was presented by Sweet and Matschinsky [36]. This metabolic model consists of rate equations for the glucose transporter, GK, hexokinase A (which however subsequently was deemed not being present in the β-cell [33]), PFK, pyruvate kinase, and pyruvate removal, and all other reactions considered to be in pseudo-equilibrium. This model predicts a high flux control coefficient for GK on the glycolytic rate. The model was later refined and supported by new experimental data to further strengthen this conclusion [37, 38].

The concept of GK being "rate-limiting" for the glycolytic flux together with the hypothesis that glucose stimulates secretion via an increased metabolism led to that GK disorders became a candidate for being culprits behind the impaired insulin secretion of NIDDM. Indeed, a subtype of NIDDM called MODY-2 was identified as being due to a family of over 30 different mutations in the GK gene [39, 14].

Phosphofructokinase and the oscillatory glycolysis

PFK, the third enzyme in the glycolysis, is like GK practically irreversible because of the coupling of the main reaction, the conversion of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (FBP), to ATP hydrolysis. However, the property of β-cell PFK that has received the greatest attention is due to the demonstration in 1995 that the isoform present in the β-cell is the M (muscle) type [40]. A significant trait of this isoform is its activation by both its products, FBP and ADP [41, 42, 40]. This is clearly a case of autocatalysis, and it is from a theoretical viewpoint well-known that this in (bio)chemical reaction systems may cause oscillatory behaviour [43]. Indeed, oscillations in glycolysis have been demonstrated in several organisms and model systems of which yeast is the most well-studied and the oscillations in all systems have been attributed as due to autocatalysis of PFK [44, 45, 46]. The time scale of glycolytic oscillations is typically in the range of minutes, which has prompted the hypothesis that they might be the mechanism behind the pulsatile insulin secretion. During the last decade, indications of an oscillatory glycolysis in the β-cell have accumulated, including observations of oscillations in cytosolic NAD(P)H concentration, oxygen consumption, glucose-6-phosphate concentration and ATP/ADP levels [47, 48, 49, 50, 51, 52, 53, 54]. Importantly, the oscillations of the latter parameter correlates with insulin secretion and $[Ca^{2+}]_i$ [55]. Therefore, a minimal hypothesis is that the glycolytic oscillations are due to the autocatalysis of the PFK reaction [20, 55], and further, that this is the cause of slow bursting and corresponding insulin secretion. Clinically, it has been shown that an inherited PFK deficiency results in loss of the oscillations of insulin secretion [56], which motivates thorough investigations of the role of PFK in the β-cell.

Theoretical modelling of oscillatory glycolytic systems has a history of 40 years, the first model being due to Higgins [57], explaining oscillations in yeast with the autocatalytic activation of PFK by FBP. Later, others elaborated on the oscillatory yeast glycolysis, and Goldbeter and Lefever [58] presented a model focused on the autocatalytic activation by the other PFK product, ADP, modelling the intricate kinetic behaviour of PFK using the Monod-Wyman-Changeux (MWC) rate equation of allosteric enzyme regulation [59]. Glycolytic oscillations were subsequently demonstrated in mammalian muscle cell extracts, and a model of glycolytic oscillations in mammalian muscle appeared in 1979 [45] which focused on the autocatalysis by FBP. A refined model of glycolytic oscillations in muscle was presented by Smolen [60], who assumed that autocatalysis by FBP is the mechanism that generates oscillations, basing his study on the experimental data of Tornheim and Lowenstein [61]. Smolen further showed that the MWC model is unsuitable for the description of mammalian M type PFK, and presented an alternative rate equation better capable of fitting the experimental data considered in the study.

A core model of the oscillatory glycolysis in the β-cell was recently presented by Westermark and Lansner [62]. This simple minimal model is schematically presented in figure 2. The simplicity is motivated by the experimental observation that the last five enzymes in the glycolysis exhibit activities an order of magnitude greater than that of the first five enzymes. In this limit, the removal of mass from the glycolytic chain is controlled solely by aldolase (FBA) and glyceraldehyde-3-phosphate dehydrogenase (GPDH).

Figure 2. A core model of β-cell glycolysis. The dashed box surrounds metabolites considered to be in pseudo-equilibrium. The dashed arrow indicates the positive feedback elicited by the PFK product FBP. In the simplest case presented here, the removal of mass from the glycolysis is controlled solely by FBA.

Here is the simple two-variable quantitative formulation of the scheme in figure 2:

$$\begin{aligned} \dot{\sigma} &= \frac{f}{S_{0.5}^{\mathrm{PFK}}}(v_{\mathrm{GK}} - v_{\mathrm{PFK}}) \\ \dot{\pi} &= \frac{1}{S_{0.5}^{\mathrm{FBA}}}(v_{\mathrm{PFK}} - v_{\mathrm{FBA}}), \end{aligned} \qquad (1)$$

We here have dimensionless variables, $\sigma = [\mathrm{F6P}]/S_{0.5}^{\mathrm{PFK}}$ and $\pi = [\mathrm{FBP}]/S_{0.5}^{\mathrm{FBA}}$. The parameters $S_{0.5}^{\mathrm{PFK}}$ and $S_{0.5}^{\mathrm{FBA}}$ represent the half-activation points for PFK (in the absence of any allosteric modifier) and FBA respectively. The glucose-6-phosphate isomerase (GPI) equilibrium has an equilibrium constant $K_{\mathrm{eq}}^{\mathrm{GPI}} = [\mathrm{F6P}]/[\mathrm{G6P}]$ which enters the expression $f = K_{\mathrm{eq}}^{\mathrm{GPI}}/(1+K_{\mathrm{eq}}^{\mathrm{GPI}})$. The rate equations of the model are:

$$v_{\mathrm{GK}} = \frac{V_{\mathrm{GK}}\varsigma^{h_{\mathrm{GK}}}}{1+\varsigma^{h_{\mathrm{GK}}}}$$

$$v_{\mathrm{PFK}} = \frac{V_{\mathrm{PFK}}\sigma^{h_{\mathrm{PFK}}}}{\sigma^{h_{\mathrm{PFK}}} + \frac{1+(q\pi)^{h_{\mathrm{PFK}}}}{1+\alpha(q\pi)^{h_{\mathrm{PFK}}}}}$$

$$v_{\mathrm{FBA}} = \frac{V_{\mathrm{FBA}}\,\pi}{1+\pi},$$

where $\varsigma = [\mathrm{glucose}]/S_{0.5}^{\mathrm{GK}}$. These rate equations are the standard Hill equation for GK and Michaelis-Menten equation for FBA, with limiting rates V and, in the case of GK, Hill coefficient h. The rate equation for PFK is the irreversible case of the reversible Hill equation with allosteric modifiers [63]. This equation was shown to be able to express all the relevant kinetic features of PFK with a minimum of parameters. Moreover, the parameters are *operationally well-defined* in a biochemical sense, i.e. measurable and non-redundant, which has the effect that an analysis of the model in terms of its parameters generates meaningful biochemical predictions. Conversely, operationally well-defined parameters facilitate the estimation of model parameters from experimental data. Thus, parameter *ranges*, estimated from a variety of literature sources, rather than fixed values were used in the model, which subsequently was analyzed in terms of the most important parameters. In another paper [64], the reversible Hill equation was generalized and shown to yield a substantially better fit to the aforementioned kinetic data used in the study by Smolen [60], than the rate equation used in that work.

This core model assumes that the mass removal is solely controlled by FBA. As shown in the paper [62], the control is effectively shared between FBA and GPDH. This does however not influence any other aspect of the analysis and we may view the FBA reaction in the model as the lumped enzyme reaction series of the FBA and GPDH reactions.

Figure 3 (a) shows the solution of the ODE system (1) at sufficiently high mass influx (i.e. glucose concentration). These oscillations exhibit the essential qualitative features experimentally seen in oscillatory glycolytic systems, i.e. sawtooth-shaped oscillations in the substrate F6P and pulses of the product FBP.

The main result of the analysis of the model is that the activities of GK *and* FBA determines the interval of glucose concentrations for which the glycolysis is oscillatory. Thus, if glycolytic oscillations underlie the pulsatile insulin secretion, the activity of FBA (and/or GPDH) and its regulation is important to investigate as well as that of GK. This has been overlooked in the biochemical characterization of β-cells. Figure 3 (b) shows the oscillatory interval of the system in a two-parameter bifurcation diagram with V_{GK} and V_{FBA} as bifurcation parameters. The near-linear borders hint that a sound characteristic of the β-cell in terms of glycolytic oscillations may be the ratio V_{GK}/V_{FBA}, a prediction which should be experimentally testable. The activity of PFK, V_{PFK}, was found to have only a limited influence on the occurrence of oscillations, but rather to modulate the oscillation period, as shown in figure 3 (c). This influence became weaker when taking into account the equilibrium between different polymeric forms of PFK, which may hint at a mechanism resulting in greater robustness.

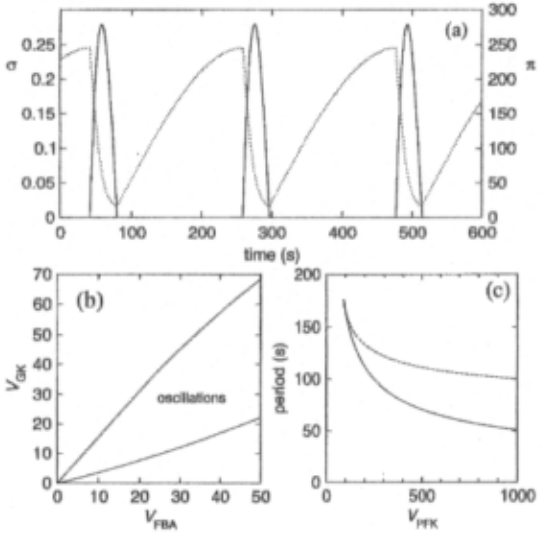

Figure 3. The glycolysis model represented by the ODE system (1) was solved using the following parameters: [glucose]= 10 mM; V_{GK} = 10 U/min, V_{PFK} = 100 U/min, V_{FBA} = 25 U/min; $S_{0.5}^{GK}$ = 8 mM, $S_{0.5}^{PEK}$ = 4 mM, $S_{0.5}^{FBA}$ = 5μM; K_{eq}^{GPI} = 0.3, h_{GK} =1.7, h_{PFK} = 2.5 – $1.5q\pi/(1+q\pi)$, $\alpha_{PFK} = 5^{hPFK}$, q = 0.5. Tissue concentrations (U) were converted to mM by dividing with a factor of 180. (a). The time course of the solution of system (1). The solid and dotted lines represent σ and π, respectively. (b). Two-dimensional bifurcation diagram with V_{GK} and V_{FBA} as bifurcation parameters. (c). Dependence of the oscillation period on V_{PFK}. The dashed line was obtained when taking into account the equilibrium between different polymeric forms of PFK.

With the oscillatory glycolysis hypothesis, the question arises how these oscillations may be propagated to $[Ca^{2+}]_i$ and insulin secretion. An obvious link is the ATP produced and ADP consumed in the glycolysis itself. However, the glycolytic ATP production is vastly below that of the mitochondria [65]. A recent model by Wierschem and Bertram [66] combines the Goldbeter and Lefever [58] model of ATP/ADP-mediated glycolytic oscillations with a phantom burster model of β-cell electrophysiology (see below). However, it is disputable whether a model of yeast PFK is appropriate for the β-cell. Moreover, this model assumes a linear dependence of ATP production on the glycolytic rate. This is a simplifying assumption which may deviate substantially from reality. Therefore, we will investigate in greater detail how the glycolysis is coupled to ATP synthesis and other signals.

The propagation of the glycolytic signal: NADH shuttles and mitochondrial metabolism

The question how the glucose signal is propagated from the glycolysis to downstream events is truly hard and multifaceted and here the mitochondria seem to play a prominent role, emphasized by the fact that clinically, 1–2 % of all cases of diabetes is attributable to mutations in mitochondrial DNA [67]. Basically, the signal consists of the transfer of the mass (mainly pyruvate) and charge (NADH) produced in the glycolysis into the mitochondria. As mentioned, it is fairly well-established that LDH has a low activity in the β-cell [28, 29] and that the diversion of glycolytic flux to the pentose phosphate pathway is limited [30]. This minimizes the mass and charge leak with respect to the mitochondria. Hence, we may speak about two input signals (mass and charge from the glycolysis) that, via the mitochondria, propagate to perhaps as many as four output signals: long-chain acyl-CoA (LC-CoA), NADPH, glutamate and ATP/ADP – see figure 4.

Let us turn our attention to one of the input signals: charge, as manifested by NADH. As in several other cell types, charge is shuttled from the β-cell cytosol to the mitochondria by two shuttles: the glycerol-3-phosphate dehydrogenase shuttle [68] and the malate-aspartate shuttle [69]. The former reoxidizes NADH and transfers the electron directly to complex II in the respiratory chain. The latter involves malate dehydrogenase (MDH) and amino aspartate transaminase (AAT) in both the cytosolic and mitochondrial compartments. It was early realized that the shuttles play an important role in GSIS [69, 70, 28]. However, the study by Eto et al. [71] must be considered as a breakthrough in this context. These investigators were the first to simultaneously inhibit both shuttles, and by doing this they discovered a great impairment of GSIS, manifested by parallel loss of glucose-induced changes in mitochondrial membrane potential, mitochondrial Ca^{2+} concentration, mitochondrial NAD(P)H

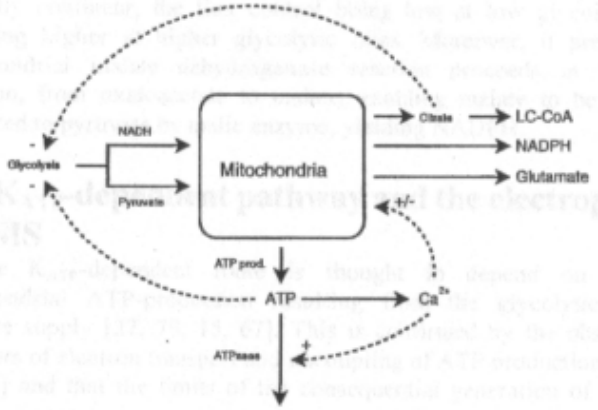

Figure 4. The mitochondria relay the glycolytic signals (pyruvate and NADH) to ATP (the K_{ATP}-dependent pathway) as well as to the K_{ATP}-independent pathway. The latter pathway consists of a yet unresolved signal, perhaps LC-CoA, NADPH, glutamate, or a combination of these compounds. Several feedback loops exist in this system. ATP and citrate inhibit glycolysis. Ca^{2+} stimulates ATPases and has a disputed effect on mitochondrial metabolism and oxidative phosphorylation.

concentration, tricarboxylic acid (TCA) cycle flux, cytosolic ATP/ADP ratio, and insulin secretion. These effects were much less marked if either one of the shuttles was inhibited separately. The conclusion drawn was that the NADH shuttles are crucial for the glucose signal propagation from the glycolysis, and that the two shuttles comprise a redundant system, at least partially. Interestingly, glucose consumption was not affected by the abolishment of the shuttles which would be the case if NADH was not reoxidized by shuttles or LDH, an enzyme which, as mentioned, has a low activity in the β-cell. This means that the NADH produced by the glycolysis is reoxidized by some unknown process when the shuttles are inactive. The authors of the study attributed the importance of the shuttles to the importance of Ca^{2+} activation of mitochondrial dehydrogenases, and argued that the ATP/ADP signal is generated as a consequence of glycolytically generated NADH hyperpolarizing the mitochondrial membrane allowing Ca^{2+} to enter the mitochondria, which via the dehydrogenases would accelerate ATP production. This is questionable, however, since the ATP/ADP signal precedes Ca^{2+} entry into the cytosol [12, 13, 14, 15].

The aforementioned possible output signals have lately received much attention, extensively reviewed elsewhere [27, 72, 73, 67, 74, 23]. We will here outline the main results and hypotheses.

The K_{ATP}-independent pathway: Different candidate mechanisms

Pyruvate enters the mitochondrial metabolism via two routes: the pyruvate dehydrogenase (PDH) or the pyruvate carboxylase (PC) reaction. Since the latter is a carboxylation reaction, its presence represents anaplerosis, a net addition of carbon to the TCA cycle, which has to be balanced by cataplerosis, the removal of carbon. It has been estimated that in the β-cell about half of the pyruvate takes the PC route [75, 29], yet both lipogenesis and gluconeogenesis, the classical fates of the anaplerotic carbons [76], exhibit low activity in the β-cell [77, 78]. Still, it has been demonstrated that the influx of Ca^{2+} to the mitochondria *together* with anaplerosis is necessary for insulin secretion [79]. Therefore the possible cataplerotic fates of the carbons have been examined as candidates for coupling factors responsible for the K_{ATP}-independent pathway. Three main hypotheses exist. The first, proposed in 1989 [80], concerns the cataplerotic export of citrate from the mitochondria. The exported citrate may be cleaved to produce oxaloacetate and acetyl coenzyme A (Ac-CoA), the latter further metabolized to produce malonyl-CoA, which inhibits the transporter CPT-1 which transports fatty acids to the mitochondria for further oxidation. Hence, cytosolic synthesis of LC-CoA levels will increase [80]. LC-CoA have been proposed to stimulate insulin exocytosis via protein acylation [81, 23] as well as via DAG formation (together with glycolytically derived glycerol-3-phosphate) [80] which via PKC activation promotes insulin secretion [82]. Recent findings shed both doubts upon [83] and gave support to [84] the hypothesis. The second hypothesis concerns the presence of malic enzyme in the β-cell [85]. This cytosolic enzyme converts malate to pyruvate while reducing NADP to NADPH. Thus, together with the TCA cycle a new cycle is formed, called the malate-pyruvate shuttle. The NADPH formed is proposed to act as a coupling factor, via direct uptake by secretory granules [86] or indirectly via further metabolism [85, 87]. The third hypothesis concerns the high glutamate dehydrogenase (GDH) activity found in the β-cell. The mitochondrial enzyme GDH catalyzes the cataplerotic conversion of 2-oxoglutarate to glutamate, which may be exported to the cytosol where it might act as a coupling factor [88, 74]. However, these findings have since their announcement in 1999 been intensely questioned [89, 90, 91]. To this day, the exact nature of the K_{ATP}-independent coupling factors remains an open question.

No modelling effort of the NADH shuttles and the propagation of the glycolytic signal to the putative coupling factors has to this date been published, although a preliminary model from our group currently is being analyzed. This model reproduces the results of Eto et al. [71] and predicts that the signals to LC-CoA, NADPH and glutamate are roughly linearly dependent on glucose with a high degree of flux control, while the signal to the ATP production is

markedly nonlinear, the flux control being low at low glycolytic rates and becoming higher at higher glycolytic rates. Moreover, it predicts that the mitochondrial malate dehydrogenase reaction proceeds in the backward direction, from oxaloacetate to malate, enabling malate to be exported and converted to pyruvate by malic enzyme, yielding NADPH.

The K_{ATP}-dependent pathway and the electrophysiology of GSIS

The K_{ATP}-dependent route is thought to depend on a stimulated mitochondrial ATP-production resulting from the glycolytic NADH and pyruvate supply [27, 79, 15, 67]. This is confirmed by the observations that inhibitors of electron transport and uncoupling of ATP production abolish GSIS [92, 93] and that the limits of the consequential generation of mitochondrial membrane potential, Ψ_m, which is negative on the inside, determines the upper limit of GSIS [94]. A consequence of an hyperpolarized Ψ_m is the influx of Ca^{2+} to the mitochondria from the cytosol [67]. As mentioned, this has been thought to modulate the K_{ATP}-dependent route in a feed-forward manner, through an increased ATP production resulting from the activation of mitochondrial dehydrogenases [95, 96, 97, 98, 71, 67]. However, much empirical data suggest that Ca^{2+} acts inhibitory, through lowering of the ATP/ADP ratio, in two different ways. One way is through the depolarization of the mitochondrial membrane which may be the consequence of Ca^{2+} influx to the mitochondria [99, 100]. This idea has been questioned [73] but has received substantial experimental support [101, 102]. The other way is by Ca^{2+} stimulating ATPases involved in ion pumping and secretion [103]. In all, Ca^{2+} may exert a biphasic effect on ATP production; first increasing it through activation of dehydrogenases and then inhibiting it by depolarization of the mitochondrial membrane and stimulating ATPases [102]. These different effects of Ca^{2+} are summarized in figure 4.

The K_{ATP}-dependent pathway acts via ATP/ADP-dependent closure of the K_{ATP} channels of the plasma membrane causing the β-cell to enter a bursting mode during which Ca^{2+} enters the cell, which in turn leads to exocytosis of insulin vesicles. But how do the dynamics of this mechanism work and what may generate the bursting behaviour of the β-cell?

Many attempts to theoretically explain the complex electrical activity of the β-cell have been made. The first mathematical formulation to appear was the model of Chay and Keizer [104]. This model featured a general K^+ leak channel, a delayed-rectifier (DR) type potassium current, a voltage-activated Ca^{2+}-current and a slow repolarizing Ca^{2+}-activated K^+ current (KCa current). The inward

Ca^{2+} current is responsible for the depolarization phase of an action potential [16], while the DR K^+-current has experimentally been shown to be responsible for the repolarization of the action potential [16, 105, 106, 107]. Together these currents are thought to be the basis of the spiking [16]. The K_{Ca} current, responsible for the negative feedback setting the slower rhythm of alternating hyperpolarized and spiking phases, produces medium bursting, as hypothesized by Atwater et al. [108]. This hypothetical model was subsequently ruled out by experiments; the large-conductance K_{Ca} channels then known to be present in the β-cell seemed to be unimportant for bursting [16]. The underlying structure of the model has however survived to this day as a working hypothesis of burst generation in the β-cell.

Reflecting this state of affairs, we here present a minimal core model in principle due to Sherman and Rinzel [109], which is essentially equivalent to the Chay-Keizer model with the general leak current substituted for the ATP-dependent K^+-current, and with the K_{Ca} current substituted for a generic slow current whose identity is discussed below:

$$\begin{aligned} \dot{V} &= -(I_{Ca} + I_{K(DR)} + I_{slow} + I_{K(ATP)})/C_m \\ \dot{n} &= \frac{n_\infty(V) - n}{\tau_n} \\ \dot{s} &= \frac{s_\infty(V) - s}{\tau_s} \end{aligned} \qquad (2)$$

This is a classical equivalent electric circuit model, where V represents the cell membrane electrical potential, C_m represents the membrane capacitance, n is a gating variable for the DR potassium current $I_{K(DR)} = g_K n(V - V_K)$ with the steady state function $n_\infty = 1/(1 + e^{(\upsilon_n - V)/s_n})$. Further, $I_{Ca} = g_{Ca} m_\infty(V)(V - V_{Ca})$ is the current of the L-type voltage-dependent Ca^{2+} channels, with the steady state activation function $m_\infty = 1/(1 + e^{(\upsilon_m - V)/s_m})$. The slow current $I_{slow} = g_{slow} s(V - V_K)$ is governed by the steady state activation function $s_\infty = 1/(1 + e^{(\upsilon_s - V)/s_s})$ and is here taken to be a K^+ channel. Finally, $I_{K(ATP)} = g_{K(ATP)}(V - V_K)$ is the ATP-sensitive K^+-current. The different parameters are conductances (g), reversal potentials (e.g. V_K), time constants (τ) and finally midpoint voltages (υ) and slope factors (s) for the steady state activation functions of the DR and slow currents.

This model captures the essential features formulated in words above. More importantly, its solution captures qualitatively the phenomenology of the electrical activity of the β-cell described earlier, i.e. bursts of spiking

superimposed on plateaus, which in turn are interspersed with hyperpolarized phases. The solutions of the ODE system (2) for two different values of τ_s are shown in figure 5 (b) and (c). The bursting is an example of what Rinzel [110] in his attempt at a systematic classification of bursters calls *type I bursting*. It can be intuitively understood if one employs *fast-slow analysis* [110, 111]. Here, we examine the limit where $\tau_s \to \infty$ and perform a bifurcation analysis of the two-variable V -n system using s as a bifurcation parameter. The resulting bifurcation diagram is shown in figure 5 (a). Starting from the left, we have a single steady state. As s increases, the system undergoes a Hopf bifurcation, resulting in a stable limit cycle representing the fast spiking during the depolarized plateau. As s increases further, the stable limit cycle intersects with the unstable steady state in a homoclinic orbit. The unstable steady state deviates down to the left and turns into a stable steady state at a saddle-node bifurcation. Now we revert back to the full three-variable system, manifested by the superimposed trajectory which is the same as in figure 5 (b). We consider the part of the trajectory under the s nullcline. Here, we find the system in its hyperpolarized phase. There, s is decreasing. As the saddle-node is passed, the trajectory flips up passing the s nullcline, and ends up at the fast limit cycle. A burst has commenced. Here, s increases, and as the stable limit cycle disappears, the trajectory is attracted back to the lower steady state representing the hyperpolarized phase. The process then repeats itself.

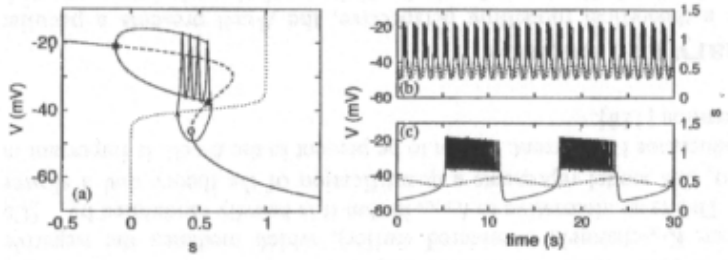

Figure 5. The core model of β-cell electrophysiology (2) was here realized with the following parameters: $g_{Ca} = 280$ pS, $g_{K(DR)} = 1300$ pS, $g_{K(ATP)} = 35$ pS, $g_s = 50$ pS; $V_{Ca} = 100$ mV, $V_K = -80$ mV; $v_m' = -22$ mV, $s_m = 7{:}5$ mV, $v_n' = -9$ mV, $s_n = 10$ mV, $v_s' = -40$ mV, $s_s = 1$ mV; $\tau_n = 7$ ms, $\tau_s = 1$ s (a, b) or 20 s (c). The bifurcation diagram, panel (a), includes stable branch (solid line) which turns into an unstable branch (dashed line) at a Hopf bifurcation point (■), and regains stability at a saddle-node bifurcation point (o). The periodic branch intersects the unstable branch at the point marked with a triangle (▲). The dotted line is the s nullcline. The solution with $\tau_s = 1$ s. is superimposed which demonstrates the validity of the fast-slow analysis.

We may think of the $I_{K(ATP)}$ conductance as determining the setpoint of the system – geometrically a decreased $g_{K(ATP)}$ (corresponding to an increased ATP/ADP ratio) translates the z-shaped steady-state curve to the right. Thus, at the resting state, when $g_{K(ATP)}$ is high, the s nullcline will intersect the z-shaped curve at the lower stable steady-state region. At higher glucose levels, the ATP/ADP levels are elevated, the z-shaped curve translates rightwards and the system enters the bursting regime depicted in figure 5 (a). This is essentially the electrophysiological action of the K_{ATP}-dependent pathway, and is also valid for the models discussed below. During the depolarized phases, Ca^{2+} enters the cell via the L-type channels and stimulates exocytosis.

The model is quite sensitive to many of the parameters. The reversal potentials may be considered as being under relatively strict homeostasis. The bursting period is effectively controlled by the time constant τ_s for the slow variable, as seen in figure 5b ($\tau_s = 1$ s) and c ($\tau_s = 20$ s). This parameter does essentially not change the bifurcation diagram, it only slows down the traversion of the trajectory in the s direction, which results in longer bursts and hyperpolarized phases. The system is very sensitive to τ_n as analyzed earlier [111]. In a model of several β-cells coupled via gap-junctions [112], it was shown how the coupling may reduce this sensitivity.

The search for the slow variable(s)

It is of course of greatest importance for our understanding of β-cell bursting to identify the nature of the slow variable s. The first candidate due to Chay and Keizer [104], which was $[Ca^{2+}]_i$ modulating K_{Ca}-channels, was ruled out since this model predicted saw-tooth shaped oscillations in $[Ca^{2+}]_i$. This prediction was experimentally falsified a few years later when the $[Ca^{2+}]_i$ oscillations were shown to more resemble square waves [113]. Another candidate for the slow variable was a slow voltage-dependent inactivation of the plasma membrane L-type Ca^{2+} channels (VDIC), which was suggested by experimental demonstration of the phenomenon [114], and an accompanying mathematical model by Chay and Cook [115]. This model predicts medium bursting from this mechanism, and on a side note, the Chay-Cook model is, with some parameter changes, able to reproduce the qualitatively different parabolic bursting applicable to *Aplysia* R_{15} neurons. The concept was elaborated by Keizer and Smolen [116] among others. Still, the application to β-cells was unfortunately found to be hampered by incompatibility with more complete experimental data on the inactivation of the Ca^{2+} channels [117], making this mechanism improbable to underlie medium bursting.

Hence, again other candidates had to be suggested. One such candidate for the slow negative feedback variable would be a slowly variable endoplasmatic

reticulum (ER) Ca^{2+} concentration $[Ca^{2+}]_{ER}$, as first proposed by Chay in two theoretical models [118, 119]. It is a well-established experimental fact that the ER acts as a Ca^{2+} store in the β-cell, the Ca^{2+} being sequestered through the action of ATP-driven Ca^{2+} pumps (SERCA), and released at least through an IP3-sensitive channel. Thus, Chay proposed in her models that a slowly variable $[Ca^{2+}]_{ER}$ may act as a negative feedback in several complementary manners, e.g. indirectly via cytosolic Ca^{2+} inactivation of the L-type channels or directly via a Ca^{2+} release activated non-selective cationic current (I_{CRAN}), a current which is activated by release of Ca^{2+} from the ER and whose presence in the β-cell has been experimentally demonstrated [120]. Via these mechanisms, the Chay models were able to reproduce a wide range of time scales of the bursting (fast, medium and slow) and thus were complete models in that respect.

An alternative road of thought pursues the idea that the negative feedback may be mediated by a Ca^{2+}-induced lowering of the ATP/ADP ratio, reopening the K_{ATP}-channels. The idea was originally formulated by Keizer and Magnus [121] and refined later [117] in models which combined this mechanism with the aforementioned VDIC mechanism. The hypothesis was further elaborated in a model where the lowering of the ATP/ADP ratio is caused by Ca^{2+} depolarizing the mitochondrial membrane, as mentioned earlier [99, 122, 100]. In some aspects, this is a very detailed model that involves a quantitative description of the mitochondrial Ca^{2+} handling, and it is able to reproduce medium bursting. This model is interesting, and might benefit from some simplifications in order to make central assumptions clear and to facilitate mathematical analysis of sensitivity of the model to different parameters. However, the time scale of metabolic processes which modulate the ATP/ADP ratio are probably too slow to mediate medium bursting [13]; rather they may underlie the slow bursting mode. Further, it is noteworthy that this model does not consider the possibility of glycolytic oscillations. Instead, it describes the glycolytic rate as solely dependent on GK, using the model of Garfinkel et al. [35].

Yet an alternative candidate mechanism for the generation of slow bursting was recently put forward by Fridlyand et al. [123]. In this model, Na^+ accumulates during the depolarized phase, which activates the repolarizing Na^+/K^+ pump. This model may also benefit from some further simplifications and elucidation of the underlying topological structure of the bursting mechanism.

A recent family of models implement the "phantom bursting" concept of Bertram et al. [124]. The authors show that by introducing two slow negative feedback variables, one which alone would generate fast bursting and one which would generate slow, all three bursting modes may be obtained. This concept provides an excellent framework for the analysis of β-cell bursting, particularly because of its power to explain medium bursting without the need for a single

process with a time constant of the medium time scale, for which there is little experimental evidence. A concrete realization of the phantom bursting concept is the "Ca^{2+} subspace" model [125, 126], in which $[Ca^{2+}]_i$ is the faster of the two slower variables, while $[Ca^{2+}]_{ER}$ is the slower one. Crucial in this model is the assumption of a compartment in the vicinity of the plasma membrane and the ER, denoted the Ca^{2+} subspace, whose Ca^{2+} concentration is passively related to that of the cytosol. This idea of separate cytosolic Ca^{2+} compartments is a variant of an earlier hypothesis [127]. The subspace Ca^{2+} concentration modulates a slow small-conductance K_{Ca}-channel (distinct from the large-conductance K_{Ca}-channels considered earlier), which mediates the negative feedback. This is an alternative to I_{CRAN} in that it is heavily modulated by $[Ca^{2+}]_{ER}$. Also, this model represents a quantification of the theory that a slower small-conductance K_{Ca} current, shown to be present in the β-cell, is important in burst generation [128].

Summary and outlook

From a theoretical modelling perspective, the β-cell presents a peculiar challenge; the metabolism and electrical activity are intimately interconnected. However, modelling of cellular electrophysiology has largely been developing among computational neuroscientists, while metabolic modelling largely has been the business of biochemists. These different schools have to be united in the theoretical and computational β-cell research. The causal connection between metabolic events and electrophysiological events is mutual. Metabolism affects the electrophysiology at least through the K_{ATP} channel, and the electrophysiology affects metabolism via Ca^{2+}. It is still unclear in what way Ca^{2+} modulate metabolism and the ATP/ADP ratio, the relationship most likely being complex and under the influence of a multitude of factors. Theoretical models have as of yet only explored the scenario where Ca^{2+} lowers the ATP/ADP ratio, and then only via decreased oxidative phosphorylation [99, 122]. A more recent model omits Ca^{2+} feedback [66]. The opposite scenario, as well as a combination of the two, ought to be investigated; which effects would this have on the dynamic behaviour of the metabolism and electrophysiology, and can their validity be experimentally tested? Also, the glycolysis is at the level of PFK subject to several feedback interactions from downstream metabolism; it is inhibited by ATP and citrate, and activated by AMP. All of these effects have yet to be incorporated into theoretical models and examined accordingly.

A fruitful strategy will be to formulate both simplified, phenomenological models amenable to mathematical analysis, as well as more detailed models with perhaps more limited scopes, and search to relate these different kinds of

models to each other in meaningful ways – in other words to work in parallel on different levels of description.

No metabolic modelling effort of the K_{ATP}-independent pathway has yet been published. This will require at least a consideration of the NADH shuttles and TCA cycle dynamics, to assess the export of citrate (for the LC-CoA pathway), malate (for the NADPH pathway), and glutamate from the mitochondria. We are currently developing a model addressing this question. Further, an interesting possibility, worthy of theoretical investigation, is the recent finding of an oscillatory mitochondrial metabolism [129]. These oscillations were demonstrated both in intact cells and in suspended mitochondria. The mechanism behind these oscillations is to this day completely unknown.

Theoretical biologists have for a long time been nourishing a hope that biological systems have a modular architecture and that they are amenable for analysis in terms of these modules [130]. Recent developments in network analysis do indeed hint in this direction [131]. Is this likely to apply also for GSIS in the β-cell? A thorough and unbiassed analysis of the structure of the extensive biochemical interaction network of GSIS might be desirable in order to gain some insights into the validity of theoretical predictions based on the modelling efforts of different subsystems of the β-cell. The slow, minute-scale oscillations in membrane potential and insulin secretion, for instance, have been hypothesized to be generated by the autocatalysis of PFK or by Ca^{2+} decreasing the ATP/ADP ratio. Is it possible to determine a single generator of the oscillations, or is the β-cell only understandable as a whole? The research and debates will continue.

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