Time-dependent Mechanisms in Beta-cell Glucose Sensing

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Abstract The relation between plasma glucose and insulin release from pancreatic beta-cells is not stationary in the sense that a given glucose concentration leads to a specific rate of insulin secretion. A number of time-dependent mechanisms appear to exist that modify insulin release both on a short and a longer time scale. Typically, two phases are described. The first phase, lasting up to 10 min, is a pulse of insulin release in response to fast changes in glucose concentration. The second phase is a more steady increase of insulin release over minutes to hours, if the elevated glucose concentration is sustained. The paper describes the glucose sensing mechanism via the complex dynamics of the key enzyme glucokinase, which controls the first step in glucose metabolism: phosphorylation of glucose to glucose-6-phosphate. Three time-dependent phenomena (mechanisms) are described. The fastest, corresponding to the first phase, is a delayed negative feedback regulating the glucokinase activity. Due to the delay, a rapid glucose increase will cause a burst of activity in the glucose sensing system, before the glucokinase is down-regulated. The second mechanism corresponds to the translocation of glucokinase from an inactive to an active form. As the translocation is controlled by the product(s) of the glucokinase reaction rather than by the substrate glucose, this mechanism gives a positive, but saturable, feedback. Finally, the release of the insulin granules is assumed to be enhanced by previous glucose exposure, giving a so-called glucose memory to the beta-cells. The effect depends on the insulin release of the cells, and this mechanism constitutes a second positive, saturable feedback system. Taken together, the three phenomena describe most of the glucose sensing behaviour of the beta-cells. The results indicate that the insulin release is not a precise function of the plasma glucose concentration. It rather looks as if the beta-cells just increase the insulin production, until the plasma glucose has returned to normal. This type of integral control has the advantage that the precise glucose sensitivity of the beta-cells is not important for normal glucose homeostasis.

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Introduction

There is an overwhelming literature, both experimental and modelling-oriented, that describes the electrophysiological events in the beta-cell and the following insulin release [1–4]. By contrast, descriptions of the mechanisms underlying glucose sensing and propagation of the sensor signal to the transportation and release of the insulin granules are sparse [5–7].

There are many indications of time-dependent mechanisms [5, 8, 9]. All of these indications point to the fact that the glucose sensitivity of the beta-cell is not a constant, but varies over time scales of minutes, hours and days in healthy persons as well as in diabetic patients. One mechanism is the translocation of glucokinase—the key enzyme in glucose sensing. Glucose-dependent translocation is well described in the liver [10–13], but recent research shows that a similar translocation takes place in the beta-cell [14, 15]. Another effect is the enhancement of insulin release after previous exposure to high blood glucose concentrations [5, 8, 9, 16]. This is supported by the observation that beta-cells exposed to high glucose concentrations over several days are observed to release more insulin in response to a given change in glucose concentration than cells exposed to low glucose [17]. Together these mechanisms lead to what can be called a dynamic glucose memory in the beta-cells [8, 9, 18]. It is obviously necessary to take this memory into account in the planning and interpretation of experimental and clinical investigations of beta-cell function.

The paper presents an analysis of the different mechanisms and provides a simplified model of the mechanisms and their consequences for insulin release. One of the most important details of the model is the condition that the net glucose flowing into the cell must match the glucose oxidation in the cell. This calls for feedback systems that can control the glucose metabolism.

Most of the considerations in this paper are new and the model is, therefore, presented in several steps, so the arguments and consequences can be elucidated in detail.

Model and Results

The model structure is illustrated in Figure 1. Glucose is transported across the cell membrane mainly by the GLUT2-transporter. Thereafter it is phosphorylated to glucose-6-phosphate (G6P) by the enzyme glucokinase (*GK*). G6P is fed into the glycolytic pathway and eventually oxidized to contribute to the cell's energy expenditure, *EE*. The metabolite *S* is regarded as a controlling factor for both a feedback inhibition of the *GK*-activity, and the translocation of glucokinase from an inactive form GK_B to an active form GK_A . The ATP/ADP ratio controls the insulin release, which is assumed also to be enhanced by the metabolite *S*.

The glucose sensor concept

The enzyme glucokinase is central in all glucose-sensing cells. Its half saturation value $K_{0.5}$ for glucose is near 8 mM, which is 50–100 times higher than most other hexokinases and in

Figure 1 The net flow of glucose into the beta cell must match its energy expenditure (EE). The most plausible way of achieving this is by an inhibition of the active glucokinase (GK_A) by a metabolite S downstream from the glycolysis. Translocation of GK is stimulated by S and insulin release is augmented by S . See text for details.

the range of normal plasma glucose concentrations [19, 20]. Glucose is phosphorylated to G6P by glucokinase. In the liver and kidney, G6P can be de-phosphorylated to glucose by G6P-phosphatase, but this enzyme is sparse or absent in beta-cells [20–22].

The transport of glucose is essentially unidirectional. The inflowing glucose must, therefore, be oxidized at a rate that corresponds to the cellular energy expenditure (EE) and, as there normally are no glycogen stores in the beta-cell [23, 24], the net transport of glucose into the cell must match the glucose oxidation.

This condition can be satisfied in essentially two different ways. The first possibility is that surplus glucose can leave the cell as one of the metabolites downstream of glycolysis. In most cells this would be lactate, but the beta-cell has a low content of lactate dehydrogenase [25]. Another candidate could be pyruvate, and other metabolites are also possible. Denoting the unknown metabolite S , and the influx of glucose J_{in} , and assuming a first order elimination of S we obtain:

$$J_{in} = EE + Loss = EE + bS,$$

where b is a constant. This immediately gives the following expression for the steady-state metabolite concentration:

$$S = \frac{J_{in} - EE}{b}. \quad (1)$$

However, there seems to be no evidence for a substantial metabolite loss from the beta-cell, so this option appears to be unlikely.

The second possibility is based on an inhibition of glucokinase by one or more metabolites, so that the concentration of metabolites regulates the glucose influx. Normally the amount of GLUT2 in the membrane is not rate limiting [19, 20, 24, 26], so the inhibition of GK becomes the main controller of glucose influx. Assuming a first order inhibition of GK by the metabolite S , this gives at steady state with similar nomenclature:

$$J_{in} \frac{K_I}{K_I + S} - EE = 0, \quad (2)$$

or

$$S = K_I \frac{J_{in} - EE}{EE}, \quad (3)$$

where K_I is a constant, and J_{in} is regarded as the uninhibited flux. With constant EE , the two expressions for S , Eqs. 1 and 2 are, in principle, equivalent, apart from some scaling factor. However, we consider the last mechanism to be more likely, so it will be adopted in the following. With S in units of K_I , the time variation of S then becomes:

$$K_I \frac{dS}{dt} = \frac{J_{in}}{1+S} - EE. \quad (4)$$

Here, J_{in} must be larger than EE to ensure a positive value of S . In principle, this means that if the glucose influx is too small, EE will go down. However, the beta-cell also has a small amount of hexokinase I [19, 20, 27], so if the concentration of G6P goes down, the hexokinase—that is normally inhibited by G6P—is activated and can participate in the phosphorylation of glucose. The cell can also get energy from other nutrients like fatty acids. The effect of this is introduced through a modification of Eq. 4 such as:

$$K_I \frac{dS}{dt} = \frac{J_{in}}{1+S} - \frac{E_0 S}{a+S}, \quad (5)$$

where E_0 is the resting value of EE and a is a constant. There is evidence to show that energy expenditure is increased during insulin secretion due to the activity of the calcium pumps [28, 29]. However, to simplify our analysis, E_0 is assumed to be constant. From Eq. 5 follows that S at steady state is given by

$$S = \frac{J_{in} - E_0 + \sqrt{(J_{in} - E_0)^2 + 4aJ_{in}E_0}}{2E_0}. \quad (6)$$

Glucose influx, J_{in}

The phosphorylation rate v of the isolated glucokinase enzyme is given by the Michaelis–Menten expression [30]

$$v = \frac{v_{max} G^h}{K_{0.5}^h + G^h} = v_{max} f_{Hill}(G), \quad (7)$$

where v_{max} is the maximal rate, G the glucose concentration, h a Hill coefficient, and $K_{0.5}$ a constant. The uninhibited glucose influx J_{in} is then

$$J_{in} = v \times GK = v_{max} f_{Hill}(G) \times GK, \quad (8)$$

where GK is the actual amount of glucokinase. For high glucose concentrations, $f_{Hill}(G)$ approaches one, and with GK_T as the total amount of glucokinase, the maximum value of J_{in} becomes $v_{max} GK_T$. Against this stands the energy expenditure E_0 . The dimensionless constant

$$k_S = \frac{v_{max}}{E_0} \times GK_T \quad (9)$$

is thus a measure of the maximal influx in terms of the cell's energy needs. If k_S is large, S must be large, in order to inhibit the glucokinase sufficiently. This would, on the other hand, imply that most of the glucokinase is inactive all the time, which appears unrealistic. Consequently, it is assumed that k_S is relatively small, i.e., $k_S = 2$ –10.

From Eqs. 6 and 8 follows that the metabolite concentration S increases with increasing G , so that S can be regarded as the output of the glucose sensor and as a measure of the glucose concentration. Figure 2 shows how the size of k_S influences the glucose sensitivity of S . It is seen that the shape of the curve mimics the activity of the glucokinase enzyme, but that it is shifted a little to the right.

First phase

When the glucose concentration is increased rapidly-*in vitro* by changing the surrounding glucose concentration and *in vivo* by an intravenous glucose infusion-the insulin release becomes biphasic with a short (2–10 min) burst of insulin, the first phase, followed by a slower rise to a steady level, the second phase [9, 31, 32]. The first phase is not well understood, and it is usually regarded as a rapid release of the so-called immediately releasable pool of granules [3, 31, 33, 34].

There are, however, three features that are not easily explained by this theory. Firstly, the magnitude of the first phase depends on how much and how fast the glucose concentration changes. This calls for a signal transduction all the way from glucose sensing to insulin release. Secondly, the magnitude is influenced by the beta-cell memory (see below), so both the first and the second phases increase after glucose exposure. Finally, the first phase is less pronounced or even absent in patients with type II diabetes.

A simple way to describe these features is to regard the inhibition of glucokinase as non-instantaneous. When the glucose concentration increases, there is a sudden increase in G6P-production and therefore also S -production. As intracellular diffusion takes time, the inhibition described in Eq. 5 cannot control the influx fast enough. Hence, there will be an initial burst in S that propagates all the way to the insulin release.

Figure 2 Steady-state relation between S and glucose, for $a = 0.2$ and various values of k_S , which is proportional to GK_T . Increasing GK_T increases the sensitivity of S to glucose. The relation between S and G mimics the activity of the enzyme (f_{Hill}) but shifted to the right.

Assuming a simple first order delay, Eq. 5 thus transforms into two coupled differential equations:

$$K_I \frac{dS}{dt} = \frac{J_{in}}{1 + S^*} - \frac{E_0 S}{a + S} \quad (10)$$

and

$$\frac{dS^*}{dt} = \gamma(S - S^*), \quad (11)$$

where S^* is the delayed signal and γ a rate constant. Eq. 10 can be transformed into

$$\frac{dS}{dt} = \alpha \left(\frac{k_{sfHill}(G)}{1 + S^*} - \frac{S}{a + S} \right), \quad (12)$$

where $\alpha = E_0/K_I$ is a constant, and where it is assumed that the amount of active glucokinase remains constant. The effect of the delay is demonstrated in Figure 3. The duration of the first phase is generally on the order of 5 min. To obtain this, γ must be around 1 min^{-1} and α somewhat larger. In Figure 3a, α is taken to be 1, 2, 5, or 10 min^{-1} . The figure demonstrates how the size of α determines the magnitude of the first phase. This opens the possibility that the lack of a first phase in patients with type II diabetes could arise from a low value of α , probably due to a large K_I . As such patients generally have a lower amount of glucokinase [19], an increase in K_I , giving less inhibition of GK , could be a way to counteract the drop in GK -activity.

The first phase also depends upon how fast the glucose concentration changes. This is illustrated in Figure 3b, where the glucose concentration is changed linearly from 5 to 15 mM within 1, 2, 5, and 10 min. The first phase is apparent in all cases, and the effect appears more or less proportional to the rate of change. The above combination of mechanisms thus provides a background for using the rate of glucose change (dG/dt) as an indicator of the size of the first phase [35–37].

Translocation of glucokinase

There is significant evidence to show that the amount of active glucokinase varies. Some GK may be bound to insulin granules, some to the nucleus, and some may be associated with larger enzymes, etc. [14, 15, 38]. The result is a varying activity of the amount of GK present in the cell. There are many possible explanations for this variation in the literature, but a general finding is a relatively fast variation in the order of 20–60 min [15, 39]. Also, the total amount may vary, but most authors find that the total amount of glucokinase in the beta-cell is relatively constant over the day [17, 40].

Both in liver and in beta-cells the translocation depends on plasma glucose. However, free glucose appears not to play a role inside the cell except for its own phosphorylation, so glucose signalling in the cell must therefore be secondary to glucose phosphorylation. The result is that it is not glucose but again some metabolite downstream of the glycolysis that controls the translocation. To simplify, we will again assume that it is S (or some metabolite proportional to S). This implies that S controls its own formation both through a negative feedback as in Eq. 2 and through a positive feedback, a so-called autocatalytic mechanism, via GK translocation.

Figure 3 The first phase of insulin release mimicked in the time course of S according to Eqs. 11 and 12 after increase of glucose from 5 to 15 mM at 10 min. Parameters are: $a = 0.2$ and $\gamma = 1 \text{ min}^{-1}$. In (a) the glucose is changed abruptly with the rate constant α varying from 1 to 10 min^{-1} . The first phase is absent for small α values. In (b) the glucose concentration is varied linearly over 1, 2, 5 and 10 min and with $\alpha = 10 \text{ min}^{-1}$. The first phase appears more or less proportional to the rate of change of glucose.

The translocation is described as a simple chemical reaction with two rate constants k_{act} and k_2 , as shown in Figure 1. Total GK_T is divided into an active form GK_A and an inactive, bound form, GK_B

$$GK_T = GK_A + GK_B. \quad (13)$$

It is assumed that the active part becomes bound with the rate constant k_2 , and the bound part is activated with a rate constant k_{act} that depends on S according to

$$k_{act} = k_1 + k_3 S. \quad (14)$$

Introducing the active fraction $X_A = GK_A/GK_T$, the time course of the translocation is described by the equation

$$\frac{dX_A}{dt} = k_{act}(1 - X_A) - k_2X_A = k_{act} - (k_{act} + k_2)X_A, \quad (15)$$

where k_{act} depends on GK_A and G via S . At steady state Eq. 15 gives

$$X_A = \frac{k_{act}}{k_{act} + k_2}, \quad (16)$$

but as k_{act} depends on GK_A via S , the relation between G and GK_A is more complex. The relation is shown in Figure 4a for different values of k_S . The other constants k_1 , k_2 , and k_3 are estimated in the following way: at steady state, only the ratios k_2/k_1 and k_3/k_1 matter. For low glucose, corresponding to small values of S , the value of X_A approaches $k_1/(k_1 + k_2)$. In the liver this appears to be a low value [41], but in the beta-cell this is taken to be 15–20%. The corresponding ratio k_2/k_1 is thus 4–6. In Figure 4 we have used a ratio of $k_2/k_1 = 5$. The value of k_3/k_1 is more difficult. It scales the influence of S on the translocation rate and on the steady-state value. It appears that, with the translocation opportunity, the inhibitory effect of S can be smaller. Hence a value of $k_3/k_1 = 5$ is chosen.

The steady-state value of S can be calculated by introducing X_A in Eq. 8 and using Eq. 6. The relation is shown in Figure 4b together with $f_{Hill}(G)$. For comparison, S is normalized by dividing by k_S . For moderate to large values of G , the curve for S approaches the shape of the Hill curve $f_{Hill}(G)$ —but shifted downwards. The shift can also be taken to mimic a right-hand shift along the G -axis, so that the main effect of the combination of a positive and a negative feedback on glucokinase is to introduce a glucose threshold of a size that depends on the different parameters.

Introducing the translocation in Eq. 12 gives

$$\frac{dS}{dt} = \alpha \left(\frac{k_S X_A f_{Hill}(G)}{1 + S^*} - \frac{S}{a + S} \right), \quad (17)$$

where X_A is given by Eq. 15. The resulting time-course of S/k_S is shown in Figure 4c. Now the figure displays both a first phase as before, but also a second phase that is slowly rising. The second phase is modelled by setting $k_1 = 0.006 \text{ min}^{-1}$, so $k_2 = 0.03 \text{ min}^{-1}$, and varying k_3 . Notice that S is small in the beginning, so that the influx of glucose is mainly determined by a low GK_A . With the constant $a = 0.2$ in Eq. 5, and a value of S/k_S from Figure 4c of 0.01 to 0.1, the GK-activity only accounts for 25–75% of the cell's energy expenditure at low glucose. With high glucose, S increases moderately to 1–3 and glucokinase can now provide some 90% of the energy expenditure EE . From this it follows that something else must provide the nutrition for the beta-cell, and that the glucokinase activity might be more related to glucose signalling than to nutrition.

Insulin release

As discussed in the previous section, we assume that the variable S controls the insulin release. The docking and emptying of the insulin granules is a complex process that includes closing of the ATP/ADP dependent K^+ -channel, bursting potential changes, and Ca^{2+} -influx [6, 42]. There is a glucose threshold for sufficient K^+ -channel closure [4, 6, 43], so S has to reach a given threshold, S_0 , to give insulin release. There is, however, a low basal insulin release R_0 independently of the bursting mechanism. R_0 also appears to

Figure 4 (a) Relation between the fraction of active glucokinase $x_A = GK_A/GK_T$ and glucose at steady state translocation. The constant k_S controls the inflection point and maximum. (b) Relation between G and S and the Hill function f_{Hill} . The main effect of the autocatalytic effect of glucokinase is a *right-hand shift*. (c) Glucose concentration is changed fast from 5 to 15 mM at $t = 10$ min. When the time-dependent translocation is introduced, the effect is, besides the first phase, a slowly rising second phase. In (a) and (b) the ratios k_2/k_1 and k_3/k_1 are set equal to 5, $a = 0.2$, $\alpha = 10 \text{ min}^{-1}$ and $\gamma = 1 \text{ min}^{-1}$. In (c) $k_1 = 0.006 \text{ min}^{-1}$, $k_2 = 0.03 \text{ min}^{-1}$, $a = 0.2$, $\alpha = 10 \text{ min}^{-1}$, $\gamma = 1 \text{ min}^{-1}$ and $k_S = 7$.

depend on the glucose concentration, etc. [44], but to simplify we will assume R_0 to be constant.

The actual insulin release R is simply taken to be a linear function of S :

$$\begin{aligned} R &= Q \cdot (S - S_0) + R_0 \text{ for } S \geq S_0 \\ R &= R_0 \text{ for } S < S_0 \end{aligned} \quad (18)$$

where Q is an amplification factor.

The presence of the threshold S_0 results in an offset in the insulin release, so that release at a rate above R_0 first begins at a given glucose concentration G_0 . The offset depends on the different parameters, particularly GK_T as shown in Figure 5 for $R_0 = 0.05$ and $Q = 1$.

Beta-cell memory

When two equal pulses of large glucose concentration are applied one after the other, the second pulse gives a much larger insulin release than the first, while the shapes of the pulses are similar [8, 9]. These findings point to an augmentation of the response to glucose, depending on the previous history of glucose concentration [16, 18]. The mechanisms behind this augmentation are not well described. One explanation could be increased GK_T , but substantial changes in GK appear to take days [17]. Another candidate could be S itself, but many authors point toward mechanisms depending on Ca^{2+} and/or insulin action via the beta-cells' insulin receptors [45–47].

In the latter cases, the augmentation depends on R itself, or rather $R_\Delta = R - R_0$, so that the amplification factor may be taken to vary according to:

$$\frac{dQ}{dt} = \beta(Q_\infty(R_\Delta) - Q), \quad (19)$$

Figure 5 Relation between the insulin release, R , and glucose. The release of insulin is highly dependent on the total amount of GK . Parameters $k_1 = 0.006 \text{ min}^{-1}$, $k_2 = 0.03 \text{ min}^{-1}$, $k_3 = 0.03 \text{ min}^{-1}$, $a = 0.2$, $Q = 1$ and $R_0 = 0.05$.

with

$$Q_{\infty}(R_{\Delta}) = Q_0 \left(1 + \frac{A \cdot R_{\Delta}}{K_R + R_{\Delta}} \right) \quad (20)$$

where β , K_R , and Q_0 are constants. The dimensionless constant A determines the maximal amplification. At low values of R_{Δ} (low glucose), the factor is close to Q_0 . When insulin is released, this can be increased to $Q_0(1 + A)$. Often very large differences are seen [8, 9], so A is here taken to be 10.

The memory effect introduces a second autocatalytic process, where the insulin release amplifies itself, but only when glucose is high enough. This phenomenon may explain the sustained increase in second-phase insulin release.

The steady-state value of R_{Δ} , R_{∞} , is found from Eqs. 18 and 20. With $S_{\Delta} = S - S_0$ and $K_R = 1$, this gives:

$$R_{\infty} = \frac{Q_0 S_{\Delta}(1 + A) - 1 + \sqrt{(Q_0 S_{\Delta}(1 + A) - 1)^2 + 4Q_0 S_{\Delta}}}{2}. \quad (21)$$

This relation is shown in Figure 6 for different values of A , and the resulting relation between glucose and insulin release is shown in Figure 7.

The effect of beta-cell memory is illustrated in Figure 8. The figure shows the insulin release from two 30 minutes pulses of glucose from 5 to 15 mM given 30 minutes apart. It is seen that the second gives a much larger insulin release, and that also the first phase is considerably larger in the second response. This is in accord with most findings in the literature [5, 8, 9].

Figure 6 The autocatalytic effect of insulin release introduces an amplification factor A . The relation between the steady-state value R_{∞} , of $R_{\Delta} = R - R_0$, and $Q_0 S_{\Delta}$ is shown for different values of the amplification factor. $K_R = 1$.

Figure 7 Steady-state relation between insulin release and glucose for different values of k_s , when the autocatalytic effect of insulin release is taken into account. $K_R = 1$, $Q_0 = 0.1$ and $\beta = 0.003 \text{ min}^{-1}$.

Discussion and Conclusion

The above results point to an interesting distinction between beta-cell glucose sensing and beta-cell metabolism. This is supported by the findings that, while glucose stimulates insulin release, pyruvate is metabolised in the beta-cell, but does not induce insulin release [48]. It

Figure 8 Demonstration of glucose memory by two 30 min. glucose pulses of 15 mM. Both the first phase and the second phase of insulin release are increased during the second glucose pulse. Parameters are $k_1 = 0.006 \text{ min}^{-1}$, $k_2 = 0.03 \text{ min}^{-1}$, $k_3 = 0.03 \text{ min}^{-1}$, $k_s = 7$, $a = 0.2$, $Q_0 = 1$, $A = 10$, $K_R = 1$, $R_0 = 0.05$, $\alpha = 10 \text{ min}^{-1}$, $\beta = 0.003 \text{ min}^{-1}$ and $\gamma = 0.7 \text{ min}^{-1}$.

is therefore not glucose as a nutrient that is important, but glucose-or rather some of its metabolites-that acts as a signal molecule and that happens to be a nutrient too. As pyruvate is the normal end point of glycolysis, our metabolite S must be part of glycolysis, or related to glycolysis.

There is no doubt that glucokinase is the key glucose sensor. It appears, however, that it undergoes a subtle regulation that enhances its glucose sensing properties, so instead of just feeding glucose into the glycolysis, the amount of active, uninhibited glucokinase is adjusted to the actual insulin requirements.

A brief pulse in blood glucose concentration is probably taken care of by the first phase. A longer variation requires additional insulin, so the second phase release begins to rise. If the glucose increase is sustained, a further rise in S and the memory effect will keep the insulin concentration high, until all extra glucose is taken up.

This regulation by three steps-and probably many more-gives a simple and reliable control of blood glucose. The different steps need not be very precise. The insulin release just increases until the glucose concentration goes down again, and everything falls back to normal. This mechanism can also explain how it is possible to have a large inter-individual variation in beta-cell function with an almost normal glucose homeostasis.

Model assumptions

The model is based on a number of conservation conditions. Glucose transport across the cell membrane is not rate-limiting, so instead it is the phosphorylation to G6P that must be matched by the oxidation or the release of metabolites to the blood. Some of the G6P or other metabolites could be lost as assumed in Eq. 1, but a much more sophisticated regulation appears with the combined regulation of GK by S , where the regulation acts as a balance between the translocation and the inhibition of GK . It is also possible that the mechanisms in Eqs. 1 and 2 are both active. However, to simplify the discussion, this is not considered here.

The model is built as a combination of saturable Michaelis–Menten relations and autocatalytic amplification processes. The autocatalytic relations are kept in place by the saturability of the processes, and the small gain of the saturable processes is enhanced by the autocatalytic ones. The result is a switching behaviour between the “resting” state with low glucose concentration and an active, insulin-secreting state at high glucose concentration.

The results indicate that there is not a simple, static relation between glucose concentration and insulin release. The glucose-insulin system rather works as a so-called integral control system, where the control signal, insulin, increases, until the controlled variable glucose is normalised. There is, however, a limit. The insulin release is not sustained forever. Some of the long-term decline may be caused by exhaustion of the granule stores and limited transport rates, but some must also be a result of changes in the glucose sensing system. These possibilities have not been considered here.

The existence of the variable S is shown indirectly. Probably several metabolites participate in the regulation, and it is not clear, how they affect the activity and translocation of GK . Future research must clarify this. It seems, however, reasonable that S is a cytoplasmic substance [49], so that the effect of S on the ATP/ADP ratio might be complex and probably involves exchange between cytoplasmic and mitochondrial NAD(P)H [48].

The distinction between glucose sensing and metabolism is interesting. At low glucose concentrations, the beta-cell must be at least partly nourished via other channels than G6P

from glucokinase. As the beta-cell appears to have a moderate amount of hexokinase I [19, 20, 27] this can take over some of the phosphorylation at low glucose concentration. This effect is more or less included in the last part of Eq. 5.

First phase

The presence of a first phase in S can be considered as a whip-lash or strobe for the first phase in insulin release. The model does not include the electrophysiological machinery and the exocytotic event, so R cannot be considered as a precise measure for the insulin release. Particularly, the presence of an immediately releasable pool [34] is likely to enhance the first phase. However, as the size of the first phase can vary considerably, the pool size cannot be considered to be constant and, as some of the variation depends on the rate of change in the glucose concentration, some signal, like S , must be transmitted all the way from glucose phosphorylation to exocytosis.

The first phase is thus the combined result of a glucose-dependent signal and a fast exocytosis of ready releasable or almost ready releasable granules. The memory effect can increase its size. On the other hand it is also possible that frequent, brief glucose pulses can exhaust the pool. The lack of a first phase in type II diabetes can therefore be a result of insufficient signalling, for example by an increased K_1 , or a defect in the exocytotic machinery—or both.

The value of 1 min^{-1} for γ in Eq. 11 accords with the findings that there is a delay between start of glucose increase and start of insulin release on the order of 1 min [34], while insulin release caused by increased K^+ is almost immediate.

Translocation

The translocation between an active and an inactive form of glucokinase appears to be similar to that in the liver, and also in the liver it results in a kind of glucose threshold [10–13]. In the liver, the activity is controlled by a glucokinase regulating protein (GKRP) which appears to be absent in the beta-cell [14, 39]. However, the dynamics is similar to that described for the beta-cell with a positive feedback controlled by a metabolite, say S , downstream of the glycolysis. It is not clear whether S has any signal effect in the liver. A possibility could be to act as a switch that induces lipogenesis, when the glycogen stores are full.

The finding that glucokinase is associated with the insulin granules indicates another possible dynamic effect. When the granules are emptied, the granule membrane is enclosed in the cell membrane [3], but it is not clear what happens with the associated glucokinase. If it is released to the cytoplasm, the effect may give another positive feedback resembling the memory effect.

The total amount of glucokinase appears to change very slowly [17, 39], although signs of transcription appear relatively fast [50]. Beta-cells grown with a high glucose concentration in the medium appear to have more glucokinase than those grown with a low concentration [17]. On the other hand, there are no indications of a fast turnover of glucokinase [51], so the total amount is taken to be constant in the present analysis. Slow variations may be introduced by changing GK_T .

The memory effect

The memory effect is generally not very well explained. Insulin is reported both to enhance [45–47] and to inhibit [52] insulin release. Another candidate is intracellular Ca^{2+} [46].

There appears to be insulin release that is not dependent on ATP/ADP-sensitive K^+ -channels. This can account for the basal insulin release, but it is not clear whether there is also a memory effect for basal release. To simplify the model, the memory effect is described as a slow increase in insulin release that depends on the release itself. This second positive feedback differs from the first by having a definite threshold corresponding to the ATP/ADP-ratio that can elicit the bursting behaviour.

The combination of translocation and the memory effect gives a nearly linear increase in insulin release after the first phase, when the glucose concentration is held constant [8, 9, 20, 53]. This phenomenon is reproduced by our model (Figure 8). The increase can continue for 1–2 h and the steady-state level is very variable [53], so also this finding indicates that a steady-state relation between plasma glucose and insulin release is irrelevant.

Incretin etc.

Both the translocation and the memory effect introduce considerable delays between glucose increase and insulin release. There are, however, several mechanisms that can overcome these delays, so the increase in blood glucose after a glucose-rich meal is moderate. Most important is probably the incretin effect. When the food reaches the duodenum, several hormones are released, among others GLP-1, that stimulate the beta-cell and enhances its response to glucose [54].

The precise mechanism is not known. Most probably it increases the effect of glucokinase either by increasing its sensitivity to glucose (smaller $K_{0.5}$) or it increases the translocation of GK. In both cases the glucose sensitivity is increased. As the release of GLP-1 is fast, it increases both the first and the second phase [54].

Also, neural mechanisms play a role [55]. Conditioned and unconditioned reflexes orchestrate the gastro-intestinal secretion and movements. This is probably the reason why insulin concentration often rises after a meal before there has been any increase in blood glucose.

In this paper we have presented a novel approach to beta-cells' glucose sensitivity and insulin secretion. The analysis was based on the regulation of the key enzyme glucokinase and its effect on insulin release. Three time-dependent mechanisms were taken into account: the first phase, the glucokinase translocation, and the memory effect. The three mechanisms all contribute to the effect that the beta-cell increases its insulin output in a controlled manner, until the plasma glucose comes down to normal. The way this is done can vary from person to person, but as long as the plasma glucose is normalized within a reasonable time, the glucose control is characterized as normal.

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