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REVIEW

The triggering pathway to insulin secretion: Functional similarities and differences between the human and the mouse β cells and their translational relevance

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ABSTRACT

In β cells, stimulation by metabolic, hormonal, neuronal, and pharmacological factors is coupled to secretion of insulin through different intracellular signaling pathways. Our knowledge about the molecular machinery supporting these pathways and the patterns of signals it generates comes mostly from rodent models, especially the laboratory mouse. The increased availability of human islets for research during the last few decades has yielded new insights into the specifics in signaling pathways leading to insulin secretion in humans. In this review, we follow the most central triggering pathway to insulin secretion from its very beginning when glucose enters the β cell to the calcium oscillations it produces to trigger fusion of insulin containing granules with the plasma membrane. Along the way, we describe the crucial building blocks that contribute to the flow of information and focus on their functional role in mice and humans and on their translational implications.

Introduction

Insulin secreted by pancreatic β cells regulates postprandial storage and interprandial usage of energy rich nutrients. A relative or absolute lack of insulin results in diabetes mellitus, a disease that affects more than 400 million people around the world, and this number is expected to increase to 640 million by 2040.[1] The burden of this disease is immense and includes public health costs of treating diabetic patients, as well as personal costs related to serious complications.[2] More than 90% of all diabetics have type 2 diabetes mellitus (T2DM), which is characterized by obesity, insulin resistance, and eventually insufficient insulin secretion.[3] A genetic susceptibility, age, obesity, dietary habits, and a sedentary lifestyle most importantly contribute to the development of the disease.[4] It has been shown that the progressive nature of T2DM can be slowed down upon or reversed by lifestyle interventions.[5,6] However, pharmacological agents are still prescribed to practically all patients, especially later during the course of the disease, to increase the deficient secretion of insulin from β cells, to improve its action on target tissues, or promote urinary glucose excretion by inhibiting its renal reuptake.[7]

β cells couple stimulation by metabolic, hormonal, neuronal, and pharmacological factors to insulin secretion by at least 3 interconnected intracellular signaling pathways (Fig. 1). After more than 4 decades of intensive research, by far the most investigated one is the so-called triggering pathway. It consists of several events starting with influx of glucose through glucose transporters, glucose metabolism increasing intracellular ATP concentration, and triggering closure of ATP-dependent K^+ channels (K_{ATP} channels). The resulting decrease in K^+ efflux causes membrane depolarization, the consequent opening of voltage dependent calcium (Ca^{2+}) channels (VDCC), and influx of Ca^{2+} ions. The resulting increase in the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_C$) is tightly coupled to membrane potential changes and typically follows a characteristic temporal pattern in the form of oscillations, activating the secretory machinery and fusion of insulin-containing vesicles with plasma membrane.[8] This triggering pathway is essential, i.e. necessary, but not very efficient without so-called amplifying pathways which mainly affect the sensitivity of the secretory machinery to $[Ca^{2+}]_C$. More
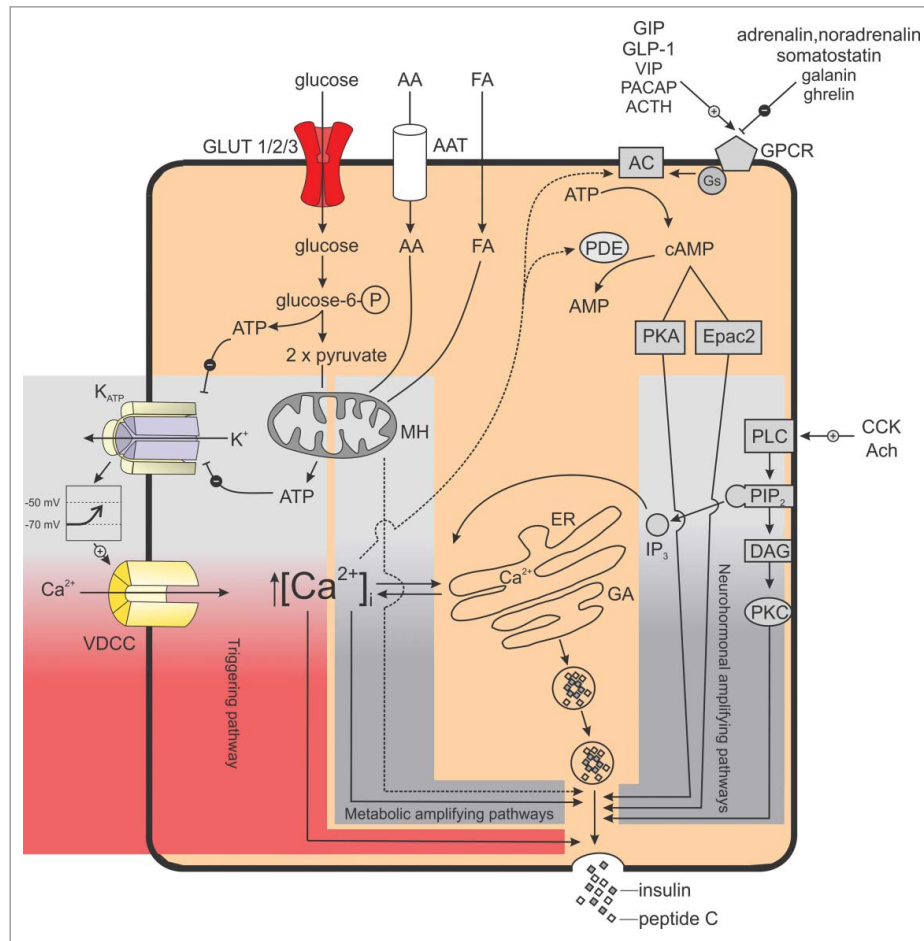
Figure 1. The three interconnected intracellular signaling pathways in pancreatic β cells. The K_{ATP}-dependent triggering pathway is indicated in red, the metabolic and neurohormonal amplifying pathways are indicated in gray.

specifically, there seem to be a K_{ATP}-independent Ca^{2+}-dependent and a K_{ATP}-independent Ca^{2+}-independent amplifying pathway.[9,10] In addition, non-nutrient secretagogues, i.e., neurotransmitters and hormones, activate membrane receptors and set into motion protein kinase A- and protein kinase C-dependent pathways (Fig. 1). To complicate things further, both glucose- and non-nutrient secretagogues shape the triggering signal by influencing uptake and release of Ca^{2+} in intracellular stores.[11]

A large proportion of our knowledge about the abovementioned pathways comes from work on rodents, especially mouse models. Although the human and the mouse pancreas and islets of Langerhans share many common anatomic and physiologic characteristics, they are not the same.[12,13] During the last decade, islets obtained from humans have been increasingly used and studies suggested many important functional differences between the human and the mouse β cells, so far mostly for the triggering

pathway. Validating findings obtained in mice and studying functional similarities and differences between β cells from humans and mice may help us understand why some therapeutic and other interventions do not yield the expected results and to better choose the experimental approach when looking for new therapeutic targets. Thus, in the present review we aim to highlight some similarities and differences between the two species at different stages of the triggering pathway, from the most proximal event, i.e., entry of glucose into the β cell, to the most distal changes in $[Ca^{2+}]_C$ (Table 1).

Glucose transporters

Influx of glucose into the β cell through glucose transporters is the first step in GSIS and in the series of signaling events collectively termed stimulus-secretion coupling (SSC). In both mice and humans, glucose enters the β cell cytosol by facilitated diffusion

Table 1. Summary of differences in the triggering pathway between mouse and human β cells.

Species	Mouse	Human
Glucose transporter	GLUT2 $K_m \approx 17$ mM	GLUT1 and GLUT3 $K_m \approx 7$ mM and 1.8 mM
Hexokinase	Glucokinase	Glucokinase
ATP-dependent K$^+$ channels	Kir6.2 and SUR1	Kir6.2 and SUR1 Kir2.1
Transient receptor potential channels	TRPC1 TRPV2 TRPA1 TRPM2, TRPM3, TRPM4, TRPM5	TRPV4 TRPM2, TRPM4, TRPM5
Voltage-dependent Ca^{2+} channels	Ca$_V$1.2 (L-type) - >50% Ca$_V$2.3 (R-type) - 25% Ca$_V$2.1 (P/Q-type) - 15% of total VDC currents	Ca$_V$2.1 (P/Q-type) - 40 - 45% Ca$_V$1.2 and Ca$_V$1.3 (L-type) - 40 - 45% Ca$_V$3.2 (T-type) - 10 - 20% of total VDC currents
Voltage-dependent Na$^+$ channels	Na$_V$1.7 - 85% and Na$_V$1.3 - 15% of total Na$^+$ currents	Na$_V$1.6 - 75% and Na$_V$1.7 - 25% of total Na$^+$ currents
Voltage- or calcium-dependent K$^+$ channels	Delayed rectifying K$^+$ channels (K$_V$2.1) Small conductance Ca^{2+}-activated K$^+$ channels (SK4) ERG1	BK channels Delayed rectifying K$^+$ channels (K$_V$2.2) Small conductance Ca^{2+}-activated K$^+$ channels (SK3 and SK4) hERG1
Pattern of membrane potential oscillations	Bursts of APs (spikes), glucose dependent, continuous firing of APs observed at higher glucose concentrations (above 16–20 mM)	Continuous or irregularly spaced APs without clear bursts or a more organized oscillatory electrical activity (for details see Table 2).
Coupling between β cells	Tight junctions and gap junctions (Cx36) The junctional conductance between cell pairs is estimated at about 200–350 pS.	Tight junctions and gap junctions (Cx36) A coupling conductance of 100–200 pS is suggested.
Pattern of [Ca^{2+}]$_C$ oscillations	Globally synchronized [Ca^{2+}]$_C$ oscillations.	Globally or locally synchronized [Ca^{2+}]$_C$ oscillations or no oscillations observed (for details see Table 3).

through insulin-independent membrane-bound glucose transporters (GLUTs). Most of the glucose transporters expressed in mouse β cells are GLUT2, the type of carrier proteins with the lowest binding affinity and highest transport capacity for glucose ($K_m \approx 17$ mM).[14] The glucose transport rate is $\approx$20-50-fold greater than the rate of glucose phosphorylation by glucokinase.[14] Genetic inactivation of GLUT2 suppresses glucose uptake and GSIS in mouse pancreatic β cells.[15]

In human β cells on the other hand, GLUT2 are almost completely dispensable, since GLUT1 and GLUT3 are far more abundantly expressed.[16] Uptake of 3-O-methylglucose was estimated at 2.2 and 2.9 mmol/min per liter intracellular space in humans[17] and at 3.0 and 4.8 mmol/min per liter intracellular space in rats at 5 and 10 mM glucose, respectively.[18] However, these data can hardly be directly compared since glucose uptake in human β cells was measured at 37°C while in rat β cells, experiments were performed at 15°C to slow down the process sufficiently to allow for accurate calculations. Glucose transport in mouse β cells has been assessed with a different protocol and compared with the rate in human β cells using the fluorescent glucose analog 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG). The uptake of 2-NBDG in

10 mM glucose at 37°C was approximately 0.8 nmol/min/5 $\times$ 10^4 cells in mice and approximately 0.9 nmol/min/1 $\times$ 10^5 cells in humans.[19]

The GLUT1 protein (encoded by SLC2A1 gene) displays 97.2% homology[1] between humans and mice and plays a principal role in human islets, since K_m in GLUT1 is lower ($\approx$7 mM) compared with GLUT2 (SLC2A2 gene, 82% homology) and more compatible with the dose-response curve for GSIS in human β cells. One of the most striking differences between mouse and human GLUT expression is the distribution of GLUT3 (SLC2A3 gene, 83.5% homology), which has the highest affinity to glucose ($K_m \approx 1,8$ mM) among all 3 glucose transporters expressed in pancreatic β cells. In mice, GLUT3 is expressed only at a background level, whereas it is as abundant as GLUT1 in human β cells, suggesting an equally important role for both transporters in the regulation of insulin secretion in humans.[20] Although GLUT 1 and GLUT3 are far more abundant in human β cells than GLUT2, the latter still seems necessary for normal GSIS. Namely, mutations in the GLUT2 gene cause the Fanconi-Bickel syndrome manifested by hepatomegaly,

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[1]Values that determine the homology of proteins are taken from the HomoloGene.

growth retardation, and renal syndrome.[21] Impaired GSIS in adults has only been reported in a few cases of Fanconi-Bickel syndrome.[22] On the other hand, a transient absolute requirement for GLUT2 for the control of insulin secretion in the first months of life exists, since neonates with Fanconi-Bickel syndrome have transient diabetes mellitus that disappears after 18 months of age.[23]

Cytosolic and mitochondrial metabolism

Rapid entry of glucose through glucose transporters ensures glucose sensing, meaning that the rate of glucose entry is proportional to blood glucose levels and it accounts for fast equilibration of glucose between the extracellular space and the cytosol. This enables glucose unrestrained access to glucokinase (GCK), the hexokinase isozyme found in both mouse and human β cells (GCK gene, 95.9% homology) that catalyzes the rate-controlling step in glucose metabolism.[24] GCK has more than a 20-times lower affinity for glucose compared with other hexokinases ($K_m \approx 6–11$ mM) and is not inhibited by its product, glucose-6-phosphate.[25] K_m values in mouse and humans are very similar, reported at 7 mM and 7.9 mM, respectively.[26] Its sigmoidal glucose dependency guarantees optimal responsiveness at physiological glucose levels, since the inflection point of the sigmoidal curve (4-5 mM) is close to the physiological threshold for GSIS in β cells. Functionally, this positive cooperativity with glucose allows the enzyme to have increased sensitivity to fluctuations in blood glucose levels.[27] The values of Hill coefficients in mice and humans are practically identical (≈ 1.7).[26] At 5 mM and 10 mM glucose glucokinase activity was 0.38 and 0.60 mmol/min per liter intracellular space in nondiabetic human β cells, respectively,[17] and similar to that seen in rodent β cells.[28] In another study, the rate of glycolysis in 6 and 12 mM glucose was estimated at ≈ 40 and ≈ 90 pmol/islet/h in mouse, respectively, and at ≈ 125 and ≈ 160 pmol/islet/h in human islets, respectively.[29]

GCK exerts a unique regulatory role in β cell metabolism because the reaction it catalyzes is virtually irreversible.[26,27] The functional reserve of GCK is low in β cells, since a decrease of only $\approx 50\%$ of enzyme activity is tolerated.[30,31] Therefore, GCK expression is indispensable for SSC in β cells. It has been demonstrated that homozygous GCK knockout mice develop lethal hyperglycemia.[32] In humans, the majority of approximately 200 known mutations in the GCK gene, including missense, non-sense, and splice site mutations, result in mild hyperglycemia that is usually discovered by routine examination. Based on these characteristics, diabetes associated with GCK mutations was included in the group of maturity onset diabetes of the young (MODY) and named MODY 2.[33] These mutations result in a GCK molecule that is less sensitive or less responsive to glucose. β cells in patients with these mutations have a normal ability to produce and secrete insulin, but expectedly a higher threshold (7-8 mM) for GSIS. In rare cases, when infants inherit mutations from 2 heterozygous parents, permanent neonatal diabetes mellitus can occur.[34] On the other hand, heterozygous activating mutations can lead to varying degrees of hyperinsulinemia and hypoglycemia.[35] These observations are the main reason GCK activators have been studied for more than a decade. Systemic GCK activators alter GCK's affinity to glucose and thereby decrease the threshold for GSIS, but their main drawback is their potential to provoke hypoglycemic episodes. Therefore, hepatoselective GCK activators that can overcome this side effect seem more appropriate candidates for treating T2DM.[36,37]

Since GCK controls the rate-limiting step in glucose metabolisms, its activity partially determines the pattern of $[Ca^{2+}]_C$ oscillations and the consequent pulsatile insulin secretion from β cells. It has been suggested that the slower component of compound oscillations with a period of approximately 5–15 minutes reflects oscillations in glycolysis, whereas the fast component is due to electrical activity in pancreatic β cells (see below). The enzyme downstream from GCK, phosphofructokinase (PFK) with positive feedback regulation by its product fructose bisphosphate, is believed to be directly responsible for oscillatory glycolysis (and slow oscillations). However, if the glucokinase flux rate is too low or too high, glycolysis will be non-oscillatory. In other words, glycolytic oscillations occur only at intermediate levels of GCK flux rate.[38,39]

Evidence for the molecular events linking glucose-6-phosphate to ATP production in mice and humans is scarce. Glucose-induced hyperpolarization of the mitochondrial inner membrane in mice[40] is consistent with the production of pyruvate, its translocation into the mitochondrion, oxidative phosphorylation, activation of the electron transport chain, and translocation

of protons across the mitochondrial membrane.[41,42] Subsequent transient increase in the mitochondrial [Ca^{2+}] is the consequence of a tightly regulated interplay between Ca^{2+} entry on one hand and Ca^{2+} buffering in the matrix and extrusion on the other hand.[41-47]

Mitochondrial [Ca^{2+}] changes are tightly coupled to [Ca^{2+}]$_C$ dynamics, at least in mice.[45,48-51] Glucose stimulation evokes an oscillatory behavior of mitochondrial [Ca^{2+}][45,50,51] that is synchronized across cells in an islet.[50] The matrix signal seems to be delayed in respect to [Ca^{2+}]$_C$ in mice,[45,48,51] implying a modulatory role of [Ca^{2+}]$_C$ on its matrix counterpart. The latter is corroborated by the observation that the frequency of electrically induced [Ca^{2+}]$_C$ modulates the amplitude of matrix [Ca^{2+}], such that matrix [Ca^{2+}] closely follows slow changes in [Ca^{2+}]$_C$ (which occur in slow bursting, see below), whereas at high frequencies of cytosolic Ca^{2+} oscillations, matrix [Ca^{2+}] is unable to follow and the frequency of cytosolic oscillations is decoded as the amplitude of increases in matrix [Ca^{2+}].[49,52] Further, there is some evidence that matrix Ca^{2+} modulates its cytosolic counterpart as impairment of Ca^{2+} efflux from the mitochondrion reduced glucose-induced cytosolic Ca^{2+} response in mice.[53] On the other hand, impairing Ca^{2+} entry into the mitochondrion in mice failed to do so.[48] A detailed recent study revealing many aspects of coupling between glucose and mitochondrial metabolism in human islets unfortunately did not compare cytosolic and matrix [Ca^{2+}] changes and thus at present, a direct comparison between the 2 species in this regard is impossible. However, the glucose- and calcium- dependent changes in ATP synthesis and insulin release indicate that the functional role of mitochondria in the signaling cascade is comparable in the two species.[54] Along this line of reasoning, we wish to point out a recent finding that is of relevance for what was stated above but also for later parts of this paper. Cytosolic ATP concentration ([ATP]$_C$) dynamics induced by the incretin hormone GLP-1 involved changes in mitochondrial potential and were found to be much better correlated between different cells in mouse than in human islets.[55] This is in contrast with the degree of GLP-1-induced coupling of ionic events, e.g., [Ca^{2+}]$_C$ changes, between β cells in human islets[56] (see below) and might also indicate a different degree of coupling between [Ca^{2+}]$_C$ and matrix [Ca^{2+}]/mitochondrial metabolism in the two species.

ATP-dependent potassium channels

Increased β cell glycolytic and tricarboxylic acid (TCA) cycle activity lead to an increased [ATP]$_C$ and decreased ADP concentration ([ADP]$_c$). In low glucose, [ATP]$_C$ is low and the K_{ATP} channel open probability is high, resulting in K^+ efflux that keeps the cell membrane hyperpolarized and prevents insulin secretion. At supratreshold glucose, [ATP]$_C$ increases due to increased metabolism, thus lowering open probability of the K_{ATP} channels. The ensuing decrease in K^+ efflux is co-responsible (together with the putatively unchanged leak conductance, see later) for depolarization and initiation of electrical activity.[57,58]

β cell K_{ATP} channels are members of the inwardly rectifying K^+ channel family, composed of 4 potassium-selective pore-forming Kir6.2 subunits (KCNJ11 gene, 95.9% homology) and 4 regulatory SUR1 subunits (ABCC8 gene, 95.4% homology).[59] The regulatory subunits are termed SURs because they bind sulfonylureas and therefore play a key role in determining the pharmacological regulation of K_{ATP} channels. Because K_{ATP} channels transport K^+ with a greater tendency into a cell then out, they are named inward rectifiers. The assembly and trafficking of the channels has been described to be precisely regulated. Assembly occurs in the endoplasmic reticulum (ER) and only completely assembled and full length complexes are then transported to the plasma membrane and expressed on the surface, while incompletely assembled complexes are retained in the ER.[60]

K_{ATP} channel subunits display differences in their sensitivity to adenosine nucleotides. Intracellular ATP acts on Kir6.2 to decrease channel open probability, while MgADP increases channel open probability through the SUR1 subunit, a member of the ATP-binding cassette superfamily.[61] Therefore, changes in [ATP]$_C$ and [ATP]$_C$/[ADP]$_C$ are believed to determine channel activity.[62] However, only fully assembled K_{ATP} channels have a normal sensitivity to ATP. The Kir6.2 isoform alone forms a weekly ATP-inhibited K_{ATP} channel (IC$_{50} \approx$100–200 μM), while reconstitution with SUR1 increases the affinity to inhibitory ATP (IC$_{50} \approx$5–10 μM).[63] Since K_{ATP} channels are not voltage dependent, their current-voltage relation is linear, although the current increases very little at

membrane potential above +20 mV. This primarily results from a voltage-dependent block of outward K^+ currents by internal Na^+ and Mg^{2+}.[64] The kinetic studies of K_{ATP} channels showed channel openings grouped in bursts. The dominant effect of ATP is to reduce the number of openings per burst and to prolong the time when the channel is closed. Furthermore, high concentrations of phosphatidylinositol phosphate (PIP_2) reduce the sensitivity of K_{ATP} channels to inhibitory ATP by increasing the number of K_{ATP} channel openings.[65] The kinetics of the K_{ATP} channel in human β cells qualitatively resemble those reported for rodent β cells.[58,66]

Due to the critical role K_{ATP} channels play in insulin secretion, it is not surprising that polymorphisms in K_{ATP} subunits alter the biophysical properties of the channel and can lead to both hypo- and hyperglycemia.[67] In humans, K_{ATP} channel mutations that result in decreased K^+ currents cause persistent hyperinsulinemic hypoglycemia of infancy (PHHI).[68] Mutations can be found in either of the subunits that make up K_{ATP} channels. Mild forms of PHHI associated with mutations in SUR1 produce K_{ATP} channels that can be inhibited by ATP, but only poorly stimulated with MgADP. Severe forms of the disease have been associated with mutations that truncate SUR1 and block expression of the K_{ATP} channels on the cell membrane. However, remarkable physiologic differences exist between human neonates missing K_{ATP} channels and the K_{ATP} channel knockout mice ($Kir6.2^{-/-}$ or $SUR6.1^{-/-}$).[61] In both PHHI and K_{ATP} channel null mice loss of K^+ currents causes an elevated membrane potential and consequently increased $[Ca^{2+}]_C$, leading to an inappropriately high rate of insulin release and producing hypoglycemia. As in PHHI, $KIR6.2^{-/-}$ and $SUR1^{-/-}$ mice display hypoglycemia immediately after birth, however their hypoglycemia is transient and persists only during the first 2–3 days of life.[69] It seems that K_{ATP} channel null mice become refractory to increased $[Ca^{2+}]_C$ and develop a K_{ATP}-independent mechanism for regulating insulin release. During adulthood, PHHI patients are hyperglycemic with high prevalence of diabetes, while K_{ATP} channel null mice display normal insulin and glucose levels when fed *ad libitum*, and are hypoglycemic upon fasting and exhibit a mild intolerance toward *i. p.* injection of glucose. $SUR1^{-/-}$ islets lack the first-phase of insulin secretion and have an attenuated second-phase after stimulation with high glucose, while $Kir6.2^{-/-}$ islets have a small first-phase of insulin secretion with no second phase.[61]

Conversely, gain-of-function mutations result in neonatal diabetes characterized by an insulin secretory deficit and hyperglycemia. The first indication that overactive K_{ATP} channels can produce neonatal diabetes came from transgenic mice expressing a Kir6.2 subunit lacking a segment of its N-terminus responsible for channel gating. Its deletion resulted in nearly constantly open K_{ATP} channels that have a reduced sensitivity to ATP and hypoglycemic sulfonylureas.[70] Severe hyperglycemia is lethal within first weeks after birth.[71] In humans, missense activating mutations associated with neonatal diabetes were also found in the gene encoding the Kir6.2 subunit of the K_{ATP} channel (KCNJ11).[67] Furthermore, activating mutations in SUR1 in mice and humans directly enhance MgATP activation of K_{ATP} channel or indirectly alter channel gating and reduce ATP inhibition at Kir6.2.[72,73]

Leak channels

The consensus model of SSC predicts that closure of K_{ATP} channels triggers membrane depolarization. However, according to the Nernst and Goldman-Hodgkin-Katz equations, closure of K_{ATP} channels alone is not sufficient for moving the membrane potential away from the equilibrium potential for K^+, as long as the membrane is permeable to K^+ only. Therefore, the presence of an additional inward current is needed to achieve depolarization by reducing K^+ permeability. Since the input resistance of β cells upon closure of K_{ATP} channels is increased, the current needed for depolarization is likely small, however the identity of this current and its properties have not yet been fully elucidated. The most likely ion channel candidates for depolarizing and hypepolarizing currents can be classified in at least 4 different groups, transient receptor potential (TRP) channels, 2-pore domain potassium or K2P channels, NALCN channels and connexins. Unstimulated β cells are to some extent permeable to Na^+ and Ca^{2+} without activation of voltage-dependent Na^+ channels and VDCCs.[10]

TRP channels are candidates for Na^+ or Ca^{2+} influx contributing to the depolarizing current. The number of different TRP channels expressed in β cells is large (TRPC1, TRPC4, TRPV1, TRPV2, TRPV4, TRPA1, TRPM2, TRPM3, and TRPM5)[74] and is likely to increase (Fig. 2). The channels are to some extent
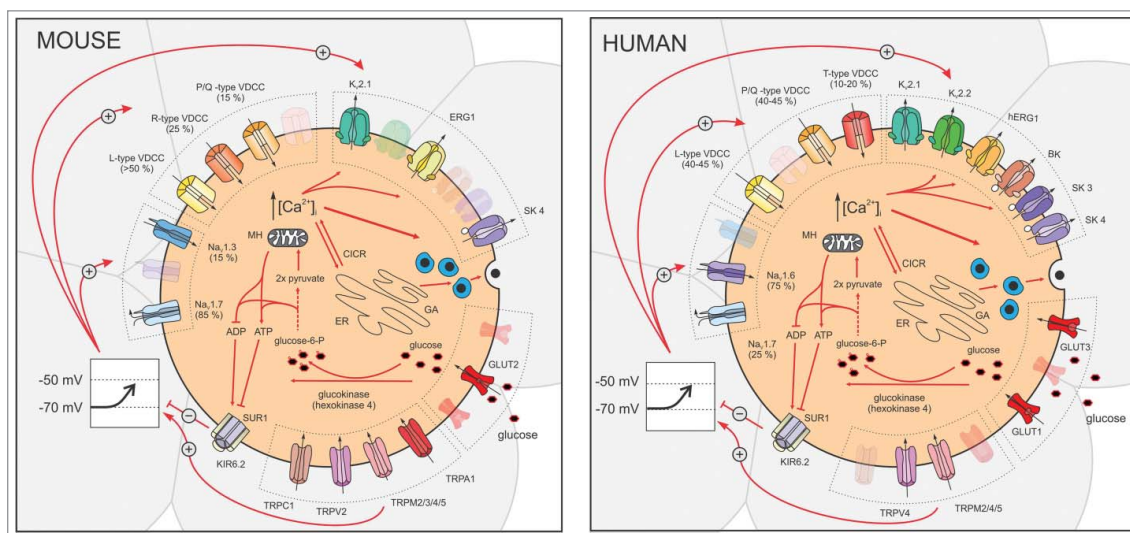
Figure 2. Ion channels involved in the triggering pathway of glucose-induced insulin secretion in mouse (left) and human (right) β cells.

differentially expressed in β cells of different species. In the following lines, only a few examples will be listed. On the one hand, they translocate to plasma membrane upon glucose stimulation and stimulation with insulin or insulin-like growth factors (TRPV2), resulting in Ca^{2+} influx and increased insulin secretion.[75] This positive feedback to increase insulin secretion may result in hyperinsulinemia, commonly found at early stage of type 2 diabetes. On the other hand, knockdown of a specific insulin receptor attenuated insulin-induced translocation of TRPV2 and knockdown of TRPV2 channels and reduces GSIS.[75]

In addition to glucose, other activators like islet amyloid polypeptide (TRPV4),[76] inflammatory mediators, glibenclamide (TRPA1),[77] pregnenolone sulfate, as well as clotrimazole and tamoxifen and structurally related compounds (TRPM3),[78-80] or steviol glycosides (TRPM5)[81] can enhance β cell function. Among all TRP channels present in β cells, the TRPM5 seems to play the most important role in insulin secretion since TRPM5 knockdown mice showed significantly reduced Ca^{2+}-activated nonselective cation current. Furthermore, glucose-induced oscillations of membrane potential and $[Ca^{2+}]_C$ were reduced, particularly due to a lack of fast Ca^{2+} oscillations.[81] Consequently, glucose-induced insulin secretion from TRPM5 knock-down mice was reduced, resulting in impaired glucose tolerance.[81,82]

Lately, another group of hyperpolarizing currents have entered the stage as fine tuners of GSIS, namely K2P channels. Inhibition of the 2-pore-domain acid-sensitive potassium channel (TASK-1) significantly stimulates both human and mouse β cells.[83] Likewise,

ablation of TWIK-related alkaline pH-activated K2P (TALK-1) also seems to result in β cell membrane depolarization.[84] These studies suggest that these 2 K2P channels play a role in limiting glucose-stimulated depolarization and insulin secretion. At least in the case of TALK-1, an intracellular binding partner, osteopontin, has recently been described, however its function is not well understood.[85] Another candidate responsible for membrane depolarization after closure of KATP channels is the so called voltage-independent non-selective Na^+ leak channel (NALCN),[86] but its precise role in insulin secretion from mouse and human β cells still has to be elucidated.

Finally, with K_{ATP} and other K^+ channels closed, the mere presence of electrical coupling between β cells via Cx36 gap junctions can serve as a source of possible electrical interference. More specifically, currents from less or more excitable neighboring cells can respectively hyperpolarize or depolarize a given cell, leading to a complex electrical activity pattern.[87-91]

Voltage-dependent calcium channels

Since both human and mouse β cells maintain a resting membrane potential at -70 mV or lower, closure of K_{ATP} channels and the subsequent decrease in K^+ efflux depolarize the membrane. When membrane potential exceeds the threshold potential ($\approx$ -50 mV), the open probability for voltage dependent calcium channels (VDCCs) increases.

A number of differences exist between mouse and human VDCCs (Fig. 2). In mouse β cells, whole-cell

Ca^{2+} currents are activated by depolarization to potentials more positive than -50 mV, increase to a maximum at about -20 mV, and reverse at about +50 mV (in the presence of 2.6 mM Ca^{2+}).[92] In human β cells, the Ca^{2+} current can be measured during depolarization to -50 mV and above with maximal amplitude at 0 mV.[93] The major VDCC subtype expressed in mouse β cells are L-type Ca^{2+} channels (Ca_v1.2), which account for more than 50% of all Ca^{2+} currents. Pharmacological inhibition and genetic ablation of the channel result in a severe reduction of GSIS during both first and second phase.[94,95] Although L-type Ca^{2+} channels play a major role in GSIS, this is not the only type of VDCCs expressed in mouse β cells. Around 25% of the Ca^{2+} current in mouse β cells can be attributed to R-type VDCCs (Ca_v2.3), which have an important role in GSIS during the second phase of insulin release, since pharmacological inhibition or genetic ablation of R-type VDCCs results in an unaffected first phase but strongly reduced second phase of insulin secretion.[96] Besides the aforementioned types of VDCCs, P/Q-type VDCCs (Ca_v2.1) are responsible for the remaining 15–25% of Ca^{2+} currents.[94,95]

Human β cells, on the other hand, do not express R-type VDCCs but T-type VDCCs (Ca_v3.2), although it was demonstrated that polymorphisms in the gene encoding R-type VDCC (Ca_v2.3) associate with type 2 diabetes and impaired insulin secretion.[97] Furthermore, P/Q-type VDCCs (CACNA1A gene, 92,2% homology) and L-type VDCCs (CACNA1C gene, 95.1% homology) contribute roughly equally (40-45%) to the Ca^{2+} current in human β cells.[98,99] In contrast to mice, human β cells possess both Ca_v1.2 and Ca_v1.3 L-type VDCCs. Since L-type VDCCs in human β cells activate quickly during depolarization to membrane potentials above -40 mV, Ca_v1.3 seems to be the dominant isoform expressed in human β cells.[93] L-type VDCCs, but not P/Q-type VDCCs, undergo Ca^{2+} dependent inactivation.[98] T-type VDCCs in human β cells is the only low-voltage activated calcium channel activated already at -60 mV, compared with high voltage-activated L-type and P/Q-type channels. T-type VDCCs reach their peak conductance at -30 mV and undergo fast voltage-dependent inactivation, which is half-maximal at -65 mV.[98] Differences between the roles VDCCs play in mouse and human β cells are illustrated in Fig. 2 and 3.

Voltage-dependent sodium channels

Mouse β cells possess Na_V1.3 (Scn3a) and Na_V1.7 (Scn9a) channels, with Na_V1.7 being the quantitatively more important type. Knockout of Scn9a lowers Na^+ current by >85%, disclosing a small Scn3a-dependent component.[100] Inactivation of Na_V1.7 current is half-maximal at ≈ -105 mV while inactivation of Na_V1.3 current is half-maximal at ≈ -50 mV and thus the latter likely contributes to action potential (AP) firing. A contribution of Na^+ channels to AP firing in mouse
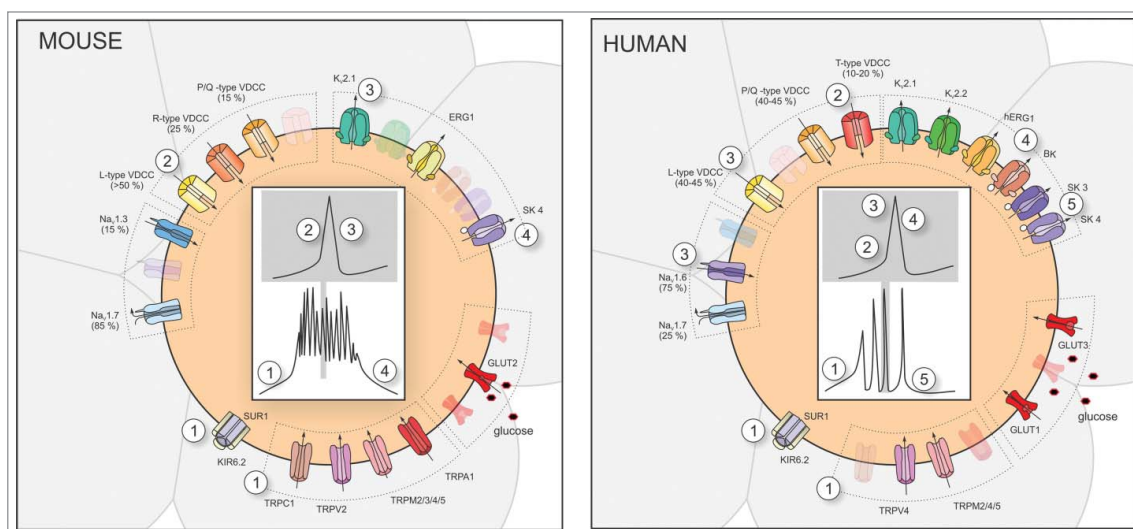

Figure 3. Ion channels involved in glucose-induced electrical activity in mouse (left) and human (right) β cells. Numbers indicate ion channels responsible for the slow depolarization phase, the upstroke and the repolarization phase of an individual spike (action potential) and for the repolarization phase between bursts.

β cells is further corroborated by the finding that the specific sodium channel blocker tetrodotoxin (TTX) reduces the height of APs.[101] Scn3a is obviously important in insulin secretion, since genetic ablation of Scn3a reduces insulin secretion, while genetic ablation of Scn9a does not affect insulin secretion.[100] Taken together, these data suggest that $Na_V1.3$ represents the functionally more important Na^+ channel subtype in mouse β cells.

Human β cells also possess voltage-dependent Na^+ channels that are responsible for the AP upstroke[102] (Fig. 3). The total Na^+ current in human β cells activates instantaneously by depolarization to -30 mV and above, reaches a maximal amplitude at 0 mV and inactivates with a time constant of 2 ms.[103] Sodium channels in human β cells undergo voltage-dependent steady-state inactivation that is half-maximal at -40 mV.[93,99,101] This is >50 mV more positive than in mouse β cells where the quantitatively more abundant Snc9a sodium channels are fully inactivated already at the resting membrane potential (-80 mV) and therefore, in contrast to humans, cannot effectively contribute to the oscillatory pattern of membrane potential (see below). $Na_V1.6$ channels probably account for the major part of Na^+ current in human β cells, and they are important for GSIS since secretion elicited by 6 and 20 mmol/l glucose is reduced by 70% and 55%, respectively, in the presence of TTX.[93] Furthermore, TTX reduces the AP amplitude by ∼10% and prolongs their duration.[104,105] Like in mice, human β cells also express $Na_V1.7$ channels (SCN9a gene, 92% homology), but they account for only 25% of all Na^+ channel transcripts,[106] inactivate at hyperpolarized voltage (≈ -105 mV), and are thus not involved in AP firing.[100]

Voltage- or calcium-dependent potassium channels

In human β cells, the voltage-dependent potassium current consist of at least 2 different components.[93] BK channels which show both voltage- and Ca^{2+}-dependence[107] are responsible for a transient component activated rapidly upon membrane depolarization above -40 mV and with peak amplitude at +30 mV.[93] Above +30 mV, amplitude decreases due to reduced Ca^{2+} entry. Blockade of BK channels increases AP amplitude in human β cells, while in mouse β cells BK channels do not seem to play a major role in electrical activity and insulin secretion.[108] The second

component of voltage-dependent potassium current is due to delayed rectifying K^+ channels. In mouse β cells mainly $K_V2.1$ are expressed, while in human β cells mRNA expression levels suggest that $K_V2.2$ channels are more abundantly expressed than $K_V2.1$[93] but latter has not been confirmed by electrophysiological measurements. In $K_V2.1$ null mice, glucose-induced AP duration is prolonged while the firing frequency (see below) is reduced.[109] Genetic silencing or pharmacological inhibition of $K_V2.1$ revealed > 70% reduction of K_V currents comparing to those from control experiments.[109-111] Furthermore, ablation of $K_V2.1$ improves glucose tolerance and enhances insulin secretion in mice.[109,110] On the other hand, pharmacological inhibition of $K_V2.x$ currents failed to reduce glucose levels *in vivo*, moreover a significant reduction of $K_V2.2$ mRNA levels were observed and glucose-stimulated somatostatin release was enhanced. The later seems to be a reason for paracrine inhibition of insulin secretion and no significant effect on blood glucose lowering *in vivo*.[110] The same mechanisms might be involved in insulin secretion from human β cells since $K_V2.x$ inhibitors, like GxTX-1E and Ry796, profoundly enhance GSIS and somatostatin release in human islets *in vitro*.[110] Furthermore, pharmacological inhibition of $K_V2.1/2.2$ currents using whole-cell patch-clamp suppresses K_V currents in human β cells[93,110] while has a week effect on electrical activity; it increases the half-width of APs, while it has no effect on action potential amplitude.[93] Regarding the effect on insulin secretion, the impact of $K_V2.1/2.2$ in human β cells is controversial. While previously mentioned study clearly shows enhanced GSIS during pharmacological inhibition of Kv2.1/2.2, no effect on insulin secretion is found in stromatoxin treated islets.[93] This different effect on insulin secretion and AP firing between mouse and human β cells may be due to the longer AP duration in mouse compared with human β cells, which results in a greater amount of $K_V2.1$ current being activated.[112] In human β cells the time constant of activation measured at -20 mV when BK channels are active and when $K_V2.1/2.2$ channels are active were ≈2 ms and >10 ms, respectively.[98] This indicates that $K_V2.1/2.2$ channels do not even activate during the AP upstroke (Fig. 3) and therefore blocking them does not affect electrical activity and insulin secretion.[98]

In addition to $K_V2.1$ and $K_V2.2$ channels, $K_V11.1$ (ERG channels) are expressed in mouse and human

β cells (KCNH2 gene is also named ether-a-go-go-related gene, 96.4% homology). Selective blockage of hERG K$^+$ channels increased firing frequency and insulin secretion in human β cells.[113] Similarly, blocking ERG1 increases $[Ca^{2+}]_C$ and therefore promotes insulin secretion also in mouse β cells.[114] Furthermore, ERG channels are responsible for long-lasting tail currents and may thus influence the intervals between spikes.[98] In the heart, hERG1 channels are the molecular basis for the so called long QT-syndrome, and some genetically determined hyperinsulinemias might involve mutations of hERG channels.[113]

Beside the abovementioned voltage-dependent potassium channels, both mouse and human β cells also possess small conductance (SK) Ca^{2+}-activated K$^+$ channels[98,112] (Fig. 2). SK3 and SK4 channels were found in human β cells.[98] Their activity is proposed as the underlying mechanism controlling the bursting pattern (see below). The silent phase between subsequent bursts is attributed to activation of potassium permeability, since blocking SK channels stimulates continuous AP firing.[115] A similar effect was observed in mouse β cells which express SK4 channels (KACNN4 gene, 87.7% homology).[116]

The pattern of membrane potential oscillations

Since the first description of membrane potential changes in mouse β cells upon stimulation with glucose,[117] numerous studies on mice using different experimental paradigms have confirmed that glucose-induced membrane potential changes in β cells occur in the form of bursts of APs (spikes), which follow a
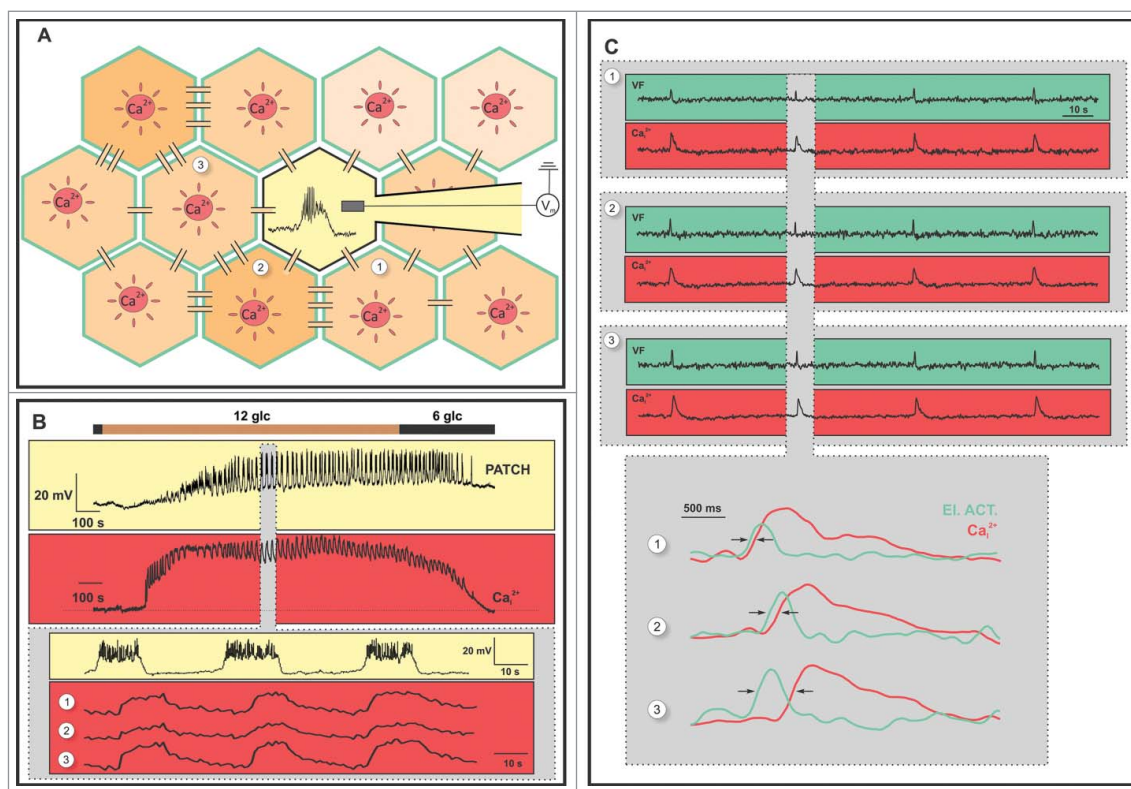

Figure 4. Coupling between membrane potential and $[Ca^{2+}]_C$ oscillations in mouse β cells. (A). A schematic representation of 2 experimental methods. The first involves a simultaneous measurement of membrane potential and $[Ca^{2+}]_C$ changes, using whole-cell patch-clamp (light yellow) and $[Ca^{2+}]_C$ imaging in the neighboring cells (red) with a CCD camera, respectively. The second involves a confocal imaging of membrane potential changes using a voltage sensitive dye (green) and $[Ca^{2+}]_C$ changes using a Ca^{2+} sensitive dye (red). Connections between cells represent gap junctions. More strongly connected cells are represented with a darker cytoplasm while less connected cells are represented with a lighter cytoplasm. (B) The upper trace on the yellow background represents oscillations of membrane potential (bursts) and the upper trace on the red background corresponds to typical $[Ca^{2+}]_C$ oscillations in a single β cell during the same glucose protocol: 6 to 12 to 6 mM glucose. The lower traces depict a close-up of the upper traces showing electrical activity from the same cell as in the upper trace and $[Ca^{2+}]_C$ from 3 β cells from the same islet. (C) Membrane potential dynamics (green) correlated with simultaneously obtained $[Ca^{2+}]_C$ dynamics (red) from 3 β cells of a single islet. The gray rectangle encloses the responses presented below in more detail. Arrows mark the delay between membrane potential and $[Ca^{2+}]_C$ oscillations.

fast, a slow, or a combined pattern coined compound oscillations (Fig. 4B).[118-121]

In human β cells, glucose-induced electrical activity is much more variable compared with mice, with continuous or irregularly spaced APs without any clear bursts reported in the majority of studies[93,104,105,122] (also termed the canine-like pattern, Fig. 5 B, upper trace), or a more organized oscillatory electrical activity with Ca^{2+}-dependent (rodent-like) or Na^+- and Ca^{2+}- dependent (distinctly human) bursts being observed in other preparations from the same studies or in other studies.[104,122,123] Of note, the bursts of APs are typically much shorter in human β cells than in mice and this pattern has also been termed complex APs or rapid bursts (Fig. 5A, upper trace).[104,118,124] In high concentrations of glucose, small amplitude oscillations of membrane potential without any clear APs (the so called wobbly pattern) have also been described (see Table 2 for details).

Irrespective of the species, oscillations in membrane potential reflect a balance between activation of Na^+ channels or VDCCs and K^+ channel activity. The shape of APs triggered in mouse β cells is largely determined by L-type Ca^{2+} channels responsible for the upstroke phase, and voltage-dependent potassium current through $K_v2.1$ channels responsible for the repolarization. In human β cells, AP upstroke is mediated by voltage-dependent sodium channels and L-type Ca^{2+} channels[102] while BK channels are mainly responsible for the repolarization phase.[93,98,104] Because of the kinetic differences between VDCCs and voltage-dependent sodium currents, APs in mouse β cells are much longer compared with human β cells. The silent phase between subsequent bursts is attributed to SK channels for mouse, and to HERG,[103,113] and SK channels for human β cells.[104] Additionally, it has been suggested that K_{ATP} and T-type Ca^{2+} channels might play a role in grouping of APs into rapid bursts in human β cells.[98] Importantly, in human β cells that do not display bursting and continuously spike, the role of SK channels seems to depend on excitability of the cell, with less excitable cells showing an increase in AP firing frequency and more excitable cells not being affected upon SK channel block.[104]

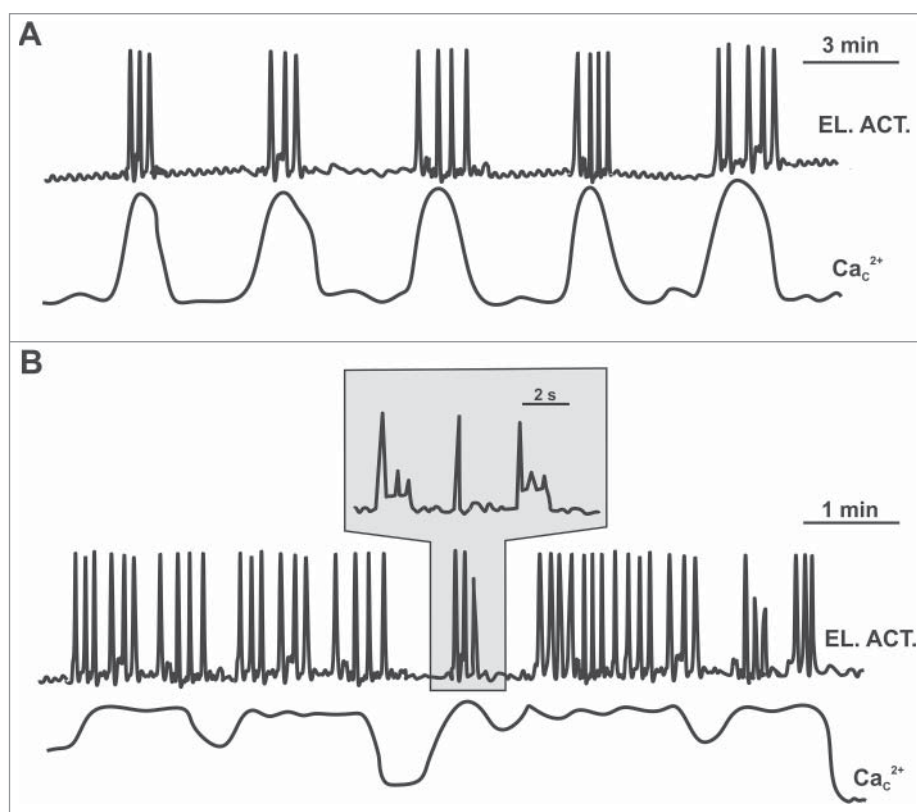In non-stimulatory glucose, β cells are electrically silent. While in mice membrane potential oscillations

Figure 5. A schematic representation of possible scenarios of coupling between membrane potential and $[Ca^{2+}]_C$ oscillations in human β cells. (A) β cells that respond to glucose with an organized oscillatory electrical activity (bursts) exhibit phase-locked $[Ca^{2+}]_C$ oscillations. (B) β cells that respond to glucose with continuous or irregular spikes do not show any clear $[Ca^{2+}]_C$ oscillations.

Table 2. Patterns of membrane potential oscillations in human β cells.

Study	Tissue	Recording	Stimulus	Response	Synchronicity
Falke et al., FEBS Lett 1989 [123]	Isolated β cells from non-diabetic organ donors. Cultured in M199 (7-10 days).	Perforated-patch clamp recordings (0,15 mg/ml amphotericin B), room temperature.	Glc (15), tolbutamide (50 μM), arginine (20 mM)	Bursting (rodent-like) in 7/11 cells (fr not assessed, ampl≈35-55 mV); the provided recordings are not long enough to reliably assess slow oscillations.	n.a.
Pressel et al., J Membr Biol 1990 [102]	Surface β cells in islet fragments. Islets isolated from cadaveric donors, cultured 2–14 d as described in Misler et al. Diabetes 1989.) [221]	Perforated-patch clamp recordings (0,15 mg/ml amphotericin B), room temperature.	Glc (3-10).	Continuous spiking with some rapid bursts (fr not assessed, ampl≈30 mV in 15 glc) in one third to one half of all cells; the provided recordings are not long enough to reliably assess slow oscillations.	n.a.
Misler et al., Diabetes 1992 [105]	Details not provided.	Perforated-patch clamp recordings (nystatin, further details not provided).	Glc (5,6,8,16)	Continuous spiking (canine-like), rapid bursting (distinctly human), or a rodent-like pattern (mentioned but not shown, fr not quantified, ampl≈30-40 mV in 5–8 glc); the provided recordings are not long enough to reliably assess slow oscillations.	n.a.
Misler et al., Diabetes 1992 [122]	Isolated islets from cadaveric donors, incubated 1–7 d in HEPES-buffered CMRL 1066 (5,5 mM glc). Used as fragments/cells.	Perforated-patch clamp recordings (further details not provided, reference to Pressel et al, see above).	Glc (0, 3, 5,10,15), tolbutamide (20 μM)	In 0 glc, resting Vm of -60 mV (6/9), in response to elevated glucose (> 5), > 20 mV depolarization with a variable configuration of APs (4/6, not further described) or without APs (2/6); or a resting Vm of -40 mV and depolarization by 5–10 mV in high glucose (3/9). All cells responded to tolbutamide with at least some APs (not further quantified). The provided recordings are not long enough to reliably assess slow oscillations.	n.a.
Barnett et al. EurJ Physiol 1995 [99]	Isolated islets from 20 cadaveric donors, incubated 1–4 d at 22–24° C in HEPES-buffered CMRL 1066, then brought to 37°C. Used as islets or fragments/cells (dispersion by dispase 0,33 mg/ml).	Perforated patch on isolated cells/clusters of cells using nystatin (0,1–0,2 mg/ml) Insulin secretion from batches of islets using RIA	Glc (3, 5, 10) Glc (3 →6/10, +1μM TTX)	In 3 glc, a resting Vm of -60-(-55) mV; in 5 glc (at background Vm < -45 mV) i) trains of single high amplitude rapid APs (8/23 cells, 4 isolated, 4 in clusters), ii) complex-shaped APs/short bursts (7/23 cells), or iii) wobbly plateau depolarizations without APs (8/23 cells). In 10 glc, membrane potential progressively rised (to a background Vm of -35 mV) and the phenotype changed from i) to iii); the provided recordings are not long enough to reliably assess slow oscillations. TTX nearly abolished rise in insulin secretion in 6, but had no effect in 10 glc.	n.a.
Rosati et al., FASEB J 2000 [113]	Surface β cells in islets and islet fragments. Islets isolated from cadaveric donors, cultured 3–15 d in Hepes-buffered CMRL-1066 (7-10 days).	Perforated-patch clamp recordings (0,15 mg/ml amphotericin B), room temperature.	Glc (15), tolbutamide (50 μM), arginine (20 mM)	Continuous spiking (fr≈3 Hz, ampl≈30 mV in 15 glc); the provided recordings are not long enough to reliably assess slow oscillations.	n.a.
Misler et al., Eur J Physiol 2005 [222]	Cryopreserved-thawed islets from non-diabetic (life-supported) cadavers. Cultured 1–2 d at 37°C, or up to 10 d at RT and then 1–2 d at 37°C. Single cells obtained by incubation in Verseneanddispase or in trypsin-EDTAandmechanical disruption. Single cells cultured up to 5 d in HEPES-buffered CMRL-1066 (5 mM glc)	Perforated patch-clamp	Glc (2, 5, 6, 15) + TTX (500 nM)	In 2 glc, Vm was stable at -60 mV, in 15 glc, background Vm was unstable at - 40 mV, with intermittent large amplitude APs (approaching 0 mV) in 7/7 cells. APs were solitary or grouped. In 6 glc, 3/4 cells showed a similar response as in 15 glc, 1/4 showed no clear response. In 5 glc (to mimic normal postprandial levels), 10/18 cells fired APs (from a background Vm of -55-(-50) mV (fr≈1/4s -2/s). In 6/10 cells APs were affected by TTX and in 4/10 they peaked at -25-(-20) mV and were not affected by TTX. Slow oscillations n.a.	n.a.

Reference	Preparation	Recording method	Conditions	Observations	Coupling / remarks
Manning Fox et al., Endocrinology 2006 [127]	β cells within isolated islets, obtained from dr. Jonathan Lakey and the JDRF Human Islet Distribution Program. Isolated using the Edmonton protocol, cultured in RPMI (5,5 mM glc), culture duration unknown.	Perforated patch using amphotericin B (0,1 mg/ml), performed at 34°C.	Glc (11,1 → 2,8)	**Individual APs** in 11,1 (−40-(−10/0) mV, **complex patterns** (−50/-40-(−15/-10) mV, similar to rapid rodent oscillations during washout in 2,8 mM. A single recording was made.	n.a.
Braun et al., Diabetes 2008 [93]	**Isolated β cells** obtained by dispersing islets from non-diabetic organ donors, using a Ca²⁺ free buffer. Cultured in RPMI 1640 (10 glc (10 glc + 2 L-gln, culture duration unknown).	Standard and perforated-patch clamp recordings, 32–33°C.	Glc (6, 10, 20), **tolbutamide** (100 μM)	Continuous **spiking** (fr not quantified, ampl≈20-40 mV in 6 glc); the provided recordings are not long enough to assess slow oscillations and the provided temporal resolution does not allow to assess rapid bursting.	n.a.
Rorsman et al., Annu Rev Physiol 2013 [98]	**β cells in intact isolated islets.** Further details not provided.	Perforated patch-clamp. Further details not provided.	Glc (6 mM), +**adrenaline** (5 μM), +**carbachol** (20 μM)	**Single APs** from a background Vm of approx. -50 mV (58% of cells); or **oscillatory electrical activity** (suggested to underlie [Ca²⁺]c oscillations) with periods from 1–10 seconds (26% of cells), in both cases large amplitudes (up to 20 mV). The phenotype in the remaining cells (16%) not further specified. Hyperpolarization (by 16 ± 4 mV) in adrenaline and inhibiton of AP firing; depolarization (by 5 ± 3 mV) and stimulation of AP firing in carbachol.	n.a.
Fridlyand et al., Islets 2013 [223]	**β cells** within isolated islets from cadaveric donors. Further details not provided.	Perforated-patch clamp recordings using amphotericin B as described in Jacobson et al., J Physiol 2010 [112]	Glc (14 mM), + **TTX** (500 nM)	**Simple spikes** and **complex spikes** (amplitude approx (−60/-50)-(−20/0) mV, further quantification not provided. Addition of TTX did not abolish AP firing but decreased the maxima of spikes by approx. 10 mV.	n.a.
Riz et al., PLoS Comput Biol 2014 [104]	**Isolated β cells** obtained by dispersing islets from non-diabetic organ donors, using a Ca²⁺ free buffer. Cultured >24 h in RPMI 1640 (7,5 glc).	Perforated-patch clamp recordings (0,24 mg/ml amphotericin B), 31–33°C.	Glc (6 mM), +**various channel blockers** (TTX, ω-agatoxin IVA, UCL¯1684, TRAM-34)	In control conditions (6 mM glucose), 3 main patterns of activity were observed: i) **continuous spiking**; ii) **rapid bursting** (1 burst/1-2 s); and iii) **slow oscillations** in AP firing (period≈5 min).	n.a.
Loppini et al., Phys Biol 2015 [135]	**β cell clusters** obtained by dispersing islets from non-diabetic organ donors, using a Ca²⁺ free buffer. Cultured >24 h in RPMI 1640 (7,5 glc).	Perforated-patch clamp recordings (0,25 mg/ml amphotericin B), 31–33°C.	Glc (6 mM), +**TTX** (0,1 μg/ml)	3/10 cells: occasional **rapid APs** (40 mV) + **small oscillations** (10 mV) around the resting MP; only small oscillations in TTX 2/10 cells: only **rapid Aps, slow oscillations** in TTX; 4/10 cells: **rapid APs**, not responsive to TTX; 1/10: silent in both control cond. and in TTX	Suggested that active and non-active cells are coupled, and that coupling serves to synchronize active cells, not to activate inactive cells. Junctional conductance between 2 cells estimated to be 100–200 pS (0,01-0,02 nS/pF). Electrical coupling sufficient to synchronize spiking and bursting cells, also to partly synchronize slow electrical oscillations.

are observed at 7 mM and above,[120] human β cells oscillate already at 5 mM glucose (seeTable 2) where resting K^+ conductance maintains the membrane potential at around -70 mV while still allowing occasional openings of VDCCs, mainly the T-type Ca^{2+} channels. This results in membrane depolarization, the subsequent opening of additional T-type channels and further depolarization which can then trigger opening of L-type Ca^{2+} channels and VD Na^+ channels. In mouse β cells, the glucose concentration needed for triggering membrane potential oscillations is higher, i.e., > 6 mM, since lower glucose does not provide enough energy to increase $[ATP]_C$ high enough to decrease K^+ conductance to such an extent that L-type VDCCs would open.[98]

In mice (Fig. 4), increasing glucose concentrations changes burst duration from 3 seconds and a frequency of 2–5 oscillations/min (i.e., period of 12–30 seconds) at the threshold concentration to a continuous spiking activity in 20 mM glucose[98,119,121,125,126] As already mentioned, in humans, the responses take several different forms at a given concentration of glucose and are much harder to predict (Fig. 5 and Table 2). For a comprehensive summary of possible patterns of membrane potential changes and factors that might influence them, in Table 2 we briefly review the main findings of all studies that have, to the best of our knowledge, described or even quantified the electrophysiological responses of human β cells to glucose and some other secretagogues (that act via the triggering pathway). All electrophysiological studies on human β cells taken together suggest that with increasing glucose, there is progressive depolarization also in human β cells and that there is at least circumstantial evidence of a trend of patterns switching from no APs to bursts of APs to continuous bursting.[99,127]

Finally, slow or the so-called glycolytic oscillations in membrane potential with a frequency of 0.2-0.4 oscillations/min are observed in mouse β cells under intermediate GCK flux rates and they are not glucose-sensitive.[38] In almost all studies on human β cells, the electrical activity was observed over periods that were too short to enable a reliable assessment of slow oscillations. However, a recent study by Riz et al. provided evidence that slow membrane potential oscillations with a period of 3–5 minutes can be present in human β cells and that they can be accounted for in the model by a glycolytic component.[104]

Coupling between β cells

Within areas of plasma membrane delimited by tight junctions, both mouse,[128,129] and human[130] β cells possess gap junctions. In addition to connexin 30.2,[131] the connexin 36 (Cx36) is considered the major form expressed in β cells in mice,[128,132] and humans[133] and is believed to be the mechanical substrate for β cell functional coupling. Functional coupling describes the finding that changes in metabolism, membrane potential, and $[Ca^{2+}]_C$, are synchronized between different cells by means of depolarization and $[Ca^{2+}]_C$ waves spreading between cells which we describe in more detail in the following chapter, and putatively also by diffusion of small signaling molecules and metabolic intermediates.

Electrophysiological approaches estimated the gap junctional conductance between 2 β cells at about 200–350 pS in mice,[128,129,134] and at about 100–200 pS in humans.[135] Taking into account that each β cell is connected to 6 - 8 neighbors,[136] the conductance between a cell and all of its neighboring cells was estimated at about 2.5-3.5 nS in mice.[137] Recently, gap junctional coupling between β cells has been explored by using fluorescence recovery after photobleaching (FRAP) and this approach suggested a quantitatively similar degree of coupling between β cells in human and mouse islets.[138]

Gap junctions are permeable to a variety of ions and metabolites.[139] The most probable candidate for gap-junctional currents in mice are Ca^{2+} ions, as their flow through gap junctions is belived to be able to spread depolarization from a cell to its neighbors,[134,140,141] although this view has been challenged.[142] Conceivably, in humans both Na^+ and Ca^{2+} ions could be implicated in waves of depolarization, but this remains to be demonstrated. Noteworthy, gap junctions are permeable to glycolytic intermediates and their diffusion could account for synchronization of metabolic oscillations in different cells, although this is not necessary and electrical coupling alone can synchronize metabolic oscillations.[143]

In addition to gap junctions, other means of cell-cell communication, may help control insulin release from β cells. First, ephrin-A-EphA ligand-receptor signaling between β cells has a role in insulin secretion as glucose stimulation enhances insulin secretion via dephosphorylation of the Eph receptor in both mice and humans.[144] In addition, disruption of neural cell

adhesion molecules (NCAM) seems to influence regulation of insulin release in mice.[145] Further, primary cilia of β cells may be involved in the control of insulin release in both mice and humans.[146,147] Moreover, there are several possible auto- and paracrine interactions in both human and mouse islets.[148] Insulin secreted by β cells binds to insulin receptors on plasma membrane and acts in an auto- or paracrine manner by upregulating insulin gene transcription[149] and triggering exocytosis.[150] Further, ATP co-secreted with insulin,[151-153] binds to membrane-bound purinergic P2X and P2Y (for a review of the expression in mice and humans, see ref. 154). In mice, purinergic stimulation seems to have either an excitatory effect via P2X1 and P2X3[155] or an inhibitory effect via P2Y1 receptors.[156,157] ATP was also suggested as an agent synchronizing β cell activity.[158] In fact, brief (15 seconds-long) stimulations by ATP were able to synchronize Ca^{2+} oscillations in isolated individual islets that were not in direct physically contact.[158] In humans, purinergic stimulation evokes a positive feedforward mechanism,[155,159] most probably via P2X3 receptors[160] and a transient increase in $[Ca^{2+}]_C$.[161] Dopamine, yet another molecule co-secreted with insulin, was shown to inhibit GSIS in both humans and mice.[162,163] In contrast, γ-Aminobutyric acid (GABA), which is also co-secreted with insulin, was ascribed a positive feedback role in humans.[164,165] Finally, glucagon secreted by α cells was found to synchronize oscillatory Ca^{2+} activity of β cells in mice, however the exact role of glucagon as a synchronizing factor needs to be explored into more detail as glucagon exhibits out-of-phase oscillations with insulin.[148,166,167] At the level of an islet, things are further complicated by the fact that somatostatin released from delta cells might critically influence the function of both β and α cells.[11] Finally, coupling between β cells from different islets might conceivably play a role *in vivo* and be brought about by means of neurotransmitters, such as acetylcholine,[168-170] or via metabolic feedback from the liver.[143]

The relationship between membrane potential and $[Ca^{2+}]_C$ changes, waves, and functional connectivity

Twenty years after the first description of glucose-induced membrane potential changes in microdissected mouse islets,[117] oscillatory $[Ca^{2+}]_C$ changes were recorded in dispersed mouse β cells,[171] but their temporal characteristics were difficult to reconcile. More specifically, dispersed β cells showed widely heterogeneous and much slower $[Ca^{2+}]_C$ oscillations compared with typical frequencies of bursts. In the early nineties, detailed studies on coupling between membrane potential and $[Ca^{2+}]_C$, as well as $[Ca^{2+}]_C$ and insulin secretion on isolated mouse islets showed that in intact islets $[Ca^{2+}]_C$ changes closely resemble and are temporally tightly coupled to membrane potential changes and that depolarization and $[Ca^{2+}]_C$ waves are probably the synchronizing mechanism.[125,172,173] In addition, the observed $[Ca^{2+}]_C$ oscillations are closely matched by pulses of insulin secretion.[174-177]

A large number of studies using different electrophysiological and imaging approaches support the view that membrane potential and $[Ca^{2+}]_C$ changes in different β cells in mouse islets are not completely in phase, but phase-locked with a temporal delay and this delay can be explained by depolarization and $[Ca^{2+}]_C$ waves spreading from cell to cell via gap junctions.[134,140,141,172,177-182] Further, both waves spread in the same direction and with the same velocity and in every β cell $[Ca^{2+}]_C$ oscillations closely follow the bursts of electrical activity but are of longer durations than the bursts (Fig. 4C).[141,183,184]

All studies that have quantified the direction and velocity of $[Ca^{2+}]_C$ waves have reported comparable values of wave velocities and also agree in that at least over the time frame of a few minutes, the direction of the waves remains rather constant.[140,141,182,185] Moreover, experiments and existing mathematical models suggest that the origin of the wave is usually a single cell or a small cluster of cells at islet periphery, also called the pacemaker region, that heterogeneity in β cell excitability and gap junction coupling is needed to fully reproduce the experimentally observed properties of synchronicity and $[Ca^{2+}]_C$ waves, that the cells in the pacemaker region are more excitable than others are, that they determine the $[Ca^{2+}]_C$ oscillation period for the rest of the islet, and that such regions might arise by chance due to heterogeneity.[182,185] As to why $[Ca^{2+}]_C$ waves usually originate at islet periphery, considering that cells with elevated excitability can probably be found in all regions of an islet, we wish to suggest that this might be due to a lower number of neighboring cells and thus a weaker hyperpolarizing contribution from less excitable cells.

The concept of depolarization and $[Ca^{2+}]_C$ waves being the synchronizing mechanism seems to be in conflict with more recent reports showing that functionally, islets behave as small world networks where signals of widely spaced β cells may be more similar to each other than to nearby cells. More specifically, if $[Ca^{2+}]_C$ oscillations are compared for every cell pair and a so called functional connection is drawn between cells whose signals are similar enough as to exceed a certain threshold, it turns out that a relatively few cells harbor a large proportion of all functional connections.[56,88] It is not intuitively clear how a spreading $[Ca^{2+}]_C$ wave could allow for such behavior. This conflict has received yet another layer of complexity with the recent report by Johnston and colleagues suggesting that hub cells are in fact *bona fide* pacemakers that convey signals to other cells called followers.[186] In comparison with the more traditional view of synchronization via waves, according to the functional coupling analysis, there seems to be more pacemakers (1-10% of all β cells) and they are not limited to the periphery but are distributed across the whole cross section of an islet.

In our opinion, the above 2 ideas are not mutually exclusive. Namely, it is possible that there is a temporal sequence of activation within the population of hubs, such that they are activated one after another and before other cells and that the depolarization and $[Ca^{2+}]_C$ wave spread to follower cells from them. A study seems to support the idea presented above.[187] Namely, cellular heterogeneity and nearest neighbor coupling, without any additional direct long range physical connections (via nerves or cilia, see above), are sufficient to account for both the properties of experimentally observed membrane potential and $[Ca^{2+}]_C$ waves and the degree of synchronization they enable,[140,141,172,178,180,182-185] and the small-world character of the functional connectivity pattern with hubs and followers.[56,88,186,188-190] In this model, in 3D there are strings of more strongly coupled direct neighbors that enable propagation of fast $[Ca^{2+}]_C$ waves over long distances, and enable a high degree of synchronicity between widely separated cells that thus assume the role of hubs when studied from the functional network perspective. The remaining cells are less well coupled to hubs and to each other and thus their signals correlate with each other to a lesser extent, making them less well connected in the functional network.[187] This model is also consistent with our report of segregated communities of nearby cells that become more and more interconnected in higher glucose, when gap junctional connectivity is believed to increase,[188] and also with reports of decreased connectivity when gap junction coupling is reduced (see below for details).[56,186]

There are certainly several open questions regarding connectivity in general and the nature of hub cells in specific and they have been recently summarized in a comprehensive review.[190] Here we only wish to point out that in accordance with predictions,[182,185] evidence is accumulating that the hub or pacemaker cells are metabolically highly active,[186,191] and display some phenotypic characteristics of immature cells, such as high levels of glucokinase, a low insulin content and a low expression of pancreatic duodenum homeobox-1 (Pdx1) and NK6 homeobox 1 (Nkx6.1). A less mature phenotype is also consistent with a lower threshold for insulin secretion.[192] Notably, immature cells can indeed be found in varying numbers in adult islets of Langerhans.[193] However, considering the role of gap junctions in β cell connectivity and the finding that adult β cells express more Gjd2 (the gene encoding Cx36) and that the expression of this gene has been linked with an increased expression of transcription factors determining a mature β cell phenotype,[194] hub cells are probably not phenotypically immature in all regards.

For several reasons, much less is known about normal patterns of $[Ca^{2+}]_C$ changes, waves, their relationship with depolarization, and also about functional connectivity in human islets.[45,195,196] First, human pancreatic tissue is more difficult to obtain than mouse tissue, and in a large proportion of the few existing studies, it has been collected from patients with pancreatic disease. Second, islets have been isolated in different ways and the culture duration and conditions have varied widely in studies on human islets, which is a possible source of phenotypic variability in their responses to glucose, as has been demonstrated convincingly for mouse islets.[197] Finally, $[Ca^{2+}]_C$ changes have been recorded by different methods and different stimulation protocols have been used in studies on human islets. Table 3 provides a comprehensive summary of the various aspects discussed above in more detail. In brief, at the one end of the spectrum of possible scenarios lies the view that human islets do not display organized fast $[Ca^{2+}]_C$ oscillations and that even if they occasionally occur,

they are synchronized only locally.[198] Putatively, the structural substrate of this behavior is the unique cytoarchitecture of human islets, where β cells are believed to be less orderly distributed within islets and make a larger number of heterotypic contacts with α and delta cells than in mouse islets.[198,199] At the other end, we have the possibility that human islets display orderly fast $[Ca^{2+}]_C$ oscillations (albeit with a lower frequency) that are at least partially[200] or even completely synchronized between different cells.[201] These 2 types of responses seem to be supported by morphological studies showing that in human islets, β cells are organized in clusters[202] or ribbon-like patterns,[203] and that in 3 dimensions they probably form uninterrupted syncytia and sometimes even islets that are similar to the stereotypical mouse mantle-core islets.[12,204,205] Between these 2 extremes lie the reports of $[Ca^{2+}]_C$ oscillations that were slower, less orderly, or were not observed in all islets included in a study (Table 3). Noteworthy, in a few recent studies synchronization of $[Ca^{2+}]_C$ signals in different cells has been analyzed in human islets and compared with mouse islets. The percentage of area synchronized[206] was similar in mouse and human islets[138] and human islets showed similar but more clustered functional connectivity.[56,186] It remains to be determined whether these different phenotypes reflect true biological variability or are at least partly due to methodological factors. Additionally, the presence of waves, the coupling between membrane potential and $[Ca^{2+}]_C$ changes, as well as between $[Ca^{2+}]_C$ and insulin secretion, remain to be investigated into more detail. Recently, evidence has been provided that β cells in human islets electrically communicate with each other similarly as in mice, but it remains to be explained how exactly the observed extracellular slow potentials relate to membrane potential oscillations.[207] Furthermore, it is difficult to understand how the described properties of $[Ca^{2+}]_C$ oscillations can be reconciled with patterns of electrical activity in human islets. The rapid bursts are too rapid to explain the observed periods of $[Ca^{2+}]_C$ oscillations and it is also unclear whether they are synchronized between different cells. The recently observed slow membrane potential oscillations seem to be a better candidate for explaining $[Ca^{2+}]_C$ oscillations observed in human islets in one study,[104,200] although they are too slow to explain the faster $[Ca^{2+}]_C$ oscillations observed in another.[201] Moreover, the release of insulin from isolated human islets occurs in a fairly regular pulsatile manner, with a period between approximately 4 and 10 minutes,[208,209] and it remains to be clarified how the irregular patterns of membrane potential and $[Ca^{2+}]_C$ are compatible with this finding. Noteworthy, the recently reported period of slow glycolytic membrane potential oscillations in β cells[104] could explain the period of insulin secretory pulse at the lower bound of the reported range.[209]. Fig. 5 illustrates the 2 abovementioned extremes of possible scenarios.

Changes in coupling, membrane potential and Ca oscillations, as well as functional connectivity in type 2 diabetes

There is currently no direct evidence for an involvement of disrupted coupling in pathogenesis of T2DM in humans. Yet, a body of circumstantial evidence suggests that disrupted coupling could play an important role. On the one hand, diminishing or abolishing gap junctional coupling by pharmacological or genetic tools in mice leads to a disrupted pattern of membrane potential and $[Ca^{2+}]_C$ oscillations, as well as to an absence of $[Ca^{2+}]_C$ waves.[87,182,185,210] Further, some studies have found an increased basal insulin secretion and a diminished amplitude of the first phase, as well as absence of oscillations in secretion during the second phase of stimulated insulin secretion from islets lacking gap junctions.[210,211] These alterations in insulin secretion from isolated islets have not been supported by all studies.[210,212] However, measurements in Cx36 knockout mice in vivo strongly support the view that first phase insulin secretion is reduced and the second phase is non-oscillatory in these animals, which could account for the observed glucose intolerance.[211] On the other hand, chronically increased glucose has been shown to decrease expression of Gjd2 and this may contribute to the effect of glucotoxicity.[213] Similarly, lipotoxicity has been shown to involve Cx36 downregulation.[56] In addition, cytokines released from the adipose tissue and in islets under hyperglycemia and hyperlipidemia are able to significantly decrease Cx36 gap junction coupling, disrupt the synchronicity and pattern of $[Ca^{2+}]_C$ oscillations, and reduce the number of hubs and correlated links.[138,186,214] Most importantly, it has been demonstrated in high-fat diet fed mice that a prediabetic milieu leads to an increase in basal insulin secretion, an impairment in Cx36 mediated intercellular

Table 3. Patterns of $[Ca^{2+}]_{iC}$ oscillations in human β cells.

Study	Tissue	Recording	Stimulus	Response	Synchronicity
Kindmark et al., FEBS Lett 1991 [161]	i) **isolated islets** (3 patients with normal glc tolerance, Whipple's resection, minced tissue, collagenase, cultured 1–4 d in RPMI 1640 (11,1 mM glc) ii) **insulinoma cells**, (1 patient)	**Fura-2** (340/380 - 515) **Spectrofluoro-meter** (fr = 1 Hz)	**glc** (3→11; 0→10 / 20) **K⁺** (25 mM) **tolbutamide** **ATP** **Diazoxide**	Only islets with an increase in Ca^{2+} considered successful (> 50% of all): i) decrease, then increase with stable (1 islet) or »fluctuating« (1 islet) plateau; ii) no initial decrease, plateau with irregular oscillations (period 70–80 s) (1 islet).	n.a.
Misler et al., Diabetes 1992 [122]	**Isolated islets** (non-diabetic life supported cadavers, age and gender unknown (collagenase + Ficoll gradient), cultured 1–7 d in 5,5 mM glc	**Fura-2** (340/380 - 505) spectrofluorometry (fr unknown) perforated patch-clamp	**glc** (3→10→20)	i) no response to glc with subsequent response to tolbutamide (4/11 islets); ii) oscillations superimposed on a plateau (4/11); iii) slow rise to a plateau without oscillations (2/11); iv) short transients on an unchanging baseline, coalescing to a spike.	n.a.
Kindmark et al., Diabetologia 1994 [224]	**Isolated islets** (9 patients, normal, impaired, and diabetic tolerance, f and m, 57–83 y (mean 65,0 yr) minced pancreas tissue, collagenase, cultured 1–4 d (in 11 mM glc)	**Fura-2** (340/380 - > 510) spectrofluorometry (fr = 1 Hz) perforated patch-clamp	**glc** (3→10 / 12; 15 mM for electrophysiology, **tolbutamide** (100 μM)	i) fairly regular slow oscillations in both glc and tolbutamide (period = 2–3 min); ii) in some a monophasic increase continuous firing of APs (5-6 Hz), no bursts.	n.a.
Hellman et al., Diabetologia 1994 [225]	i) **isolated cells,** ii) **clusters,** iii) **isolated islets** (8 cadaveric donors, 39 ± 5 yr, gender unknown, ductal distension and Ficoll gradient, cultured 3–7 d in 6,1 mM glc, sent by air and stored for another 1–3 d in 5,6 mM glc)	**Fura-2, Indo-1** PMT or video camera, fr unknown	**glc** (3→20 glc, 3→3+1mM **tolbutamide,** 1 mM **tolbutamide**+20 mM **glc,** addition of **glucagon** (10 nM or **glycine** (10 mM) in isolated islets **glc** 3→20	In isolated cells and clusters large amplitude oscillations from the basal level, fr = 0,1-0,5/min (type a) and superimposed on an elevated Ca (type b), slow oscillations were also present in tolbutamide, glucagon and glycine transformed type a to a plateau. In islets plateau with superimposed oscillations (period 2–5 min).	Analyzed in clusters, well synchronized slow oscillations, no pacemakers. In isolated islets well synchronized, different amplitudes, no quantification of synchronization. Good overlap with type a oscillations and insulin release. Higher glucose affected amplitude, not frequencies. In isolated islets, tybe b pulses of insulin wered described (but not Ca).
Martin et al., Cell Calcium 1996 [201]	**Isolated islets** 2 cadaveric donors (age unknown) and 2 patients (44 and 80 yr), Tissue minced and collagenase, hand picking, cultured overnight in 5,5 mM glc	**Fura-2** (340/380-510) **CCD camera**, fr. = 0,167 Hz)	glc 3→11, 11→16,7; **tolbutamide** 50 μM	A triphasic response (31/42 islets), decrease, a rapid transient increase, then oscillations (fr. = 1 ± 0,3/ min), or (11/41 islets), slow rise to a plateau w.o. osc.; Upon elevating glc, prolonged duration or slow oscillations (0,15 ± 0,2/min) (14/17) or plateau (3/17). Tol: reversible plateau-like increase (7/7).	Synchrony in all 3 phases in all regions of an islet (13/15 islets) or regions 10–15 s out of phase (2/ 15).

Reference	Preparation	Method / sensor	Stimulus	Observations	Synchronization
Cabrera et al., PNAS 2006 [198]	Isolated islets (5 cadaveric donors, 48 ± 7 y of age), liberase + purification, cultured in CMRL (5,5 mM glc)	Fura-2 (340/380-510) CCD camera, fr. unknown.	glc 3→11 mM	No general oscillatory responses (> 70 islets from 10 preparations), only localized oscillations (single cells or clusters). In dispersed cells, slow oscillations were observed, period = 5min (not further quantified).	Not quantified in detail, qualitatively assessed that there is no sync.
Quesada et al., Diabetes 2006 [200]	Isolated islets (5 cadaveric donors (22,6 ± 5,3 yr, gender unknown; minced pancreas tissue, then collagenase, recorded acutely)	Fluo-3 (488/505-530) Ca^{2+} Confocal microscopy at the equatorial plane (t = 8 μm, fr = 0,5-0,25 Hz	glc via perifusion, 3→11, 11→16,7	6/41 β cells (10 islets): sustained increase without oscillations 35/41 β cells: oscillations in 11 mM (0,45 ± 0,02 /min)	Assessed qualitatively, in clusters only, sometimes more widely distributed after several minutes (4/7 islets) or a more general but weak coupling (3/7 islets). Heterogeneity in the degree of coupling, even from the same preparation.
Hodson et al. J Clin Invest 2013 [56]	Isolated islets (21 normoglycemic donors, isolation centers (UK, I, CH), cultured in RPMI in 5,5 mM glc	Spinning-disk confocal; Fluo-2 (491-525/25) CCD, fr = 0,5 Hz	glc 3→11 GLP-1 (20 nM)	Stochastic fast oscillations, large deflections in a subpopulation of β cells upon GLP-1.	Moderate correlation in 11 mM glc, large synchronous deflections in Ca upon GLP-1.
De Marchi et al., J Biol Chem 2014 [54]	Isolated islets (2 donors, purchased (Tebu-bio), cultured in RPMI in 5,5 mM glc	Genetically encoded cameleon sensor YC3.6$_{cyto}$ (430 - 480/40 and 535/30), CCD, fr = 0,5 Hz	glc via perifusion, 1→16,7 mM, inhibition by oligomycin (2,5 μg/ml) and diazoxide (100 μM)	Transient increase, then stable plateau with occasional superimposed »spikes."	n.a.
Johnston et al. Cell Metab 2016 [186]	Isolated islets (from distant isolation centers (I, CH), cultured in RPMI in 5,5 mM glc	Spinning-disk confocal; Fluo-2 (491-525/25) CCD, fr = 2-8 Hz	glc 3→11	Not shown.	Complex network theory analyses. Similar topological characteristics as mouse islets, more clustered/ compartmentalized synchrony.
Farnsworth et al. J Biol Chem 2016 [138]	Isolated islets (from distant isolation centers (I, CH), cultured in RPMI in 5,5 mM glc	Wide field microscope; Fluo-4 (490/40-525/36) CCD, fr = 1 Hz	glc 11	Not shown.	Percentage area synchronized ≈90% in controls.

coupling,[215] as well as to disorganized $[Ca^{2+}]_C$ oscillations in islets isolated from these mice.[56] Similar findings have been obtained on islets from ob/ob [216] and db/db [217] mice. In sum, it seems that the series of events connecting altered gap junctional coupling with impaired glucose tolerance via a disrupted pattern of synchronization of membrane potential and $[Ca^{2+}]_C$ oscillations operate both ways in mice. In other words, diminished coupling can result in glucose intolerance and vice versa.

Regarding the relevance of this series of events for humans, disruptions to insulin dynamics similar to the ones described above are observed in humans with prediabetes and T2DMs.[218,219] Moreover, chronic exposure of human islets to elevated free fatty acids reduces Cx36 expression, disrupts the synchronicity of the $[Ca^{2+}]_C$ response, functional connectivity, and insulin release in response to GLP-1 added to stimulatory glucose (but not to stimulatory glucose alone). Importantly, donor BMI seems to be negatively correlated with islet responses to GLP-1.[56] It was further demonstrated that proinflammatory cytokines decrease Cx36 gap junction coupling in isolated human islets, albeit in a spatially more uniform manner that in mouse islets,[138] and that glucolipotoxicity induces hub failure in isolated human islets, i.e., it reduces the number of hubs and functional links between β cells.[186] The above recent lines of evidence suggest that similar although not same pathophysiological mechanisms linking glucose intolerance and gap junctional coupling might be at work in humans. It has been proposed that inherent structural differences between human and mouse islets[12] might explain the differences in the spatial pattern of cytokine-induced Cx36 downregulation,[138] and that in addition to the inherent differences in islets, patterns of meal consumption might account for the differential responsiveness of human and mouse islets to glucose and incretins.[56] To get a more complete picture of β cell connectivity as a target of various pathological processes and future therapies, see some resourceful recent reviews.[91,189,190,196,220]

Conclusions

At all levels of the triggering pathway to insulin secretion, from the entry of glucose into β cells to the pattern of $[Ca^{2+}]_C$ changes that are believed to trigger insulin release, there are important functional differences between mice and humans. At present, our knowledge about these differences is largely limited to the types and functional properties of transporter and channel proteins being expressed in the 2 species, whereas there is still much confusion regarding the normal or prevailing patterns of membrane potential and $[Ca^{2+}]_C$ changes and intraislet synchronization of these 2 crucial signals in the signaling cascade, especially in human islets. Even less is known about the mechanisms of interislet synchronization in mouse and human islets that are believed to govern the *in vivo* oscillations in plasma insulin concentration. In addition, we deliberately limited ourselves to functional aspects in adults and developmental facets were only touched upon. The accumulating evidence regarding interspecies similarities and differences in other pathways to insulin secretion and the role of neurotransmitters, paracrine factors from other islet cells, and hormones, such as incretins, deserves separate reviews.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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