Pulsatile Basal Insulin Secretion is Driven by Glycolytic Oscillations

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Summary

Mathematical modeling suggests that glycolytic oscillations coupled to insulin secretion via metabolic factors mediate oscillations in basal insulin levels.

Abstract

In fasted and fed states, blood insulin levels are oscillatory. While this phenomenon is well studied at high glucose levels, comparatively little is known about its origin under basal conditions. We propose a possible mechanism for basal insulin oscillations based on oscillations in glycolysis, demonstrated using an established mathematical model. At high glucose, this is superseded by a calcium-dependent mechanism.

Introduction

In response to a meal, blood glucose levels rise, and this is sensed by the β cells of the islets of Langerhans distributed sparsely throughout the pancreas, which then secrete the hormone insulin (1). Once entering the circulation, insulin acts to increase glucose uptake into muscle and adipose tissues while it inhibits hepatic glucose production (2). Glucose-induced insulin secretion is thus a key player in whole body glucose homeostasis, and its dysregulation is a major contributor to type 2 diabetes (3).

The insulin secretory component of this homeostatic control system is regulated by an exquisite set of mechanisms in β cells (1) (Fig. 1). In contrast to many other hormones, insulin secretion is regulated directly by the rate of glucose metabolism, which serves as a surrogate for the concentration of glucose in the blood (4). Glucose enters the β cells through glucose transporters and is metabolized in glycolysis and then by mitochondrial respiration, increasing the ATP/ADP ratio. Insulin secretion is then stimulated via two major pathways: the triggering pathway, which mediates the rise in intracellular Ca^{2+} needed to trigger exocytosis, and the amplifying pathway, which increases docking and priming of insulin-containing granules, and brings them into close proximity to Ca_V channels, enhancing the efficacy of Ca^{2+} in driving secretion.

Central to the triggering pathway are K_{ATP} potassium channels, which close in response to increases in the cytosolic ATP/ADP ratio. K_{ATP} channels are open in low glucose, maintaining the cells at a negative membrane potential, but when they close, the cells depolarize, opening voltage-dependent calcium (Ca_V) channels and initiating Ca^{2+} entry (c in the diagram shown in Fig.1). The reciprocal activation of Ca_V and K_V channels generates action potentials (spiking), much like the excitable membranes of neurons and muscle cells. Ca^{2+} then triggers the exocytosis of insulin granules, primarily those located near the Ca_V channels.

In addition to generating ATP, glucose metabolism gives rise to one or more metabolic signals, called amplification factors (AF in diagram), that mediate the amplifying pathway introduced above. The identity of the AF remains unclear, but some of the suggested candidates include ATP, glutamine, and NADPH (4, 5). A recent review highlights possible roles of reactive oxygen species, lipids and phosphoenolpyruvate (6). The AF is physiologically important, as it is responsible for about half the insulin secretion (7).

The picture described above contains the basic information needed to understand β-cell function but is incomplete, as blood insulin levels *in vivo* are not steady but oscillatory, with a period of about five minutes, as has been observed in humans, rodents, non-human primates, and canines (8-11). Figure 2 shows an example of insulin levels recorded from the portal vein in a human (panel A), with insulin secretion rate reconstructed by deconvolution, as shown below the raw

secretory data (panel B) (12). Similar data have been recorded from the portal vein in rats (13) and in dogs (14). The latter study looked at oscillations before and after glucose ingestion, indicating an average increase of 400% in pulse amplitude and 40% in frequency. The pulsatile nature of insulin release, which resembles in a general manner that of other hormones (15) is believed to be necessary for the efficacious action of the hormone (16); for a review see (17).

The insulin oscillations observed in the circulation are driven by insulin secreted in pulses from the islets. Pulsatile insulin secretion from islets *in vitro* has been shown for both humans and mice (18-24). Notably, the oscillations of isolated islets have the correct period and respond to increasing glucose concentration with increases in insulin pulse amplitude. Despite the pulse-generating capability of individual islets, important questions remain about how the hundreds of thousands of islets within the intact pancreas synchronize their secretory output to generate the insulin pulses of portal blood. Suggested synchronizing signals include acetylcholine, ATP and nitric oxide (NO), which have been studied by pharmacologically inhibiting the neurons that innervate the pancreas or by vagotomy, but these studies failed to reach a consensus (25-28). While it is also possible that inter-islet synchronization is different under basal conditions, we assume in the absence of evidence to the contrary that the core oscillation mechanism still resides within individual islets. We proceed to focus here as a first step on the oscillation mechanisms of individual islets.

At higher glucose levels (7 – 15 mM) corresponding to post-prandial or diabetic conditions, oscillations in islet insulin secretion are driven in large part by oscillations in cytosolic free Ca^{2+} (29). These Ca^{2+} oscillations arise in turn from a second role of Ca^{2+} in addition to stimulating the exocytosis of insulin granules shown in Fig. 1, namely the negative feedback exerted by Ca^{2+} on its own entry. This occurs by two complementary mechanisms. The first mechanism proposed was activation of K_{Ca} channels, similar to the situation in many other endocrine cells and neurons. This causes the spikes to cluster into bursts rather than occurring continuously (look ahead to panels showing simulation of V and c in Fig. 4) and was the basis of the earliest mathematical models of Ca^{2+} oscillations in beta cells (30). A number of other models were quickly proposed based on this idea or variations on it, as reviewed in (31, 32).

A second, more subtle form of negative feedback was subsequently appreciated: Ca^{2+} entry and accumulation activate Ca^{2+} pumps in the ER and plasma membranes (SERCA and PMCA, as shown in Fig. 1). These pumps restore cytosolic Ca^{2+} levels in between bursts of spikes but also consume ATP; see (33) for experimental evidence that raising Ca^{2+} lowers ATP/ADP. This would reopen some of the K_{ATP} channels that were closed when glucose was elevated and could contribute to the termination of each burst of spikes, as first proposed in (33). This is a much slower process than activation of K_{Ca} channels and is a more appropriate explanation of the five-minute oscillations of insulin observed in the circulation. Current models in which K_{ATP} channels either drive Ca^{2+} oscillations (34) or are limited to setting the glucose threshold for oscillations

driven by other ion channels (35, 36) are reviewed and contrasted in (37, 38). A further point, which will take on heightened significance below, is that if metabolism oscillates in phase with Ca^{2+}, the AF will also oscillate and reinforce the pulses of secretion (39).

We now come to the heart of the matter: oscillations in secretion are also seen in basal glucose *in vivo* (4 – 5 mM) (12, 14, 40, 41) and from isolated human (24) and mouse islets *in vitro* (42, 43). In fact, it has been reported that as much as 70% of the total insulin secreted from the pancreas under basal conditions, where both constant and pulsatile release are observed, occurs in pulses (44). Yet, *in vitro* data from islets exposed to these low glucose concentrations indicate that the beta cells are not electrically active and do not exhibit large amplitude oscillations in cytosolic free Ca^{2+} concentration in this case. This raises the question: *What is the mechanism for oscillatory insulin secretion at basal glucose levels?* This is not a purely academic question, because blood glucose is within its basal range most of the time. The high concentrations commonly used in *in vitro* experiments are typically only experienced by people or animals with diabetes.

Regulation of basal insulin secretion has clinical significance, as elevated basal insulin is a good predictor of future diabetes (45). It occurs years before basal glucose increases and is a marker of insulin resistance, as embodied in the widely used indices HOMA-IR and QUICKI (46, 47). It has also been suggested that in addition to being a marker of insulin resistance, elevated basal insulin may be a cause of insulin resistance (48, 49) as well as obesity (6, 50). A recent review, however, concludes that the preponderance of evidence is against hyperinsulinemia as a primary cause of diabetes in most cases; a good introduction to this debate is the review (51) and the commentary responding to it (52, 53).

While the mechanism of elevated basal insulin secretion and its contribution to diabetes is not established, various hypotheses have been proposed. Examples include dysregulated basal insulin release due to the interaction between reactive oxygen species (ROS) and long chain fatty acids (as reviewed in (6)), abnormal levels of cardiolipin in mitochondria due to its altered biosynthesis with concomitant changes in mitochondrial respiration (54), changes in the regulation of sweet taste receptors on the beta cell that normally act as a brake on basal secretion (55), elevation in basal secretion due to fatty acids that does not involve ATP synthesis, mitochondrial lipid oxidation or ROS but does involve Ca^{2+} (56), and increased proton leak across the mitochondrial inner membrane mediated by fatty acids independently of ATP synthesis (57), and lastly a novel Ca^{2+} influx pathway activated by ER stress under low glucose conditions, leading to more insulin secretion (58).

Calcium oscillations have in fact been seen in low glucose (e.g., at 6 mM, just below threshold levels of glucose needed to trigger full blown oscillatory activity) and in islets treated with high glucose and mannoheptulose to inhibit glycolysis (Fig. 3) (59). However, these small-amplitude Ca^{2+} oscillations are too small to plausibly engage the Ca^{2+} feedback mechanisms acting on K_{Ca}

or K_{ATP} channels oscillations described above, and it is unlikely that they alone could be the driver of insulin oscillations in basal glucose.

We hypothesize instead that metabolism can oscillate at the level of glycolysis despite low levels of Ca^{2+}, as described in detail below. The possibility that glycolytic oscillations can occur in low glucose is supported by recordings of oscillations in K_{ATP} channel conductance at 3 mM glucose (60) as well as by simulations carried out using mathematical models (61). We further hypothesize that when subthreshold, small-amplitude Ca^{2+} oscillations (henceforth referred to here as *subthreshold oscillations*) are coupled to coincident oscillations in metabolism, their effect is amplified sufficiently by the AF to produce small amplitude secretory oscillations. Oscillations in secretion driven by oscillations in metabolism with Ca^{2+} fixed, albeit at high glucose levels, have been seen experimentally (62), making this plausible. Finally, we demonstrate here using a current mathematical model of oscillatory activity in mouse islets that as glucose is increased, the oscillations in the free cytosolic Ca^{2+} concentration, membrane potential and insulin secretion transform naturally into the patterns that are observed at high glucose. Although our goal is to explain basal insulin oscillations in humans, the model for mouse is the best developed for addressing the interplay between oscillations driven by metabolism vs. Ca^{2+}, and we expect the general principles to apply.

The Integrated Oscillator Model

Over the past two decades we have developed a mathematical model that can account for most of the oscillatory activity patterns observed in beta cells. This model, the Integrated Oscillator Model (IOM) (37, 63, 64), has been very helpful for generating hypotheses that were subsequently tested in experiments, and in the design of those experiments. Here, we use the IOM to illustrate our hypothesis for the origin of oscillatory insulin secretion at basal glucose levels and demonstrate its feasibility. An earlier version of the model demonstrated that subthreshold Ca^{2+} oscillations were indeed possible and would convert to full amplitude oscillations at higher glucose (59, 61), but we did not examine their relationship to secretion oscillations using that model. The model used in this article is closely related to the version previously described in (64), with the addition only of a previously published set of equations to translate oscillations in Ca^{2+} and metabolism into oscillations in insulin secretion (39). Computer codes for the model are available at https://www.math.fsu.edu/~bertram/software/islet/ as well as the public repository Figshare (https://doi.org/10.6084/m9.figshare.17063984.v2).

We hypothesize that glycolytic oscillations drive pulsatile insulin secretion at basal glucose levels

Using the IOM, we demonstrate the transitions in electrical activity and secretion that occur as glucose is ramped from basal to supra-threshold levels (Fig. 4). At subthreshold glucose it is

possible to produce oscillations, albeit small, in insulin secretion (see the first 15 minutes of the ISR panel in Fig. 4). In this case, the model β cell is nearly electrically silent, so the dramatic opening and closing of ion channels that would occur when the cell is electrically active do not take place. In fact, the Ca^{2+} channels that allow Ca^{2+} entry into the cell are mostly closed since the membrane potential (V) is relatively hyperpolarized, so the small changes in the Ca^{2+} concentration (c) observed in this scenario reflect small fluctuations in V and associated changes in the driving force for Ca^{2+} current. These Ca^{2+} oscillations are not by themselves sufficient for basal insulin secretion oscillations.

In the model, the oscillations in the insulin secretion rate (ISR) are driven instead by oscillations in glycolysis, represented here by the intermediate metabolites fructose 6 phosphate (F6P) and fructose 1,6 bisphosphate (FBP). These lead to oscillations in the ATP/ADP ratio and in turn to small oscillations in V and c via changes in K(ATP) channel conductance. Additionally, the oscillations in glucose metabolism lead to robust oscillations in the metabolic amplification factor described above (AF in Figs. 1, 4), which enhances the efficacy of Ca^{2+} to trigger the exocytosis of insulin granules. The model calculation in Fig. 4 suggests that even small excursions of the Ca^{2+} concentration, when combined with large pulses in AF, can result in meaningful oscillations in the secretion rate.

Insulin pulse amplitude increases with glucose and activity patterns change

As glucose increases above the threshold for electrical activity (about 5 mM), repetitive bursts of action potentials appear (starting at around 17 minutes in Fig. 4), mediating large oscillations in the intracellular Ca^{2+} concentration (c in Fig. 4). The oscillations in FBP and AF persist, but do not increase dramatically in amplitude; the large increase in ISR seen here is due mainly to the increased amplitude of the Ca^{2+} oscillations.

As glucose rises further towards the range usually studied *in vitro* (8 – 11 mM), oscillations in V, c, and ISR continue but intensify. The active, spiking phases of the bursts become longer, which increases the average level of Ca^{2+}, which in turn combines with glucose-dependent increases in AF to increase the amplitude of the ISR pulses. Note that oscillation frequency does not change much, consistent with the experimental data in Fig. 2.

A subtle but important change in the character of the FBP oscillations in Fig. 4 also appears at these higher glucose levels: instead of a discrete pulse that precedes each burst and falls nearly to 0, the FBP oscillations become sawtooth in shape, rising slowly throughout the silent phase and falling during the active phase. This is a good sign of the fidelity of the model, as measurements of FBP oscillations at higher glucose levels generally have a sawtooth shape (65, 66). Closer examination elsewhere (37, 63) has revealed that the pulse-like metabolic oscillations do not require oscillations in Ca^{2+}, whereas the sawtooth oscillations cease if Ca^{2+} is held constant. We

denote these as Active Metabolic Oscillations (AMOs) and Passive Metabolic Oscillations (PMOs), respectively, to distinguish the two cases as previously described in (37, 63). The existence of AMOs at basal glucose is critical to our hypothesis, as PMOs require large amplitude Ca^{2+} oscillations to occur that would not be possible at basal glucose.

How do β cells orchestrate such a wide range of activity patterns?

Work dating back to the 1970s has demonstrated that metabolic oscillations could be produced in glycolysis, the first stage of glucose metabolism that precedes aerobic respiration. First in yeast (67), and later in muscle extracts (68, 69), it was shown that glycolysis can sustain long-term oscillations. These oscillations are believed to be due to the actions of a key allosteric enzyme, phosphofructokinase (PFK), that converts the substrate fructose-6-phosphate (F6P) into fructose-1,6-bisphosphate (FBP). Importantly, the product FBP is a positive regulator of PFK, and that positive feedback causes a buildup of FBP at the expense of the substrate F6P. At the same time, AMP, which increases the affinity of PFK for F6P, also falls due to increased ATP production downstream in glycolysis and the mitochondria. When F6P and AMP fall too low, PFK activity largely shuts down, ending the positive feedback cycle and resetting the system. This is the basis of the FBP oscillations in Fig. 4 that underlie oscillatory insulin secretion at low glucose levels. This oscillation mechanism was first proposed in the context of stimulatory glucose levels (69), but we believe that it is actually most important at subthreshold levels.

The PFK isoform that has highest affinity for FBP, PFKM, was long assumed to be the critical player in this scenario, but we and others have found that slow Ca^{2+} oscillations persist in PFKM KO mice (70, 71). Furthermore, model simulations suggested that other PFK isoforms can take over in the absence of PFKM (70). Regardless of the details regarding this enzyme, as long as glycolytic oscillations can occur independent of Ca^{2+}, our basic hypothesis that they are responsible for subthreshold oscillations in insulin secretion would remain viable.

Although we have described how glycolytic oscillations can produce Ca^{2+} oscillations, the reverse can also happen: Ca^{2+} can influence glycolytic oscillations. This is the key to the transformation from AMOs to PMOs as glucose increases, as illustrated in Fig. 4 and discussed above. At high glucose, glycolysis loses the ability to oscillate independently of Ca^{2+}. This is partly due to the intrinsic glucose sensitivity of glycolysis: at high glucose the substrate F6P remains high, so PFK activity remains high, and oscillations are lost. In addition, Ca^{2+} activation of pyruvate dehydrogenase (PDH, in Fig. 1) contributes by increasing the consumption of pyruvate by PDH, which in turn increases the consumption rates of glycolytic metabolites, including FBP. This shuts down the positive feedback of FBP onto PFK and inhibits glycolytic oscillations. The signature of this in Fig. 4 is the conversion of the FBP waveform from pulses into a sawtooth, as FBP slowly responds to the rise and fall of Ca^{2+}.

Nonetheless, oscillations in metabolites, such as FBP and ATP/ADP, can still occur, by a mechanism discussed in the Introduction. When Ca^{2+} is high, ATP is consumed by Ca^{2+} pumps, including the sarco/endoplasmic reticulum Ca^{2+} ATPase and plasma membrane Ca^{2+} ATPase (labeled as SERCA and PMCA in Fig. 1). This lowers ATP/ADP, allowing some K_{ATP} channels to reopen, which turns off Ca^{2+} entry. The drop in Ca^{2+} allows ATP/ADP to recover, which again closes K_{ATP} channels, and electrical activity resumes. Glycolytic metabolites, such as FBP, also oscillate because of the Ca^{2+} dependence of PDH mentioned above – when Ca^{2+} is high, flux through glycolysis and cellular respiration is increased – and also because of feedback of ATP and AMP onto PFK. These repeated cycles of ATP consumption and production underlie the oscillations in Ca^{2+}, metabolites, and insulin secretion at high glucose, shown in the simulation in Fig. 4 starting at around 25 minutes. *In vitro* data support this mechanism for PMOs (65).

Summary and future directions

We have presented here the hypothesis that oscillations of basal insulin secretion are driven by metabolic oscillations, specifically, oscillations in glycolysis that do not require, but can be modified by, Ca^{2+}. We have used model simulations of oscillations in membrane potential, Ca^{2+} and insulin secretion to illustrate and support the feasibility of the hypothesis. Models in which oscillations of metabolism only occur secondary to oscillations in Ca^{2+}, which likely occur for the secretory oscillations produced at elevated glucose, cannot account for oscillations observed under basal glucose.

The hypothesis could be tested in islets *in vitro* by looking for small amplitude oscillations in Ca^{2+} at low glucose, say in the range of 3 – 7 mM, while simultaneously recording oscillations in metabolites, such as ATP/ADP, FBP, or NAD(P)H. It may take some trial and error to find the right conditions, as the simulations show that the prevailing Ca^{2+} levels may determine whether metabolic oscillations occur at a particular level of glucose.

There are ample data in the literature demonstrating oscillations at stimulatory glucose levels in multiple metabolites, including oxygen consumption (72), mitochondrial membrane potential (73), ATP/ADP (74), and NAD(P)H (75), which are synchronized with membrane potential and Ca^{2+} (65). In addition, a FRET sensor has been developed called "PKAR", for Pyruvate Kinase Activity Reporter, based on the glycolytic enzyme pyruvate kinase (PK). PK is allosterically activated by FBP and PKAR FRET is thus an assay to dynamically measure FBP concentration. Fluorescence measurements from islets showed that at stimulatory glucose levels PKAR FRET responses were oscillatory (76) and coincident with Ca^{2+} oscillations (65). Furthermore, metabolic oscillations persisted under conditions where oscillations in membrane potential and Ca^{2+} were abolished with diazoxide, demonstrating the existence of AMOs at high glucose (65). No study has yet been performed using PKAR to look for AMOs at basal glucose levels, so it is not known if oscillations exist in this case. *We predict that they would exist for cases in which basal insulin secretion*

oscillations are present. We also predict that the concentration of ATP would oscillate. As an alternative to PKAR, the fluorescent reporter Perceval-HR, which provides a readout of the cytosolic ATP/ADP ratio, could be used to demonstrate metabolic oscillations in basal glucose.

In addition to offering a plausible mechanism for oscillations in basal insulin secretion, the simulations described here also help resolve a conundrum about how the IOM works. Slow oscillations in Ca^{2+} in the model can result from either AMOs or PMOs. The simulations here suggest that these two modes of operation are most appropriate at different glucose levels. AMOs are the only candidate mechanism of which we are aware that can generate secretory oscillations in basal glucose, and they transition in an orderly way to PMOs as glucose is increased. This was not apparent from previous modeling studies in which glucose was held fixed while other parameters were varied. It is still not apparent why AMOs give way to PMOs at higher glucose, as either mechanism could operate effectively in this range in theory, raising the question of what benefit islets might derive from having two seemingly redundant mechanisms. Nonetheless, AMOs appear to persist in high glucose in at least some islets, as demonstrated by the PKAR measurements mentioned above (65), as well as by the existence of compound oscillations (e.g., slow oscillations that have superimposed fast bursts), which so far have only been plausibly explained by AMOs. However, the observed and detailed characteristics of the slow oscillations indicate that PMOs predominate at higher glucose levels (63, 77).

An analogy for the division of labor between AMOs and PMOs that we find useful is the gas-electric hybrid car, which has two motors. At low speeds, the car is powered by the battery, which energizes the car's electric motor, while at higher speeds, typically 25 – 40 mph, depending on the rate of acceleration and battery capacity, the internal combustion engine takes over. This arrangement seems complicated at first glance but is an effective way to exploit the characteristics of each type of engine to produce high fuel efficiency. If pulsatility is important for the efficiency of insulin action (17), it would seem appropriate to maintain such pulsatility over a range of glucose.

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Author Contributions:

PAF performed mathematical simulations and analysis. IM provided conceptual advice on the model. ASS, LSS and RB provided resources and supervision. All authors contributed to writing and editing the paper.

Figure Legends

Graphical Abstract: Insulin secretion is pulsatile, driving oscillations in circulating insulin with a period of about 5 min. In the postprandial state (left), glucose is elevated, and the secretion oscillations are triggered by large calcium oscillations in the β cells of the pancreas. However, insulin in the basal state (right) is also oscillatory with the same period but reduced amplitude. In this condition, β cell calcium oscillations are small. We propose that oscillations in glycolysis produce a metabolic signal that is able to drive oscillatory insulin secretion despite small calcium oscillations.

Fig. 1: Diagram illustrating the key cellular components and molecular mechanisms involved in oscillations of Ca^{2+} and insulin secretion in the pancreatic β cell. Glucose enters the cell through glucose transporters and is metabolized in glycolysis and the mitochondria. ATP, mostly produced by the mitochondria, closes K_{ATP} channels in the plasma membrane, leading to depolarization. Additional ion channels, including voltage gated Ca^{2+} (Ca_V) and K^+ channels (K_V), and Ca^{2+}-activated K^+ channels (K_{Ca}), support excitability of the plasma membrane potential (V) and underlie bursting activity at postprandial glucose levels. Depolarization of the membrane leads to Ca^{2+} (c) influx through CaV channels, while cytosolic Ca^{2+} levels are reduced by activity of Ca^{2+} ATPase pumps (SERCA and PMCA). Exocytosis of insulin-containing granules is triggered by Ca^{2+} and amplified by one or more metabolic signaling factors (AF) that promote the movement of insulin granules towards a releasable state.

Fig. 2: Basal and glucose-stimulated insulin secretion are oscillatory in humans. A) Insulin oscillations measured from the hepatic portal vein at basal glucose (4.4 mM; left) and high glucose imposed via hyperglycemic clamp (nominally 8 – 9 mM; right). The ~5 min period of the oscillations does not change dramatically in response to glucose, but insulin rises about 2-fold in the higher glucose condition (note the difference in scale). B) Deconvolution analysis resolves the underlying pulsatile nature of the insulin secretion rate, demonstrating that glucose stimulates a large increase in insulin pulse mass. Reproduced with permission from (12).

Fig. 3: Oscillations in intracellular Ca^{2+} concentration, the main trigger for insulin exocytosis, are observed in islets *in vitro*. A) Upon partial block of glycolysis using D-mannoheptulose, large amplitude glucose-stimulated Ca^{2+} oscillations in a mouse islet give way to small amplitude oscillations unlikely to be sustained by ionic mechanisms alone. B) A similar result observed in response to a reduction in glucose from a supra-threshold (11 mM) to a subthreshold (6 mM) concentration. Reproduced with permission from (59).

Fig. 4: The Integrated Oscillator Model with modification can account for basal insulin oscillations. A model simulation of a ramped increase in glucose from basal (3 mM) to post-prandial to the high levels typically studied *in vitro* (11 mM). Key dynamic variables shown include the glycolytic metabolites F6P and FBP, AMP, ATP/ADP ratio, membrane potential (V), cytosolic Ca^{2+} concentration (c), an amplifying factor (AF) that enhances exocytosis, and the insulin secretion rate (ISR).

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